ENCYCLOPEDIA of GEOBIOLOGY
Volume Editors
Joachim Reitner is Professor of Paleontology, Head of the Department of Geobiology, and Managing Director of the Museum, Collections and Geopark, at the University of Göttingen, Germany. He is also Editor-in-Chief of Lecture Notes in Earth Sciences (Springer), Co-Editor of Facies (Springer), and Associate Editor of the Geomicrobiology Journal (Taylor & Francis). Dr. Reitner’s research focuses on the interplay between organisms and their metabolic processes with various abiotic parameters. Many geological processes can be understood as geo-physiological processes, allowing chemical reactions to proceed that would never occur under standard thermodynamic conditions. Therefore, a major thrust of Dr. Reitner’s research is the investigation of the evolution of these processes, which are visible in biosignatures and biomineralization patterns, and in their interaction with biogeochemical cycles. Among his many honors and accolades, Dr. Reitner is the recipient of the G. W. Leibniz Award from the Deutsche Forschungsgemeinschaft.

Volker Thiel is Professor of Organic Geochemistry in the Geoscience Center at the University of Göttingen, Germany. Dr. Thiel has been involved in geobiological research for some 15 years, with a focus on the use of organic molecules as chemical tracers (biomarkers) for biogeochemical pathways. His research interests include lipid biomarkers as indicators for biogeochemical processes, molecular fossils, biological formation, and turnover of methane, and microbial control on mineral formation. The results of his studies have significantly contributed to the characterization of microbial processes associated with methane turnover in modern and ancient environments. Much of Dr. Thiel’s current work is devoted to new approaches to enhance the spatial resolution of biomarker analysis in geobiological systems. He is member of the Editorial Board of the journal Geobiology (Wiley-Blackwell).

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ENCYCLOPEDIA OF GEOBIOLOGY

edited by

JOACHIM REITNER
VOLKER THIEL
University of Göttingen
Germany
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Preface

Geobiology is a highly cross-disciplinary field that explores the present and past relationships that life has with non-living matter. “Biosphere meets Geosphere” perhaps most parsimoniously describes the fundamental concept of Geobiology. In 1991, Peter Westbroek, a Dutch paleontologist and influential protagonist of Geobiology defined the field in a book entitled “Life as a Geological Force: Dynamics of the Earth”, thus motivating a new way of thinking in the geosciences. His fundamental work on processes of biomineralization in coccolithophorid algae (Westbroek and de Jong, 1983) greatly contributed to the understanding of metabolic processes controlling mineral formation. Westbroek’s thinking was influenced by James Lovelock’s Gaia concept (Lovelock, 1988) which advocated the importance of biological processes with regard to global change over time. Other early pioneers of the Geobiology concept were the Russian scientist Georgy Adamovich Nadson (1903), who recognised microorganisms as geological agents, and the Swiss geologists Johannes Neher and Ernst Rohrer who discovered the role of microbes in dolomite formation (Neher and Rohrer 1958) and their presence in the deep biosphere of crystalline rocks (Neher and Rohrer 1959). In 1971, the German geoscientist Gerd Lüttig introduced a new discipline that merged aspects of geology and biology and called it “Lithobiontik”. This was the first time that research on geological and biological interactions received a well-founded definition:

“Die Erdgeschichte ist umschreibbar als eine ständige Auseinandersetzung zwischen Gesteinswelt (lithos) und Lebewelt (bios). Die Gesamtheit der entsprechenden Vorgänge zu erforschen, ist Aufgabe der Lithobiontik, einer Forschungsrichtung im Grenzgebiet zwischen Geologie und Biologie.” — Earth history can be described as a permanent interaction between the geosphere (lithos) and life processes (bios). To investigate these processes is the mission of Lithobiontics, a new research discipline between Geology and Biology.

Kenneth Nealson and William Ghirose colleagues provided, in a conceptual review written for the American Academy of Microbiology (Nealson et al. 2001), a modern and concise perception of Geobiology as “Exploring the interface between the Biosphere and the Geosphere”.

The interplay between biological and geological processes has shaped the Earth and driven the evolution of its biodiversity from the early dawn of life, some four billion years ago. Since then, organisms have been responding to a changing global environment and in turn, have themselves altered the chemical and physical settings on our planet. Geobiology strives to identify these cause-and-effect chains in both modern environments and the geological record. Its goal is to provide, on different time and spatial scales, an ‘organismic’ biological perspective on Earth’s environmental evolution (Knoll and Hayes, 1997).

Shifting their focus from traditional morphological studies made by paleontologists, geobiologists continue to develop their ability to relevant chemical and molecular signatures within living and non-living materials, and to interpret these signatures to better understand the geological record and better predict our course into the future. A key issue in most geobiological studies is the elucidation of ancient environmental states, and to understand the evolution of biological processes and their geological consequences. Meaningful signatures that reveal such processes are not limited to visible remains, but also encompass for instance, organic molecules, minerals, petrofabrics and isotope patterns. Organisms may alter their chemical environment, thereby giving rise to the production or destruction of minerals, rocks, atmospheric gases and even fossil fuels. An understanding of these processes creates enormous potential with respect to issues of environment protection, public health, energy and resource management. Geobiological research and
education is therefore rapidly growing, with topics becoming more common in University and High School curricula. With Geobiology issues including many spectacular aspects (e.g., early life, deep biosphere, gas hydrates, black smokers etc.), public interest also increases concomitantly.

Moving beyond the borders of classical core disciplines, scientists from a broad range of disciplines are actively involved in geobiological studies. There is no common perception of a ‘typical’ geobiologist, but as an underlying requirement, scientists need to adapt concepts and utilize methodologies from other disciplines to exploit the full potential of Geobiology. Fields united under the umbrella of geobiology include, but are not limited to: geology and paleontology, mineralogy, microbiology, molecular biology, genomics, organic and inorganic geochemistry, oceanography, astrobiology and (paleo)ecology. This incomplete list provides an impression of the implications of geobiological research, and that the super-discipline of Geobiology is ‘greater than the sum of its parts’ (Nealson et al. 2001).

The Encyclopedia of Geobiology was compiled to provide clear explanations of current geobiological topics. It is not structured as a student textbook, but rather to quickly access particular terms and concepts in self-contained entries. We hope that this volume will also tempt the casual reader to browse and become curious about the different facets and foci of Geobiology - following the philosophy of the late Founding Series Editor, Rhodes Fairbridge, we will be most content if an original discovery may emerge from perusing a variety of entries.

We are thankful to our authors, both distinguished ‘old hands’ and young researchers from five continents, who have synthesized the particular subdisciplines for this volume. The preparation of this book was greatly aided by the input we received from our Editorial Board members Hans-Joachim Fritz, Andreas Kappler, Kurt Konhauser, Pamela Reid, and Xingliang Zhang, and we wish to express our sincere thanks to them for their invaluable support and encouragement.

Göttingen, October 2010
The Editors-In-Chief
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References


ACETOGENS

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Synonyms
Homoacetogens

Definition
Acetogens are defined as anaerobic prokaryotes that use the acetyl-CoA pathway for the (a) reductive synthesis of the acetyl moiety of acetyl-CoA from CO₂, (b) conservation of energy, and (c) assimilation of CO₂ into biomass.

Introduction
Acetogens utilize the acetyl-CoA “Wood–Ljungdahl” pathway as a terminal electron-accepting, energy-conserving, CO₂-fixing process. The reductive synthesis of acetate from CO₂ differentiates acetogens from organisms that synthesize acetate by other metabolic processes. Although the production of acetate as a sole reduced end product is not a part of the definition, because the acetogen might not form acetate in situ or when cultured in the laboratory.

The first acetogen, Clostridium aceticum, was isolated from soil by the Dutch microbiologist K. T. Wieringa in 1936. This spore-forming, mesophilic bacterium was shown to grow at the expense of H₂–CO₂ and synthesizes acetate according to the following stoichiometry (Wieringa, 1939–1940):

\[ 4\text{H}_2 + 2\text{CO}_2 \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O} \]

In 1936, this reaction constituted a unique mechanism for the fixation of CO₂. Unfortunately, the culture of C. aceticum was lost and no further work was done until it was re-isolated in the early 1980s (Braun et al., 1981). In 1942, F. E. Fontaine and coworkers isolated the second acetogen, Clostridium thermoaceticum (later reclassified as Moorella thermoacetica), a spore-forming, thermophilic bacterium that catalyzed the near stoichiometric conversion of glucose to acetate (Fontaine et al., 1942):

\[ \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 3\text{CH}_3\text{COOH} \]

C. thermoacetica became the most extensively studied acetogen and was used to resolve the enzymology of the acetyl-CoA pathway in the laboratories of two biochemists, H. G. Wood and L. G. Ljungdahl (Drake and Daniel, 2004).

The demonstration that \(^{14}\text{CO}_2 \) was incorporated equally into both carbons of acetate by C. thermoacetica was the first \(^{14}\text{C}\)-study in biology (Barker and Kamen, 1945). The synthesis of acetate from two molecules of CO₂ was later confirmed by Wood using \(^{13}\text{CO}_2 \) and mass spectrometry (Wood, 1952). Both studies demonstrated that acetogens featured a new autotrophic mechanism for the fixation of CO₂. The acetyl-CoA pathway, also referred to as the Wood–Ljungdahl pathway, is now recognized as a fundamental component of the global carbon cycle, important to primary production in subsurface ecosystems, and essential to the metabolic potentials of not only acetogens but also methanogens, sulfate-reducers, and anammox bacteria (Wood and Ljungdahl, 1991; Drake, 1994; Schouten et al., 2004).

The acetyl-CoA Wood–Ljungdahl pathway

The acetyl-CoA pathway is a reductive, linear, “one-carbon” process (Figure 1) in contrast to cyclic CO₂-fixing processes (Calvin cycle, reductive tricarboxylic acid cycle, and hydroxypropionate cycle). The two branches of the acetyl-CoA pathway merge at the synthesis of acetyl-CoA that is converted to either acetate or...
assimilated into biomass (Drake, 1994; Drake et al., 2006). Acetyl-CoA synthase (ACS) not only catalyzes the reduction of CO₂ to CO and the synthesis of acetyl-CoA but also oxidizes CO to CO₂. Thus, the acetyl-CoA synthase is also termed CO dehydrogenase (CODH). Energy conservation occurs during the reductive synthesis of acetate by substrate-level phosphorylation and chemiosmotic processes. For each glucose that is converted to three acetates, four molecules of ATP are produced by substrate-level phosphorylation (ATPSLP). However, when growth occurs under autotrophic conditions, there is no net gain in ATPSLP, and growth is dependent upon translocation of protons or sodium ions.

The relatively simple features of the linear acetyl-CoA pathway and the fact that metals and metal sulfides do the biochemical work of CO₂ fixation in the key enzymes of the pathway (i.e., CODH and ACS) suggest that the acetyl-CoA pathway might have been the first autotrophic and early respiratory process on earth and, thus, important in the evolution of life (Wood, 1991; Miyakawa et al., 2002; Russel and Martin, 2004). It has been proposed that biochemistry started when marine CO₂ from volcanoes and hydrothermal H₂ met at a hydrothermal vent rich in metal sulfides, where an analogue of the exergonic acetyl-CoA pathway catalyzed the synthesis of organic precursors to fuel primordial biochemical reactions.

**Phylogenetic diversity of acetogens**

Over 100 acetogenic bacterial species have been isolated to date from very diverse habitats, including marine and freshwater sediments; salt lake soda deposits; tundra, forest, and agricultural soils; the subsurface; fecal material; and marine and salt marsh plants (Drake et al., 2006, 2008). Acetogens display extreme genetic diversity, having genomic G + C contents that vary between 22 mol% (Clostridium ljungdahli) to 62 mol% (Holophaga foetida). Acetogens have been assigned to 22 different bacterial genera including Aciditomaculum, Acetoanaerobium, Acetobacterium, Acetohalobium, Acetonema, Bryantella, “Butyribacterium,” Caloramator, Clostridium, Eubacterium, Holophaga, Moorella, Natroniella, Natronincola, Oxobacter, Ruminococcus, Sporomusa, Syntrophococcus, Tindalia, Thermoacetogenium, Thermoanaerobacter, and Treponema (the name in quotation marks has not been validated). The diverse habitat range of acetogens demonstrates that they are adapted to a broad range of in situ conditions. Many acetogens are sporeformers (e.g., Byrer et al., 2000), a feature that aids in surviving unfavorable in situ conditions, and some acetogens have connecting filaments (Küsel et al., 2001) that might facilitate communication. The usage of the acetyl-CoA pathway for autotrophic assimilation of carbon and acetate utilization by methanogens suggests that Archaea also can grow via acetogenesis. Indeed, the methanogen Methanosarcina acetivorans C2A uses the acetyl-CoA pathway to convert carbon monoxide to both acetate and methane, that is, methane is not the sole reduced end product of this methanogen (Rother and Metcalf, 2004; Lessner et al., 2006). Similarly, the archaea Archaeoglobus fulgidus VC16 can grow via CO-dependent acetogenesis (Henstra et al., 2008).

Only some acetogenic genera, like Moorella and Sporomusa, are monophyletic, but many acetogens are phylogenetically dispersed within genera that contain non-acetogenic species. Thus, the phylogenetic position of 16S rRNA gene sequences is usually inadequate for resolving the functional identity of a potential acetogen. To date, only some highly specific 16S rRNA-based probes and primers have been designed to target subsets of acetogenic taxa (Küsel et al., 1999). Molecular approaches based on the analysis of functional genes central to the acetyl-CoA pathway (e.g., a gene for formyltetrahydrofolate synthetase) are still limited due to problems of probe specificity (Leapheart et al., 2003).

**Functional diversity of acetogens**

Acetogens utilize a wide variety of electron donors and electron acceptors and can engage alternative terminal
electron-accepting processes when challenged with O₂ (Drake et al., 2006). Many acetogens can utilize one or more terminal electron-accepting processes in addition to acetogenesis. For example, nitrate is a preferred electron acceptor for *M. thermoacetica* and is dissimilated to nitrite and ammonium (Seifritz et al., 1993), and perchlorate is a preferred terminal electron acceptor for *Moorella perchloratireducens* (Balk et al., 2008). The engagement of diverse redox couples enables acetogens to form junction points within and between biological cycles at the ecosystem level (Drake and Küsel, 2005). Acetogens are known to use the following alternative terminal electron acceptors that yield diverse end products listed below:

Fumarate → succinate  
Methoxylated phenylacrylates → methoxylated phenyl propionates  
Nitrate → nitrite  
Nitrite → ammonium  
Thiosulfate → sulfide  
Dimethylsulfoxide → dimethylsulfide  
Perchlorate → chloride  
Chlorate → chloride  
Pyrurate → lactate  
Acetaldehyde → ethanol  
Protons → hydrogen gas

CO₂, H₂, carbohydrates, alcohols, carboxylic acids, dicarboxylic acids, aldehydes, substituent groups of various aromatic compounds, and certain halogenated substrates are examples of substrates that acetogens can oxidize. Although hexoses or pentoses are utilized by most of the acetogens isolated to date, none of these isolates appear to be able to degrade high molecular weight polymers, such as cellulose and lignin. Most acetogens have the capacity to produce more than acetate as their sole reduced end product, and this capacity is dependent on the availability of both reductant and terminal electron acceptors, including CO₂. Thus, referring to acetogens as homoacetogens is usually a misnomer.

In anoxic habitats, acetogens compete with primary fermentors for monomeric compounds that are derived from the initial breakdown of cellulose and lignin and with secondary fermentors for fermentation products. Thermodynamic considerations suggest that acetogenesis should not be a highly competitive microbial process. Nonetheless, acetogens can outcompete methanogens for H₂ in freshwater sediments with low pH and low temperature and in the hindgut of certain termites (Conrad et al., 1989; Breznak and Kane, 1990; Drake et al., 2006). Microbially produced acetate can provide up to 100% of the energy requirement of wood-feeding termites. The attachment of H₂-consuming acetogenic spirochetes to H₂-producing protozoa provides H₂ concentrations above the known H₂-threshold values for acetogens.

Another ecological advantage might be the low oxygen sensitivity of certain acetogens (Figure 2). Acetogens have been classically referred to as obligate, if not strict, anaerobes, and many enzymes central to acetogenesis are extremely sensitive to O₂. However, acetogens have been isolated from oxic habitats, like soils and the rhizosphere of macrophytes that release O₂ through their roots, indicating that such acetogenic species must cope with periods of oxidative stress. Acetogens contain numerous enzymes that can reductively remove O₂ and its toxic by-products (e.g., superoxide and peroxide), when the concentration of O₂ is relatively low (Drake et al., 2006). These enzymes include peroxidase, NADH-oxidase, rubredoxin oxidoreductase (a superoxide reductase), rubrerythrin (a peroxidase), superoxide dismutase, catalase, and cytochrome bd oxidase (Das et al., 2001, 2005; Küsel et al., 2001). Acetogens can shift the flow of reductant away from the acetyl-CoA pathway to alternative terminal electron-accepting processes that are less sensitive to O₂ and operate at higher redox potentials than the very low standard redox potential of the CO₂/acetate half-cell reaction (−290 mV). For example, *Clostridium glycolicum* RD-1 (isolated from seagrass roots) is an aerotolerant acetogen that switches from acetogenesis to

![Diagram](https://example.com/diagram.png)

**Acetogens, Figure 2** Mechanisms by which acetogens cope with oxidative stress. **X**, products (e.g., H₂, formate, lactate) that are derived from the partial oxidation of carbohydrates (in some cases, short-chain polymers [e.g., stachyose] that are not substrates for the acetogen); e⁻, electron. (Modified from Müller et al., 2004, and used with kind permission from Horizon Bioscience.)
classic fermentation in response to O₂ (Küsel et al., 2001). Acetogens can also form symbiotic relationships with O₂-consuming microaerophiles and aerotolerant fermenters (Gößner et al., 1999). The microaerophile can be a fermentative non-acetogen that has the capacity to consume O₂ and forms fermentation products (e.g., lactate, formate, and H₂) that can be used by the acetogen for acetogenesis. Such interactions can protect acetogens from oxidative stress and form trophic linkages in habitats with fluctuating redox conditions.

Conclusions

It has been estimated that approximately 10¹² kg of acetate is synthesized per year via H₂-driven acetogenesis in sediments and the hindgut of termites (Breznak and Kane, 1990), a number that is fivefold greater than the annual amount of methane produced via the methanogenic reduction of CO₂. Such estimates accentuate the potential importance of acetogens and acetogenesis to the global carbon cycle. However, in situ acetate turnover measurements are complicated and acetogens catalyze a large number of redox reactions, suggesting that their in situ activities are not restricted to acetogenesis. Thus, although acetate forms an important trophic link in a wide variety of ecosystems, in situ information on acetogens and their activities are often theoretical.

Bibliography


Cross-references

Anaerobic Transformation Processes, Microbiology
Bacteria
Carbon (Organic, Cycling)
Fermentation
Hydrogen
Methanogens
Microbial Communities, Structure, and Function
Microbial Degradation
Origin of Life

ACID ROCK DRAINAGE

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Synonyms
Acid mine drainage; AMD; ARD

Definition
Acid mine and acid rock drainage (AMD/ARD) refer to the extremely acidic (pH < 3), metal-rich waters that are derived from the weathering of sulfidic minerals when exposed to air, water, and microorganisms (Figure 1; Nordstrom and Alpers, 1999; Bond et al., 2000):

Acid Rock Drainage, Figure 1. An AMD stream at a nickel-copper mine in northern Ontario, Canada. The orange and red colours in the stream are Fe-oxyhydroxide and Fe-sulphate mineral precipitates.

Environmental significance
AMD/ARD is considered to be the most important and widespread global-mining-industry-related pollution problem (Rowe et al., 2007). The main characteristics of ARD/AMD are (1) low pH, (2) high concentrations of dissolved heavy metals, and (3) high concentrations of sulfate (SO4\(^{2-}\), Tsukanoto et al., 2004). Total dissolved metal concentrations as high as 200,000 mg/L and dissolved SO4\(^{2-}\) as high as 760,000 mg/L have been reported to be associated with AMD (Nordstrom et al., 2000).

AMD/ARD generation
AMD is generated through a combination of chemical and biological (microorganism) processes by which sulfidic minerals are converted to sulfates and iron oxyhydroxides.
when exposed to water and oxygen (see *Sulfide Mineral Oxidation*). The overall oxidative dissolution of pyrite (FeS$_2$), a widespread common mine constituent, and associated with AMD generation is given by *Equation 1*:

$$\text{FeS}_2 + 15/4\text{O}_2 + 7/2\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_3(s) + 2\text{SO}_4^{2-} + 4\text{H}^+ \tag{1}$$

This process progressively increases water acidity, resulting in the mobilization of metals from mine wastes. AMD generation is typically amplified when the reactions involved in the overall process, described in *Equation 1*, are catalyzed by aerobic Fe- and S-oxidizing bacteria, such as *Acidithiobacillus* (formerly *Thiobacillus*, e.g., *Acidithiobacillus ferrooxidans*; see *Biomining (Mineral Bioleaching, Mineral Biooxidation)*; *Sulfide Mineral Oxidation*). However, factors such as levels of microorganism activity, pH, sulfide mineral area, crystallography, type of sulfide mineral, temperature, and oxygen concentration all interactively influence the rate of AMD generation (Berghorn and Hunzeker, 2001).

*Figure 2* provides a schematic illustrating the overall AMD generation process. Initiation at neutral pH occurs through the release of ferrous iron (Fe$^{2+}$) and acid (H$^+$) into solution (*Equation 2*):

$$\text{FeS}_2(s) + 7/2\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + 2\text{SO}_4^{2-} + 2\text{H}^+ \tag{2}$$

At pH values <4, increased solubility of ferric iron promotes Fe$^{3+}$ pyrite oxidation (*Equation 3*) at far greater rates than those associated with O$_2$ oxidation:

$$\text{FeS}_2(s) + 14\text{Fe}^{3+} + 8\text{H}_2\text{O} \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+ \tag{3}$$

At pH values greater than 4, O$_2$-driven oxidation of ferrous iron to ferric iron (*Equation 4*) results in Fe oxyhydroxide formation (*Equation 5*) and iron removal through precipitation:

$$\text{Fe}^{2+} + \frac{1}{4}\text{O}_2 + 2\text{H}^+ \rightarrow \text{Fe}^{3+} + 3\text{H}_2\text{O} \tag{4}$$

$$\text{Fe}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_3(s) + 3\text{H}^+ \tag{5}$$

In addition, as the pH decreases below 4, iron-oxidizing bacteria, many of which are adapted to pH values of 2–4, substantively increase rates of Fe oxidation (*Equation 4*) by factors greater than 10$^6$. For these reasons, the oxidation of Fe$^{2+}$ to Fe$^{3+}$ is often referred to as the rate-determining step in the acid generation process. Thus, once the system reaches pH values less than 4, a self-perpetuating cycle of Fe$^{3+}$-driven oxidation of pyrite and regeneration of Fe$^{3+}$ through Fe oxidizers is initiated, producing more acidity and Fe$^{2+}$ until either ferric iron or the pyrite waste is depleted (*Figure 2*).
Treatment of acid mine drainage

AMD waters must be treated to remove metals and raise the pH before they are discharged to a receiving environment (Neculita et al., 2007). Numerous approaches, often characterized as active or passive, exist to treat AMD. Active processes refer to operations that require a relatively high degree of management and continuous input of consumables, while passive processes theoretically require minimal management and other costs once they are established (Johnson, 2006). The most widespread method is active treatment involving the addition of a chemical neutralizing agent or base (i.e., lime or Ca oxide, CaCO₃, Na/Mg carbonates, Na/Mg hydroxides) to increase water pH and precipitate metals as hydroxides and carbonates. Over the last 20 years, there have been considerable new developments for AMD treatment including constructed wetlands (Johnson and Hallberg, 2002), bioreactors, and permeable reactive barriers (Blowes et al., 2000), which take advantage of the increasing knowledge of microbially driven reactions. Much of the biotechnology developments rely on sulfate-reducing bacteria (SRB) to treat AMD. Fundamentally, bioreactors use SRB to drive sulfide precipitation, removing metals from the AMD solution (Equations 6 and 7):

\[
2\text{CH}_2\text{O} + \text{SO}_4^{2-} \rightarrow 2\text{HCO}_3^- + \text{H}_2\text{S} \quad (6)
\]

\[
\text{H}_2\text{S} + \text{M}^{Z+} \rightarrow \text{MS}_{(a)} + 2\text{H}^+ \quad (7)
\]

where M\text{Z}⁺ is a cationic metal such as Cu or Ni.

In addition, SRB will drive alkalinity generation (Equation 6) (Koschorreck and Tittel, 2007). However, all emerging treatment approaches are challenged with regard to long-term efficiency. In large part, this reflects the challenges in identifying the complex and dynamic reactions involved in the overall AMD process. The exact mechanisms and controls involved in reactions as well as an understanding and identification of the microbial flora required to efficiently drive desired reactions for bioreactors over the long-term have not yet been elucidated (Edwards et al., 2000; Bernier and Warren, 2005; Bernier and Warren, 2007). In particular, growing evidence of the importance of consortia or mixed microorganism communities in driving AMD-associated processes underscores the need to more fully investigate the interactive roles of bacteria, system pH, [Fe], [O₂], and other [metals] involved in the process to narrow the gap in determining effective long-term strategies to mitigate AMD.

Summary

AMD/ARD refer to metal-rich acidic waters that are generated through the exposure of sulfidic minerals in mine wastes to water, oxygen, and microorganisms. These discharges are a significant global pollution issue associated with mining activities. Both sulfur- and iron-oxidizing bacteria are involved with Fe-oxidizing bacteria playing a key role in the increased rates of AMD generation compared to abiotic (nonbiologically stimulated oxidation) sulfidic wastes. AMD must be treated for the high levels of acidity and metals before it can be released to the environment. New treatment strategies are emerging that include some combination of chemical and biological approaches; however, all are challenged with regard to long-term efficacy. New research evaluating the roles of environmentally mixed communities of microorganisms demonstrates the opportunities to harness environmentally occurring microorganisms to combat AMD.

Bibliography


ACIDOPHILES

“Acidophiles” are organisms thriving in environments below pH 5. For details, see entries “Extreme Environments,” “Biomining (Mineral Bioleaching, Mineral Biooxidation),” “Hot Springs and Geysers,” “Hydrothermal Environments (Marine) and “Acid Rock Drainage.”

ACRITARCHS

Acritharchs are organic-walled acid-resistant microfossils known from the Proterozoic and throughout the Phanerzoic. The position of these microfossils is still uncertain. A number of acritarch genera have been assigned to Green Algae; others are considered to bear some resemblance to the cysts of dinoflagellates. For more information, please refer to entries “Algae, Eukaryotic” and “Protozoa.”

AEROBIC METABOLISM

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Synonyms
Aerobic respiration; Oxygen metabolism

Definition
Aerobic metabolism comprises the reduction of molecular oxygen as electron acceptor of aerobic respiration, its use as cosubstrate in the degradation of certain compounds, and reactions leading to the detoxification of partially reduced oxygen species.

Introduction
Life has evolved in the absence of molecular oxygen (see Chapter Early Earth). Therefore, it is not surprising that basic metabolic pathways involved in growth and cell division (like DNA replication or protein synthesis) do not depend on the presence of molecular O₂. The evolution of the oxygen-producing (oxygenic) phototrophs dramatically changed the situation of living organisms. Molecular oxygen raised the redox potential of the environment and enabled microorganisms to respire with a much higher energy yield than before. Although oxygen is involved in a limited number of reactions only, its presence or absence has fundamental impact on biogeochemical processes.

Aerobic degradation of organic matter
Aerobic respiration consumes a major part of the photosynthetically produced O₂ and about 90% of the organic matter (see Chapter Carbon Cycle). Aerobic (oxygen-metabolizing) organisms normally oxidize their substrates completely to CO₂. In most cases, O₂ is not directly involved in this process. Substrate oxidation generates reduced electron carriers by means of the respiratory chain. Oxygen is reduced as the last step of the respiration process.

Respiratory chain
Respiration is a membrane-bound process that generates a chemiosmotic proton gradient across the membrane (Mitchell, 1966). Respiratory chains are composed of series of enzymes, which are organized in multienzyme complexes and use cofactors that allow the stepwise transport of reducing equivalents to oxygen (Figure 1). In eukaryotes (plants, animals, fungi) it takes place within the mitochondria, which are assumed to have evolved from endosymbiotic bacteria. In prokaryotes (Bacteria, Archaea), the respiratory chain is located in the cytoplasmic membrane. The respiratory chain of the bacterium Paracoccus denitrificans appears to have many similarities to the mitochondrial type (Harold, 1986). However, many bacteria have variations compared to the mitochondrial type presented in Figure 1.

Energetics and ATP conservation
Most reducing equivalents are fed into the respiration process via NADH₂, which has a standard redox potential (E₀°) of −0.32 V. The terminal electron-accepting redox pair O₂/H₂O has redox potential of +0.82 V. Thus, the usable redox potential is about 1.14 V. From this redox potential one can calculate a standard ΔG°' of −440 kJ per mol of O₂. Assuming that ATP conservation requires about −75 kJ per mol, the conservation of 5 to 6 ATP per O₂ reduced is possible. As a consequence of the high ATP yield, aerobic organisms can form more biomass from the same amount of substrate than anaerobic organisms.

Use of oxygen as cosubstrate
Molecular oxygen is used as a cosubstrate in some catabolic and anabolic reactions (Lengeler et al., 1999). The enzymes involved are called mono- or di-oxygenases, depending on the number of oxygen atoms transferred to their substrate. Oxygenases are used by aerobes to activate certain substrates that do not have functional groups that allow a simple degradation. They catalyze the initial hydroxylation of hydrocarbons to yield alcohols that can easily be metabolized further. They are also involved in the ring cleavage of aromatic compounds and in the first oxidation step of ammonia oxidation. Anaerobic organisms are either unable to oxidize the substrates activated...
by means of oxygenases or possess different degradation pathways.

Anabolic reactions making use of molecular oxygen are the biosynthesis pathways of tetrapyrroles, ubiquinones, pyrimidines, sterols, and unsaturated fatty acids. Again,
Eukaryotic algae are a collection of extremely diverse, nonrelated organisms that perform photosynthesis in plastids, permanent organelles of green, brown, or bluish colors derived from endosymbiosis. In contrast to plants, algae do not form embryos.

Plastids are known from plants and many algal groups (Williams and Keeling, 2003). Plastids are the organelles of plants and eukaryotic algae that harbor photosynthesis and synthesize many chemical compounds also important for other biochemical pathways (e.g., aromatic amino acids, heme, isoprenoids, and fatty acids); nonphotosynthetic plastids are known from plants and many algal groups (Williams and Keeling, 2003). Recently, from strategies of synthesizing various information, that is, mainly DNA sequence analyses of concatenated genes as well as the incorporation of discrete characters such as insertions, deletions, and gene fusion events, and consideration of morphology and biochemistry, a phylogeny of eukaryotes has emerged which consists of five “supergroups” (Keeling, 2004). Algae are scattered among four of the five major eukaryotic groups. The “Plantae” comprises exclusively eukaryotes with plastids derived from primary endosymbiosis, that is, they are bound by two membranes, derived from the inner and outer membranes of a cyanobacterium which was transformed into an organelle through uptake and retention by the host cell followed by the loss of much of its genome (Keeling, 2004). The Plantae are formed by three distinct but related lineages, the viridiplantae (which comprises all green algae and land plants or embryophytes), the rhodophytes (red algae), and the glaucophytes. The “Chromalveolates” comprises large and abundant algal groups such as the heterokont algae (e.g., diatoms and brown algae), the haptophytes, the alveolates (which include the dinoflagellates and apicomplexans), and the cryptomonads. Only a single lineage of each of the “Excavates” and “Rhizaria” includes algae, the euglenoids and chlorarachniophytes. Except for the Plantae, all other eukaryotic algal lineages acquired their plastids through secondary endosymbiosis (for reviews see McFadden, 2001; Keeling, 2004; Palmer, 2003). Secondary plastids are surrounded by four or three membranes which resulted from phagocytosis of a primary alga, which was either a red alga or a green alga. In secondary plastids of both red and green origin, the primary algal nucleus is highly reduced (in the cryptomonads and chlorarachniophytes) or more commonly lost altogether (e.g., in the heterokonts and haptophytes; Keeling, 2004).

**Cross-references**

- Anaerobic Transformation Processes, Microbiology
- Archaea
- Bacteria
- Carbon (Organic, Degradation)
- Critical Intervals in Earth History

**ALGAE (EUKARYOTIC)**

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**Algæ (eukaryotic)**

Eukaryotic algae are a collection of extremely diverse, nonrelated organisms that perform photosynthesis in plastids, permanent organelles of green, brown, or bluish colors derived from endosymbiosis. In contrast to plants, algae do not form embryos.

Algæ is a term of convenience and refers to a collection of highly diverse organisms that undertake photosynthesis and/or possess plastids (Keeling, 2004). Many authors even include the prokaryotic cyanobacteria into the algae, because they exhibit a life-style rather similar to their eukaryotic counterparts and often share the same habitat with eukaryotic algae. Cyanobacteria form the origin of plastids (for reviews see McFadden, 2001; Keeling, 2004; Palmer, 2003). Plastids are the organelles of plants and eukaryotic algae that harbor photosynthesis and synthesize many chemical compounds also important for other biochemical pathways (e.g., aromatic amino acids, heme, isoprenoids, and fatty acids); nonphotosynthetic plastids are known from plants and many algal groups (Williams and Keeling, 2003). Recently, from strategies of synthesizing various information, that is, mainly DNA sequence analyses of concatenated genes as well as the incorporation of discrete characters such as insertions, deletions, and gene fusion events, and consideration of morphology and biochemistry, a phylogeny of eukaryotes has emerged which consists of five “supergroups” (Keeling, 2004). Algae are scattered among four of the five major eukaryotic groups. The “Plantae” comprises exclusively eukaryotes with plastids derived from primary endosymbiosis, that is, they are bound by two membranes, derived from the inner and outer membranes of a cyanobacterium which was transformed into an organelle through uptake and retention by the host cell followed by the loss of much of its genome (Keeling, 2004). The Plantae are formed by three distinct but related lineages, the viridiplantae (which comprises all green algae and land plants or embryophytes), the rhodophytes (red algae), and the glaucophytes. The “Chromalveolates” comprises large and abundant algal groups such as the heterokont algae (e.g., diatoms and brown algae), the haptophytes, the alveolates (which include the dinoflagellates and apicomplexans), and the cryptomonads. Only a single lineage of each of the “Excavates” and “Rhizaria” includes algae, the euglenoids and chlorarachniophytes. Except for the Plantae, all other eukaryotic algal lineages acquired their plastids through secondary endosymbiosis (for reviews see McFadden, 2001; Keeling, 2004; Palmer, 2003). Secondary plastids are surrounded by four or three membranes which resulted from phagocytosis of a primary alga, which was either a red alga or a green alga. In secondary plastids of both red and green origin, the primary algal nucleus is highly reduced (in the cryptomonads and chlorarachniophytes) or more commonly lost altogether (e.g., in the heterokonts and haptophytes; Keeling, 2004).

**Green algae (Plantae supergroup)**

The green algae are photosynthetic eukaryotes with double membrane-bound plastids (derived from primary endosymbiosis), stacked thylakoids, and the chlorophylls $a$ and $b$. Their other accessory pigments are the carotenoids, beta-carotene, and xanthophylls. The main reserve polysaccharide is starch which is deposited inside the plastid (Lewis and McCourt, 2004; Pröschold and Leliaert, 2007). A few genera (e.g., *Prototethea*) contain nonphotosynthetic plastids which lost their pigments secondarily. Apart from plastid-associated features, a unique stellate structure linking nine pairs of microtubules in the flagellar base of those green algae that are motile or produce motile stages is characteristic, as well as cell walls composed of cellulose which is present in most green algae (Graham et al., 2009). Most of these characters are shared with embryophytes (land plants) and, therefore, the green algae are difficult to define to the exclusion of the latter. The green algae have evolved in two major lineages (Friedl, 1997; Karol et al., 2001; Lewis and McCourt, 2004). One lineage, the chlorophyte lineage (Chlorophyta sensu Bremer, 1985; Sluiman, 1985) comprises the majority of green algal diversity (i.e., the green algae in a more narrow sense or the majority of what have been traditionally called green algae) with a bewildering array of morphologies attributable to at least five different morphological organizations (Pröschold and Leliaert, 2007). Examples of microscopic green algae reported from freshwaters with high calcification levels are rather small coccoid forms (e.g., *Chlorella*-like, Figure 1a; *Desmochloris*, Figure 1d; *Pseudendocloniopsis*, Figure 1f), coccoids that form coenobia (e.g., *Scenedesmus*, Figure 1b) or are of filamentous organization which may easily disintegrate into unicells (e.g., *Stichococcus*, Figure 1e) or form small thalli of branched filaments (e.g., *Pseudendoclonium*, Figure 1e). Also some monadoid (flagellated unicells) green algae and the siphonochladoïd *Cladophora* are well known from highly calcifying freshwater habitats. Several marine green algae of coenocytic organization precipitate carbonate and belong to the
calcaneous algae, for example, the orders Dasycladales (Kingsley et al., 2003) and Caulerpales (Stanley et al., 2010). Whether the aforementioned freshwater green algal taxa are involved in carbonate precipitation is still unknown, but microscopic freshwater green algal genera with calcite deposits on their surfaces are several members of Zygematales (e.g., Oocarcium) and Gongrosira (Rott et al., 2009; Freyert and Verrecchia, 1998). The other major
Rhodophytes – red algae (Plantae supergroup)

Red algae are a very large and diverse group of microscopic algae and macroalgae. They are best known for their economic and ecological importance. Though they are present in freshwater with several genera, the majority of red algal genera occur on tropical and temperate marine shores, where they play important ecological roles (Graham et al., 2009). Certain calcified red algae, known as corallines, form hard, flat sheets that consolidate and stabilize coral reef crests, that is, coralline red algae protect reefs from wave damage and, thus, they are regarded as important keystone organisms. Of well-known economic importance are the species of Porphyra and other red algal species which are grown in mariculture operations for use as human food. Several other marine genera are cultivated or harvested for the extraction of gelling polysaccharides such as agarose and carrageen (Graham et al., 2009).

Corallines are known from fossil records 500 million years old (Lower Ordovician; Riding et al., 1998). The oldest fossil record of a (non-coraline) red alga may represent the fossil taxon Bangiomorpha pubescens which was described from the 1,200-million-year-old Hunting Formation in the Canadian Arctic (Butterfield, 2000). It is most notable that B. pubescens even represents the earliest putative record for sex and taxonomically resolvable complex multicellularity among eukaryotes (Saunders and Hammersand, 2004). For a review of fossil records of the various taxonomic groups of red algae, see Saunders and Hammersand (2004).

The plastids of red algae lack chlorophyll b and c, but contain phycobilins (allophycocyanin, phycocyanin, and the red pigment phycoerythrin) as accessory pigments which are located in phycobilisomes on the outer surface of the thylakoids. The red algal plastids are bounded by two membranes and derived from a cyanobacterial primary endosymbiosis (Keeling, 2004). They produce floridean starch which is deposited in the cytoplasm. The rhodophytes lack flagella and centrioles in all stages of their life history (Graham et al., 2009).

The red algae (Rhodophyta) are a distinct eukaryotic lineage, whose members are united in the phylogenetic analyses of a nuclear, plastid, and mitochondrial genes (for review see Yoon et al., 2006). They belong to the Plantae supergroup of eukaryotes (Keeling, 2004) and are believed to form a sister group with the green line (Martin et al., 2002). Their divergence has been estimated ca. 1.500 million years ago (Yoon et al., 2004). Based on molecular evidence, the red algae are partitioned into seven classes, six of which have a rather simple body structure and reproduction (Yoon et al., 2006). The florideophytes (class Florideophyceae) are the monophyletic group of red algae that exhibit more complex bodies and reproduction (Saunders and Hammersand, 2004). The ecologically significant corallines, the economically important genus Chondrus (used as food and for the extraction of carrageen; Graham et al., 2009), and the vast majority of other modern red algal species are florideophytes; they are divided into several monophyletic groups (subclasses; Saunders and Hammersand, 2004; Le Gall and Saunders, 2007).

Coralline red algae represent not only a large carbon reservoir, but they also consolidate sediment and build landforms, and they occupy harder substrate in the...
world’s oceans than any other group of photosynthetic organisms. Unlike most other calcified algae that deposit carbonate as aragonite, the coralline red algae form calcite and it is believed that each family of coralline red algae is defined by only one crystalline modification of calcium carbonate (Borowitzka, 1977). The red algae show evidence of a surprising variety of calcification mechanisms, but the location of deposition is even more diverse. Mineralization may occur throughout the walls of nearly all vegetative cells, it may occur within the walls of only specialized cells, or it may be located in an intercellular organic matrix between cells, but not within the cell walls (Pueschel et al., 1992). Calcification in corallines is poorly understood, however, it seems clear that corallines deposit high magnesium calcite in a definitive pattern within their cell walls via the membrane pumps that control the flux of calcium and carbonate with the control of crystal orientation by organic wall components (Zankl, 2007). Coralline red algae can be the deepest algae (−50 to −110 m, Dullo et al., 1990) and many species occur in great abundance into the Arctic and subarctic. Coralline red algae in coral reefs are ubiquitous and dominant (Glynn et al., 1996; Keats et al., 1997), and their abundance in cryptic and shaded environments can be greatly underestimated (Littler, 1972). In temperate systems, the accumulation of unattached living or dead coralline algae forms large maerl beds (Martin et al., 2006). They also provide food for herbivores with hardened mouthparts (Steneck and Dethier, 1994) and surfaces for the settlement of invertebrate larvae (Adey, 1998). Coralline algal abundance, size, shape, and species composition in maerl beds vary considerably depending on their location, and several environmental factors influence coralline algal distribution. Maerl beds are highly sensitive to desiccation and are found from the low intertidal zone to depths of 150 m (Foster, 2001). Furthermore, coralline species are sensitive to seasonal variations in temperature inducing changes in their physiology, for example, Lithothamnion spp., growth rate increases with water temperature (Potin et al., 1990). Despite their ecological importance in temperate systems, primary production and calcification of coralline algae has been mainly investigated in tropical areas (Chisholm, 2003; Payri et al., 2001). A number of different associations between sponges and several species of red macroalgae have been described in literature (Trautman et al., 2000, 2002). The symbiotic association between the haplosclerid sponge Haliclona cymiformis and the red macroalga Ceratodictyon spongiosum is common on shallow coral reefs of the Indo-West Pacific, where it has been described as being one of the most conspicuous organisms found in these areas (van Soest, 1990). This symbiosis seems to be an obligatory association as neither sponge nor alga has ever been identified growing independently or in association with other species (Morrissey, 1980).

Glaucophytes (Plantae supergroup)
Glaucophytes (or glaucocystophytes) form a small group of microscopic algae exclusively found in freshwater environments; only about 13 species of glaucophytes have been described so far. They represent the third lineage of the plant supergroup, which is defined by plastids bound by a double membrane, that is, which is derived from primary cyanobacterial endosymbiosis (Keeling, 2004). Glaucophyte plastids are unique among plastids in that they are retaining the prokaryotic peptidoglycan layer between their two membranes. Therefore, they may represent an intermediate in the transition from a cyanobacterial endosymbiont to plastid; molecular phylogenetic analyses show the glaucophytes as the earliest divergence within the plant supergroup (Keeling, 2004; Martin et al., 2002). Glaucophyte plastids share the accessory photosynthetic pigments, phycobilins, which are organized in phycobilisomes (small particles on the outer surface of thylakoid membranes) with cyanobacteria and rhodophytes. For a review on glaucophytes, see Bhattacharya and Schmidt (1997) and Steiner and Löffelhardt (2002).

Photosynthetic stramenopiles (chromalveolates)
Stramenopiles form a monophyletic group of photoautotrophic as well as heterotrophic organisms which are characterized by swimming cells possessing at least one flagellum with distinct tripartite tubular hairs (Graham et al., 2009; Andersen, 2004). Most stramenopiles have two distinctly different flagella and, therefore, are also known as heterokonts. They have a long flagellum with tripartite hairs (tinsel or immature flagellum) to pull the cells through the water and a shorter smooth one (whiplash or mature flagellum) that lacks tripartite hairs, often with a light-sensing flagellar swelling at its base (Graham et al., 2009; Andersen, 2004). Photosynthetic stramenopiles or heterokont algae usually appear brown or golden brown (and are thus sometimes referred to as chromophytic algae) due to the presence of characteristic accessory pigments, fucoxanthin or vaucheriaxanthin, and at least one form of chlorophyll c (except in eustigmatophytes). The plastids are derived from a secondary endosymbiosis event with a red alga involved and surrounded by four membranes (McFadden, 2001; Keeling, 2004; Palmer, 2003). In most photosynthetic stramenopiles, a chloroplast endoplasmatic reticulum is present, that is, plastid and nucleus are structurally connected. The photosynthetic stramenopiles comprise a tremendous morphological diversity: a large variety of unicellular or colonial algae, including the diatoms with silica frustules as walls, multicellular simple filaments (e.g., in the xanthophytes) as well as the macroscopic complex brown algae forming a brown canopy at sea shores. Based on detailed analyses of pigment composition, ultrastructure and molecular phylogenies more than a dozen of different classes of photosynthetic stramenopiles have been described (Graham
et al., 2009; Andersen, 2004). Some photosynthetic stramenopiles do not form mineralized cysts or scales and, therefore, they have no fossil record. Examples are the brown seaweeds (Phaeophyceae), the yellow green algae (Xanthophyceae), and the inconspicuous eustigmatophytes. In the context of geobiology also xanthophytes and eustigmatophytes may be important, as they have been reported from freshwaters with high calcite precipitation (see Figure 1g, e; Arp et al., 2010). However, their role in the calcification processes is still unclear. Therefore, in this entry, only those classes of photosynthetic stramenopiles will be discussed that produce mineralized cell coverings which are important in fossil deposits and, therefore, may be of particular interest in geobiology. Siliceous skeletons from silicoflagellates (class Dictyochophyceae) and silica scales and cysts are commonly formed in synurophytes and chrysophytes.

**Silicoflagellates** are unicellular, flagellated, and photoautotrophic stramenopiles which are characterized in at least one life cycle stage by the formation of distinctive, one-piece external silica skeleton that is highly perforate. They form the order Dictyochales, one of three orders that have been described for the class Dictyochophyceae (Daugbjerg and Henriksen, 2001; Graham et al., 2009). Silicoflagellates are commonly in fossil deposits dating back to the middle Cretaceous (ca. 120 million years ago). The skeletal remains in fossil material also indicate that species diversity reached a peak in the Miocene (ca. 23–25 million years ago), with more than 100 described species, but the diversity of silicoflagellates has decreased thereafter, leaving behind only two genera (Daugbjerg and Henriksen, 2001; Tappan, 1980). Biostratigraphic zonations have been developed for silicoflagellates in all oceans throughout the Cenozoic, though the zones typically range over longer intervals of time than foraminiferal or diatom zones (McCarterney and Wise, 1990; McCartney and Harwood, 1992). Silicoflagellate skeletons may have considerable potential as ecophenotypic indicators (McCarterney and Wise, 1990). Modern silicoflagellates are widespread in the oceans with a tendency to be more abundant in colder waters where they can even grow up to form blooms (Graham et al., 2009). Dictyochophytes occur in both marine and freshwater habitats (Moestrup, 1995; Moestrup and O’Kelly, 2000).**S**ynurophytes and **c**hrysophytes (often summarized as “Chrysophyte Algae”) produced an exceptional fossil record owing to the high preservation potential of their siliceous stomatocysts and scales (Smol, 1995; Andersen, 2004; Graham et al., 2009). In addition, they are highly sensitive to an array of limnological parameters. Synurophytes and Chrysophytes are close relatives in molecular phylogenies (Andersen, 2004); both share the capability to form stomatocysts, silica-walled resting stages, which arise sexually or asexually under unfavorable environmental conditions. The walls of stomatocysts are so heavily silicified that they resist silica dissolution processes and thus accumulate in the sediments of lakes (Graham et al., 2009). Fossil records of stomatocysts extend back to the Lower Cretaceous (at least 150 million years ago). Synurophytes and chrysophytes are able to switch between autotrophy, heterotrophy, and even phagotrophy, which puts them at a distinct advantage in aquatic ecosystems that are low in nutrient concentrations and have reduced light penetration (Betts-Piper et al., 2004). From patterns of stomatocyst assemblages, past environmental conditions can be inferred making stomatocysts very important for paleoecology (Duff et al., 1995; Siver, 1995; Wilkinson et al., 2001; Zeeb and Smol, 2001).

Synurophytes are unicellular or colonial silicified flagellates and despite they are photoautotrophic, many species are also capable of phagotrophy. Synurophycean cell surfaces are covered by overlapping silica scales, sometimes with spiny bristles, and are perforated (Graham et al., 2009). The scales’ perforation patterns are used as taxonomic features to delimitate species of synurophytes. A number of adhesive polysaccharides are involved in the scale attachment. The scales are produced in certain vesicles located on the surface of one of the plastids. In contrast to diatoms, synurophytes can continue to divide and function also in the absence of an external silica covering. In cultures of naked cells most cells will recover a complete cell covering when silicate is resupplied to silica-depleted cells (Leadbeater and Barker, 1995). Fossil scales similar to those of modern synurophytes have been reported from deposits of the middle Eocene (about 47 million years ago; Siver and Wolfe, 2005). Synurophyte scales can be used like stomatocysts for tracking environmental change using lake sediments (Zeeb and Smol, 2001; Wolfe and Perren, 2001). Synurophytes are abundant in neutral to slightly acidic freshwaters. Some species are regarded as indicators of low levels of pollution, but others are characteristic of eutrophic lakes (Graham et al., 2009).

Chrysophytes are golden-brown microalgae that are mostly unicellular or colonial which occur as flagellates or nonmotile cells; they include many unique and interesting morphologies (Graham et al., 2009). In contrast to synurophytes, scales covering the cell surface are absent in chrysophytes. Chrysophytes typically favor slightly acidic freshwaters of moderate to low productivity; their abundance and species richness increase with lake eutrophic status (Elloranta, 1995). Chrysophytes may have strong ecological impacts because many are mixotrophs, able to take up and metabolize dissolved organic compounds and particulate food as well as photosynthesize (Graham et al., 2009). Several chrysophytes are associated with the formation of undesirable blooms because living cells of certain species can produce toxic fatty acids that affect fish or excrete aldehydes and ketones into the water which can give it an unpleasant taste and odor.
Coccolithophorids and haptophyte algae (chromalveolates)

Haptophyte algae are a monophyletic group that includes all photosynthetic organisms with a haptonema. However, haptophytes also include some nonphotosynthetic relatives, and some that have secondarily lost the haptonema (Andersen, 2004). The haptonema, from which the group derives its name, is a microtubule-supported appendage that lies between two approximately equal flagella (for a review, see Inouye and Kawachi, 1994). Most haptophytes are marine, occurring in significant abundances even at greater water depth (up to 200 m). Haptophyte algae share many features, for example, pigment composition, presence of a chloroplast endoplasmatic reticulum, and plastids derived from red algal secondary endosymbiosis with the photosynthetic stramenopiles (Andersen, 2004). Based on structural and molecular evidences the haptophytes form a monophyletic lineage, treated as phylum Haptophyta which is divided into two classes (Edwardsen et al., 2000; Andersen, 2004). The larger group, class Coccolithophyceae, comprises the coccolithophorids which are haptophytes with a cell coat of mineralized scales (coccoliths) and two equal flagella or lack flagella altogether. Coccoliths are largely composed of calcium carbonate crystals in the form of calcite. There are two types of coccoliths, holococcoliths and heterococcoliths. In case of holococcoliths, an organic scale consisting largely of cellulose and produced by the Golgi body is secreted to the cell surface and then calcite crystals which are held together by organic material are deposited on the scale. In contrast, heterococcoliths develop on organic scales before being secreted from the cell, that is, calcite crystal deposition is already within the cell. The calcite crystals grow and interlock gradually forming the complex shape of the mature coccolith (deVrind-deJong et al., 1994; Probert et al., 2007; Graham et al., 2009). Coccoliths do not degrade at normal ocean pH and thus accumulate on the ocean floor. Therefore, coccolithophorids have produced huge amounts of sedimentary carbonates.

Coccolithophorids remove large quantities of atmospheric CO₂ through their photosynthesis and calcification and, therefore, are an important component of the global carbon cycle (McConnaughey, 1994), accounting for a substantial part of the ocean floor limestone sediments. Coccolithophorids contribute at least 25% of the total annual vertical transport of inorganic carbon to the deep ocean (Rost and Wiebesell, 2004). Coccolithophorids have an excellent fossil record with fossil coccoliths well over 200 million years old. The abundance of coccolith fossils peaked during the late Cretaceous (63–95 million years ago) when extensive chalk deposits were laid down. Coccoliths are widely used as stratigraphic indicators and fossil coccoliths are widely used as bioindicators in the oil industry and as indexes of past climate and ocean chemistry conditions (Young et al., 1994).

Haptophytes are ecologically significant in terms of both biotic interactions and biogeochemistry. Haptophyte algae are considered as high-quality foods for zooplankton; several species contain nutritionally important polyunsaturated fatty acids which make them also commercially valuable for the production of fish in aquaculture systems. Other coccolithophytes may produce toxins that destroy cell membranes or produce copious amounts of organic slime and foam (Graham et al., 2009). Many coccolithophorids form blooms in ocean waters and produce large amounts of a volatile sulfur-containing molecule, dimethyl sulfide (DMS) that enhances cloud formation and increases acid rain (Malin and Steinke, 2004). Another cooling effect on the climate comes from the coccoliths which readily reflect light, thereby increasing reflectance of the ocean’s surface.

Dinoflagellates (chromalveolates)

The dinoflagellates are an important group of phytoplankton in marine and freshwaters. Their adaptation to a wide variety of environments is reflected by a tremendous diversity in form and nutrition and an extensive fossil record dating back several hundred million years (Graham et al., 2009). Dinoflagellate cells have two structurally distinct flagella whose motion causes the cells to rotate as they swim, but also nonflagellate unicells and filamentous forms of dinofytes are known. Two different cell types can be distinguished on the basis of the cell-wall covering or theca. The “naked” or unarmored forms have an outer plasmalemma surrounding a single layer of flattened vesicles (membrane sacs or alveoli). Armored dinoflagellates have cellulose or other polysaccharides within each vesicle, giving the cells a more rigid, inflexible wall. These cellulose plates are arranged in distinct patterns which are extensively used as taxonomic “fingerprints” (Hackett et al., 2004). Cells of armored dinoflagellates display conspicuous anterior–posterior and dorsal–ventral differentiation. Their cells consist of two parts, separated by a groove that encircles the cell and contains the transverse flagellum which is flattened, ribbon-shaped, and with a single row of hairs. A smaller groove is mostly extending into the posterior cell part and contains the longitudinal flagellum which has two rows of hairs; it is directed posterior and emerges from the cell (Graham et al., 2009; Hackett et al., 2004).

Success of dinoflagellates as phytoplankton may be due in part to unique behavior patterns, including duel vertical migration and their capability to live phagotrophic (feeding on particles such as the cells of other organism) in addition to photoautotrophy (mixotrophic life-style). Some of the armored or thecate heterotrophic dinoflagellates have developed a remarkable pseudopod-like structure that is extruded from the cell and flows around the prey, enveloping it so the contents can then be digested (Hackett et al., 2004). Some dinoflagellates produce toxins that are dangerous to man, marine mammals, fish,
seabirds, and other components of the marine food chain (Van Dolah, 2000). Others are bioluminescent and emit light; some function as parasites or symbionts that rely on host organisms for part of their nutrition. Many photosynthetic dinoflagellates live as endosymbionts with reef-building corals. Stimulated by chemical signals from their animal hosts, dinoflagellate endosymbionts provide the corals with essential organic food.

Therefore, the dinoflagellate symbionts are critical components of the coral reef ecosystems whose loss during stress-related “bleaching” events can lead to mass mortality of coral hosts and associated collapse of reef ecosystems (Baker, 2003). Also, the high rates of calcification and production that characterize typically oligotrophic coral reef ecosystems are largely credited to the mutualistic symbiosis between the scleractinian corals and photosynthetic dinoflagellates belonging to the genus *Symbiodinium* (Apprill and Gates, 2007). Corals with a dinoflagellate symbiont calcify much faster than those without—an effect linked to photosynthetic fixation of CO₂ by the dinoflagellates (Marshall, 1996). A significant amount of photosynthetic product is excreted by the symbiotic dinoflagellates, primarily as glycerol. Up to 50% of the fixed carbon may be transferred to the host (Paracer and Ahmadjian, 2000), in which it is converted mainly to lipids and proteins. On the dinoflagellate side, many of these symbioses occur in oligotrophic waters in which nutrients are scarce in the water column (Hackett et al., 2004). In other symbiotic dinoflagellate associations, the hosts include foraminifera, radiolarians, flatworms, anemones, jellyfish, and even bivalve mollusks (Hackett et al., 2004).

Undisputed structural fossils of dinoflagellates occur some 200 million years or so ago (Graham et al., 2009). These fossils, known as hystrichospheres, resemble resting stages of some modern dinoflagellates. Some modern dinoflagellates produce resting cysts or zygotes with an organic compound resistant to decay and thus aid the survival of dinoflagellate fossils, of which a considerable diversity has been described (Fensome et al., 2003). Other chemical compounds thought to be specific to dinoflagellates have already been found in Proterozoic (Precambrian) rocks and such chemical fossils suggest that dinoflagellate-like organisms might have existed already more than 600 million years ago (Graham et al., 2009).

Many dinoflagellates are photosynthetic and, through endosymbiosis, have acquired a wide diversity of plastids from distant evolutionary lineages. The most common plastid in dinoflagellates is golden brown with a unique accessory pigment, peridinin; they also contain chlorophyll c. Such peridinin plastids are bounded by three membranes and are derived from red algal secondary endosymbiosis (Delwiche, 2007; Hackett et al., 2004). A number of dinoflagellates have plastids from haptophytes, diatoms, green algae, or cryptomonads which may have replaced preexisting peridinin plastids (tertiary endosymbiosis; McFadden, 2001; Keeling, 2004; Palmer, 2003).

A small group of dinoflagellates with peridinin plastids produce calcareous structures, formed during the life cycle or found in vegetative stages (Gottschling et al., 2005). The potential to produce calcareous structures has been considered as apomorph within alveolates (Kohring et al., 2005), arguing for the monophyly of the family Calciodinellaceae which comprises approximately 30 (recognized) extant species that are distributed in cold through tropical seas of the world. Calcareous cysts are deposited in both sediments coastal (Montresor et al., 1998; Persson et al., 2000) and oceanic (Vink, 2004; Zonneveld et al., 1999). According to the fossil record, calcareous dinoflagellates originate in the Upper Triassic (Janofske, 1992) and are highly diverse during the Cretaceous and throughout the Tertiary (Keupp, 1991; Kohring, 1993; Willems, 1994), thus many fossil species (namely their cysts) have been described (Gottschling et al., 2005). Based on morphological and molecular data, calcareous dinoflagellates (Thoracosphaeraceae, Peridiniales) are a monophyletic group comprising three major clades (Gottschling et al., 2008).

Other chromalveolates

*Cryptomonads*. Recent molecular phylogenetic analyses indicate that the cryptomonads and the haptophyte algae together form a monophyletic group (Hackett et al., 2007) and, therefore, also belong to the Chromalveolates supergroup. Cryptomonads form an abundant group of marine and freshwater unicellular flagellates that contain a plastid derived from red algal secondary endosymbiosis with chlorophyll* a* and* c* surrounded by four membranes. Cryptomonad plastids have received some attention because they are one of only two groups in which the primary algal nucleus has not been completely lost; they retain a small relict nucleus called a nucleomorph (McFadden, 2001; Keeling, 2004; Palmer, 2003).

*Apicomplexa* are a large group composed entirely of obligate intracellular parasites, including several that cause significant diseases such as malaria. As intracellular parasites, the discovery of a relict plastid (or apicoplast) in apicomplexa bounded by four membranes has drawn a great deal of attention as an evolutionary novelty and possible drug target (Keeling, 2004). There is current evidence indicating that this plastid is derived from a red alga and that the apicomplexa share a monophyletic origin with all other groups with plastids from red algal secondary endosymbiosis. The apicomplexa may be most closely related with the dinoflagellates (Moore, 2008). For a review of apicomplexa relating to their plastids, see Foth and McFadden (2003).

Algal groups with plastids derived from green algal secondary endosymbiosis

*Chlorarachniophytes* (Rhizaria supergroup). Chlorarachniophytes are a relatively rare group of marine amoeboflagellates and flagellates that contain a green algal plastid (chlorophyll* a* and* b*) bounded by four membranes. They
contain a relict nucleus, nucleomorph, which has been retained from the green algal symbiont (McFadden, 2001; Keeling, 2004; Palmer, 2003). Chlorarachniophytes form a monophyletic group within the Rhizaria supergroup of eukaryotes (Ishida et al., 1999).

**Euglenids** (Excavates supergroup). Euglenids are a diverse group of common marine and freshwater flagellates, about half of which contain a plastid derived from a green algal secondary endosymbiosis which is bounded by three membranes and contains chlorophyll b as accessory pigment. The remainder of the group are osmotrophs or heterotrophs that feed on bacteria or other eukaryotes. Molecular phylogenies show that photosynthetic euglenids may have acquired their plastids from a green alga relatively late in evolution, despite the Euglenozoa may be a rather old group. Euglenids are closely related to the parasitic trypanosomes (Kinetoplastids), together with diplomids, making up the monophyletic Euglenozoa. Distinguishing features of euglenids are that they produce a storage carbohydrate which is a β-1,3-linked glucan (paramylon) in their cytoplasm and that they display a unique surface structure composed of parallel ribbon-like proteinaceous strips. For reviews of euglenids, see Leedale and Vickerman (2000), Milanowski et al. (2006) or Ciugulea and Triemer (2010).

**Summary**

Algae is a term of convenience and refers to a collection of highly diverse organisms that undertake photosynthesis and/or possess plastids. In addition, the photoautotrophic cyanobacteria are regarded as algae by some authors. In a recent phylogeny of the eukaryotes, the algae are scattered among four of the five recognized supergroups. A single primary endosymbiosis with a cyanobacterium may have been the start of all eukaryotic algae which first diverged into the green algae, red algae, and glaucophytes (Plantae supergroup). The plastids of all other algal lineages derived from secondary endosymbiosis involving either a red algal cell (algae of the Chromalveolates supergroup) or green algal cells (euglenids and chlorarachniophytes, Excavates and Rhizaria supergroups). Many algae form symbioses with ciliates and some metazoa. Algae are very important primary producers in almost every aquatic or terrestrial habitats and are important key players within the carbon and silica cycles for the major part of Earth history. Many lineages have produced significant fossil records due to their ability to precipitate calcium carbonate on their cell surfaces (red algae, some dinoflagellates) or form calcified (haptophytes) or silicified body scales, silica endoskeletons, resting stages, or cell walls (photoautotrophic stramenopiles, including dictyochohytes, synurophytes, chrysophytes, and diatoms). Due to their high sensitivity to certain environmental parameters and their high preservation potential, these algae also play important roles as sedimentary bioindicators in tracking environmental changes.

**Bibliography**


Cross-references
Cyanobacteria
Diatoms
Early Precambrian Eukaryotes
Fungi and Lichens
Microbialites, Modern
Microbialites, Stromatolites, and Thrombolites
Photosynthesis
Protozoa (Heterotroph, Eukaryotic)
Symbiosis

ALKALINITY

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The term “Alkalinity,” commonly denoted by TA (also AlK, and others) and then called titration alkalinity or total alkalinity, refers to a very important chemical concept in aquatic chemistry. Total alkalinity is one of the few measurable quantities in natural waters that allows, together with other properties, to calculate concentrations of single species of the carbonate system such as CO2, HCO3, CO32, H+, and OH− (Wolf-Gladrow et al., 2007), and further on, the saturation state of the waters with respect to certain carbonate minerals. Therefore, alkalinity represents a hydrogeochemical key parameter for the understanding of carbonate precipitation, either biologically mediated or not, and its variation through the geological history.

Definition
From its determination by titrimetric techniques, alkalinity has historically been defined as the number of equivalents of a strong acid required to neutralize 1 L of water at 20°C. The obtained end-point, corresponding to the second equivalence point of the carbonate system, was operationally defined and results were then commonly given as eq L−1. The recent definition of alkalinity is based on the implementation of the Brønsted–Lowry concept of acids and bases by Rakestrow (1949) who defined alkalinity as the excess of bases (proton acceptors) over acids (proton donors). Considering seawater, alkalinity is mostly determined by the ions bicarbonate and carbonate, also by the borate ion, and to a smaller extent by OH and H+ from dissociation of water. Alkalinity then refers to the proton condition with reference to a zero level of protons defined by the species H2CO3, B(OH)3, and H2O, where the proton acceptors are HCO3−, CO32−, B(OH)4−, and OH− and the proton donor is H+. That is, alkalinity is the equivalent sum of bases that have one or two protons less than the reference species minus H+ (or H3O+) that has one proton more than H2O (Dickson, 1981; Stumm and Morgan, 1996; Zeebe and Wolf-Gladrow, 2001). The expression for the total alkalinity is thus presented as

$$\text{TA} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B(OH)}_4^-] + [\text{OH}^-] + [\text{H}^+ - \text{H}^+]$$

where conceptually the right hand term can be divided up in carbonate alkalinity (CA = [HCO3−] + 2[CO32−]) as the most dominant contributor, borate alkalinity, and water alkalinity.

Natural waters may contain various acid–base systems that can accept and donate protons other than or in addition to carbonic acid or boric acid. These complications have been taken into account currently in the most precise definition of total alkalinity of Dickson (1981) (compare also Dickson, 1992; DOE, 1994; and recent extension by Wolf-Gladrow et al., 2007). The definition is unequivocally based on a chemical model of occurring acid–base processes and to be independent of temperature and pressure expressed in gravimetric units (mol kg−1). In Dickson’s concept, the total alkalinity (TA) is defined as the number of moles of hydrogen ion equivalent to the excess of proton acceptors over proton donors with respect to the proton condition defined by the value pK = 4.5 in 1 kg of sample. Bases formed from weak acids with a dissociation constant $K \approx 10^{-4.5}$ are considered proton acceptors, acids with $K > 10^{-4.5}$ are proton donors, and the chemical species defining the zero level of protons of the various acid–base systems are H2CO3, B(OH)3, H2O, H2PO4−, H4SiO4, NH4+, H2S, SO42−, F−, and NO3−. From this definition the expression for the total alkalinity is presented as

$$\text{TA} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B(OH)}_4^-] + [\text{OH}^-] + [\text{HPO}_4^{2-}] + 2[\text{PO}_4^{3-}] + [\text{H}_2\text{SiO}_4^-] + [\text{NH}_3] + [\text{HS}^-] + \ldots - [\text{H}^+] - [\text{H}_2\text{O}] - [\text{H}_3\text{PO}_4^-] - [\text{HNO}_2] \ldots$$

where the ellipses stand for additional acid–base species (Wolf-Gladrow et al., 2007).

An alternative definition for alkalinity derives when considering the charge balance of natural waters (Stumm and Morgan, 1996). From the principle of electroneutrality total alkalinity can be expressed in terms of the charge imbalance between the equivalent sum of conservative cations (base cations of strong bases) and the sum of conservative anions (conjugate bases of strong acids). For the total alkalinity of sea water dominated by
HCO\textsubscript{3}^-, CO\textsubscript{3}^{2-}, and B(OH)\textsubscript{4}^-, the expression may be presented by

\[
TA = [\text{Na}^+] + [\text{K}^+] + 2[\text{Mg}^{2+}] + 2[\text{Ca}^{2+}] - [\text{Cl}^-] - [\text{Br}^-] - [\text{NO}_3^-] - 2[\text{SO}_4^{2-}],
\]

which is equivalent to the above definition in terms of proton acceptors and donors

\[
TA = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B(OH)}_4^-] + [\text{OH}^-] - [\text{H}^+].
\]

In the charge balance expression quantities are defined to be conservative with respect to mixing and changes in temperature (T) and pressure (P) provided that concentrations are on the gravimetric unit scale (mol kg\textsuperscript{-1}). Thus, from the TA expression entirely in terms of conservative ions TA is a conservative quantity too. In contrast, the concentrations of individual species of, e.g., the carbonate system may change with temperature and pressure because of the T–P dependency of the equilibrium constants.

Following the charge balance approach, Zeebe and Wolf-Gladrow (2001) and Wolf-Gladrow et al. (2007) have derived an expression in terms of total concentrations of conservative ions and conservative total concentrations of the various acid–base species under concern in the definition of Dickson (1981) in terms of an acid–base balance. This derived expression for TA is denoted as the explicit conservative alkalinity expression (TA\textsubscript{ec}).

\[
TA_{\text{ec}} = [\text{Na}^+] + [\text{K}^+] + 2[\text{Mg}^{2+}] + 2[\text{Ca}^{2+}] + \ldots - [\text{Cl}^-] - [\text{Br}^-] - [\text{NO}_3^-] - \ldots - 2[\text{SO}_4^2^-] - [\text{THF}] - [\text{TPO}_4^3^-] - [\text{THNO}_2^-] + [\text{TNH}_3^+],
\]

where total concentrations terms represent concentrations sums of the species of the acid–base systems

\[
\text{TSO}_4^2^- = [\text{SO}_4^{2-}] + [\text{HSO}_3^-], \quad \text{THF} = [\text{F}^-] + [\text{HF}].
\]

\[
\text{TPO}_4^3^- = [\text{H}_3\text{PO}_4^+] + [\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{2-}] + [\text{PO}_4^{3-}],
\]

\[
\text{THNO}_2^- = [\text{NO}_2^-] + [\text{HNO}_2], \quad \text{TNH}_3 = [\text{NH}_3] + [\text{NH}_4^+],
\]

and ellipses stand for additional ions with minor concentrations.

The definition of TA\textsubscript{ec} has the advantage that each term in the expression is conservative with respect to T–P changes, even though single species in the total concentration terms may be not. In particular, the approach is very convenient to derive changes of TA due to certain physicochemical and biogeochemical processes as nutrient uptake in biological production and release during remineralization.

**Sources, changes, and ranges**

The primary source of alkalinity in surficial (and subsurface) waters is carbonic acid-controlled chemical weathering of carbonates, aluminum-silicate minerals like feldspars or Mg-Fe-silicates. The overall concentration and subsequent changes in alkalinity of water are therefore determined by the geology of the watershed, the predominant weathering reactions and ion exchange in the soils, and concomitant biogeochemical processes.

TA as a conservative quantity will first alter with changes in salinity as a result of precipitation, evaporation, and mixing. Contrariwise to its increase from dissolution of limestone carbonates TA decreases when biogenic or non-biogenic CaCO\textsubscript{3} or other carbonate minerals precipitate. Further important changes are due to various biogeochemical processes. It has to be noted that total alkalinity does not change as a result of CO\textsubscript{2} exchange with the atmosphere. Invasion or degassing of CO\textsubscript{2} affects the adjustment of the carbonate species (ratio of [HCO\textsubscript{3}^-] to [CO\textsubscript{3}^{2-}]) and the pH but not TA. Therefore, it is also not the consumption of CO\textsubscript{2} during photosynthesis or release during aerobic respiration that brings about a change in TA, but the utilization or remineralization of nitrate and phosphate involved in the processes. Nitrogen assimilation in form of ammonia decreases TA and no change of TA results from molecular nitrogen uptake (Stumm and Morgan, 1996; Wolf-Gladrow et al., 2007). Several microbially mediated processes lead to a change in alkalinity where deviation in the charge balance is compensated by yield or consumption of H\textsuperscript{+} or OH\textsuperscript{-}. When oxygen is consumed in processes like nitrification, oxygenation of soluble ferrous iron to ferric oxide or sulfide oxidation from HS\textsuperscript{-} or pyrite total alkalinity decreases. Under anoxic conditions, remineralization of organic matter by denitrification, reduction of iron and manganese oxides and sulfate reduction frequently result in large increases of total alkalinity (Lerman and Stumm, 1989; Stumm and Morgan, 1996).

Depending on the rock mineralogy of the catchment area and prevalent vegetation total alkalinity in streams and inland lakes commonly ranges below 3 mmol kg\textsuperscript{-1}. Very low alkalinity is bound to tributaries and lakes in crystalline rock areas or to acidic bog and peatland waters. Here, H\textsuperscript{+} released by biomass exceeding H\textsuperscript{+} consumption by soil weathering results in an excess of protons that is referred to as “mineral acidity” or in turn “negative alkalinity” (Stumm and Morgan, 1996). On the other hand, in hard-water creeks that gain their alkalinity preferably from biogenic CaCO\textsubscript{3} or other carbonate minerals precipitate. Even higher total alkalinity is typical for highly alkaline salt lakes, so-called soda lakes, which receive an equivalent load of HCO\textsubscript{3}^- and CO\textsubscript{3}^{2-} in excess to Mg\textsuperscript{2+} and Ca\textsuperscript{2+} from their riverine inflows. Here, TA can rise to
more than $10^{-1}$ mol kg$^{-1}$ and pH increase above 10 in the course of evaporation and subsequent Ca-carbonate precipitation while alkali cations, in particular sodium and potassium, increasingly maintain the charge balance of bicarbonate and carbonate ions (e.g., Lerman and Stumm, 1989; Kempe and Kazmierczak, 1994; Reimer et al., 2009).

### Alkalinity and carbonate precipitation

Alkalinity, together with any of the three other measurable properties of the carbonate system — pH, dissolved inorganic carbon (DIC or $C_T = [CO_2] + [HCO_3^-] + [CO_3^{2-}]$), or $P_{CO_2}$ (partial pressure of CO$_2$) — can be used to compute the remaining quantities including concentrations of...
single species such as HCO$_3^-$ and CO$_3^{2-}$. From CO$_2^{2-}$ and Ca$^{2+}$ concentrations (or more precise activities) saturation with respect to carbonate minerals is determined, where saturation $\Omega$ is given by the product of the actual ion activities ($\text{IAP} = \{\text{Ca}^{2+}\} \times \{\text{CO}_3^{2-}\}$) divided by the solubility product of the carbonate ($K_{\text{mineral}}$) at ambient temperature and pressure ($\Omega_{\text{mineral}} = \text{IAP}/K_{\text{mineral}}$). A commonly used alternative notation is the saturation index (SI) which is defined as $\log \Omega$ ($\text{SI}_{\text{mineral}} = \log (\text{IAP}/K_{\text{mineral}})$.

The oceanic CaCO$_3$ saturation state is primarily determined by variation of the CO$_3^{2-}$ ion concentration, because the Ca$^{2+}$ concentration closely depends on salinity and does not vary substantially. In the upper ocean, CO$_2$ removal by primary production increases CO$_3^{2-}$ concentrations resulting in an almost fivefold supersaturation (SI = 0.7) with respect to calcite. On the other hand, biologically controlled calcification of calcareous plankton or reefal organisms is responsible for the lower alkalinity of about 2.2–2.3 mmol kg$^{-1}$ in surface seawater. In turn, supersaturation decreases with depth when CO$_2$ is released by remineralization of sinking organic matter when temperature and pressure changes. In the deeper ocean, total alkalinity then increases to about 2.4–2.5 mmol kg$^{-1}$ reflecting the dissolution of sinking calcium carbonate particles in response to the lower saturation state (e.g., Broecker and Peng, 1982; Morse and Mackenzie, 1990).

As one of the major factors in the oceanic carbonate system, alkalinity distribution and behavior in the oceans has thoroughly been investigated during the last decades. Dissolution-preservation patterns of biogenic carbonates in surface to deep oceans and the changes brought about by a rising atmospheric CO$_2$ level (Feely et al., 2004) have been assessed within global ocean research programs, as well as general feedbacks between the oceanic carbonate and the global climate system. Contrary to this rising knowledge on the modern marine environment, estimates of ocean alkalinity throughout geological history are rare and, in the lack of direct analytical proxies, mainly rely on model assumptions. Ocean alkalinity comparably high as modern values is suggested for the Cenozoic and late Mesozoic (Tyrell and Zeebe, 2004; Ridgwell, 2005). During periods of the Mesozoic and Paleozoic, alkalinity about two to three times higher than recent can be assumed according to the evolution of the Phanerzoic atmospheric CO$_2$ (Ridgwell, 2005), fluctuations in the rate of continental weathering (Locklair and Lerman, 2005), or the more widespread occurrence of anoxic basins (Kempe and Kazmierczak, 1994). Elevated alkalinity concentrations of some tenths of mmol kg$^{-1}$ are suggested for the Earth’s primordial ocean in favor of a slightly acidic to neutral Na-Cl-dominated chemistry under an early CO$_2$-rich atmosphere (Grotzinger and Kasting, 1993; Morse and Mackenzie, 1998). Alternatively, even higher alkalinity has been postulated for a Precambrian alkaline “Soda Ocean” in analogy to modern Na-carbonate-dominated soda lakes (Kempe and Degens, 1985).

In principle, precipitation of CaCO$_3$ is kinetically unfavorable even at the fivefold supersaturation of the modern ocean (e.g., Morse and He, 1993). Mechanisms to overcome this kinetic inhibition evolved at the Precambrian–Cambrian boundary with the advent of controlled biomineralization that became a major regulator of carbonate precipitation since the Mesozoic proliferation of the calcareous plankton. Prior to the Cambrian, carbonate deposition was characterized by sea-floor encrustations, cement beds, and the widespread occurrence of microbialites (Grotzinger, 1990; Riding, 2000), which are attributed to calcification of biofilms and microbial mats. In the lack of direct metabolic control on carbonate production, these Precambrian deposits are thought to have been formed under the pressure of a higher supersaturated environment, possibly still accounting for the dominance...
of microbial carbonates in the early to middle Paleozoic (Riding and Liang, 2005). Today, the occurrence of carbonate deposits comparable to ancient microbialites is confined to particular environments such as restricted evaporational marine settings, freshwater habitats like tufa stromatolite forming hardwater creeks, or alkaline salt lakes (Figure 1). Although at first glance very different, these environments in common exhibit a significantly higher alkalinity than recent ocean values and a 10–15 fold supersaturation (i.e., SI = 1–1.2) with respect to calcite. Maintaining elevated carbonate supersaturation as found in these recent environments and as suggested for certain periods in Earth’s history calls for variation in alkalinity in paleo-ocean model calculations (Locklair and Lerman, 2005). Therefore, changes in alkalinity may be considered as an underappreciated hydrogeochemical key mechanism with regard to carbonate supersaturation and carbonate deposition in its various forms through geologic times.

Cross-references
Calcite Precipitation, Microbially Induced
Carbonate Environments
Carbonates
Saline Lakes
Salinity History of the Earth’s Ocean
Soda Ocean Hypothesis

AMBER

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Synonyms
Fossil resin; Resinite
Definition
Fossilized plant resin from various botanical sources.

Introduction
 Amber is a fossilized plant resin from various botanical sources and of different geological ages, ranging from the Carboniferous to the Pliocene, although it is particularly frequent in the Cretaceous and in the Eocene to Miocene. The term “amber” is used most commonly as a synonym for fossil resin of every provenance, geological, and palaeobotanical source. A more reductive meaning of amber has been considered, referring only to succinite, the mineralogical species occurring in the Baltic coast deposits and neighboring localities containing significant amounts of succinic acid (up to 8%). However, most commonly, amber and fossil resin are the terms used interchangeably for indicating macroscopic material (Anderson and Crelling, 1995; Langenheim, 2003). Another term used by coal geologists for fossil resins is resinite, which is a microscopic material often found in coal maceral. Amber, fossil resin, and resinite, according to Anderson and Crelling (1995) should therefore be considered “solid, discrete organic material found in coals and other sediments as macroscopic or microscopic particles, which are derived from the resins of higher plants.”

The term amber derives from the Arabic word anbar, which was first used to indicate ambergris (a waxy substance secreted by the sperm whale, used in perfumery as a fixative) and then also fossil resin, both natural products with the common feature of being carried ashore by sea waves. The Romans called amber succinum (from succus, literally “juice,” “sap”). The ancient Greek name for amber is elektron, that means “shining thing,” a term which was related to the Sun God Elektor (the awakener), and the Moon, called Elektris (Stoppani, 1886). The modern term electron was coined in 1891 by the Irish physicist George Johnstone Stoney, using the Greek word for amber because static electricity could be generated on the surface of rubbed amber. The German word Bernstein (or Börnstein), the Dutch Barnsteen, and the Swedish bärnsten mean “burning stone” because amber may burn when exposed to flame.

Geological names are often attributed to fossil resins on the basis of the locality of discovery or of the discoverer, and these names are not based on specific knowledge of the composition; however, they are often used and also quoted in the scientific literature as common names. For example, Simetite is for amber collected in Sicily (Italy) near the Simeto river, Burmite, for amber found in Burma (Myanmar), and Rumanite for that found in Romania; Beckerite and Stantienite were named for Becker and Stantien, two developers of amber dredging and mining operations in the Samland region in the 1800s. Over a hundred of different fossil resins have been described, so far.

Fossil resins are considered the archetype of a “chemical fossil” (chemical evidence of life processes preserved in the fossil record), and besides their property of preserving embedded organisms, they can also maintain particular details of their original composition better than any other sedimentary material.

Amber has been known by humans since prehistorical times and most bizarre hypotheses about its origin and composition have been formulated. In the antiquity amber has been considered even as produced by the rays of the Sun (Pliny the Elder, Book 37: Nicis solis radiorum sucum intellecti voluit hoc) and that was not such a wrong idea, since amber, as a resin of ancient trees, is a metabolite of plants, originated from products of photosynthesis, that is, from the energy of the Sun.

Amber composition and formation
Several different parameters can play relevant roles in amber composition and consequently, they all should be considered in the study and comparison of different amber samples. These are for example:

1. Original environment: temperature, insolation, and climate influence the resin composition before it is embedded in the sediment (bistratonomic processes);
2. Age: complex chemical arrangements occur with time; in general, low molecular weight components disappear with increasing age;
3. Plant source: resin produced by different plants exhibit different chemical composition;
4. Diagenetic history: following the embedding in the sediment, resin maturation is affected by temperature, pressure and permeating fluids which can modify the chemical composition and the physicochemical properties of fossil resin;
5. Alteration processes after amber mining (of relevance especially in archaeological context): direct sunlight and exposition to air and heat may change the color and physical properties (and, consequently, the chemical composition) of the sample.

Resins consist of secondary components of plants, that is, chemicals occurring only in some groups of plants, without an apparent role in primary physiological or metabolic pathways. Most natural resins are derived from terpenes, a large and varied class of hydrocarbons, which are chemicals composed by structures based on the isoprene unit (CnH_{2n}) (Figure 1). The basic molecular formula of terpenes can be expressed as multiples of isoprene, (C_{5}H_{8})n, where n is the number of linked isoprene units. The isoprene units can link together “head to tail” to form linear chains, or alternatively they can arrange to form rings. The overall terpene structure undergoes oxidation or rearrangement of the carbon skeleton, producing compounds, which are generally indicated as terpenoids or isoprenoids. Mono-, sesqui-, di-, and tri-terpenoids are polymers formed by two, three, four, and six isoprene units, respectively.

The biosynthesis of resins starts from simple molecules, namely mevalonic acid, which can be considered the precursor of isoprene units. Photosynthetically
produced carbohydrates are broken down to give pyruvate, which represents the basis for the synthesis of mevalonic acid. Mevalonic acid is converted into the activated form of isopentenyl pyrophosphate, which gives origin to geranyl pyrophosphate (C10), the basis for the monoterpene synthesis, or can further undergo condensation into farnesyl pyrophosphate (C15) for the production of sesquiterpenes, or geranyl-geranyl pyrophosphate (C20), which yields diterpenes and tetraterpenes, or squalene (C30), which is the basis of triterpenes (Figure 2).

Fluid resins, after their exudation from plant, are characterized by a large volatile fraction, up to 50%, mainly constituted by monoterpenes and sesquiterpenes, while the nonvolatile fraction is composed of di- and tri-terpene acids (carboxylic acids with double bonds and functional groups), with alcohols, aldehydes, and esters. The volatile components of the resins are mainly involved in the defensive role of the resin against pathogens and insects. Evaporation of volatile components, following polymerization of the remaining nonvolatile constituents, mainly presenting dienic functions, causes the hardening of resin, which, if allowed to be embedded in appropriate sediment, poses the basis for transformation into a fossil resin.

Diterpenoid resins, mainly composed of diterpene acids (C20), are produced by gymnosperms (conifers) and angiosperms (mostly in the Leguminosae family), and they are particularly prone to polymerization and thus being preserved during fossilization into amber.

Gymnosperm diterpenes are mainly represented by diterpene acids of the abietane, pimarane, and labdane types (Figure 3). Abietane- and pimarane-type diterpene acids, such as abietic and pimaric acids, abundant in the Pinaceae family, tend to remain unpolymerized; on the contrary, labdane-type acids (e.g., agathic and communic acids, common in the Cupressaceae family), easily polymerize thanks to conjugated diene compounds (Langenheim, 2003). Resins of the Araucariaceae family may contain all three diterpene groups.

Also the nonvolatile fraction of angiosperm resins, for example, from the genus *Hymenaea* of the Leguminosae (Fabaceae) family, which yields copal, is constituted by diterpenes of the labadiene group. Resins from other angiosperms, such as Dipterocarpaceae, contain mainly triterpenes.

Therefore, resins containing particular diterpenes, such as abietic acid, cannot form amber *sensu stricto* because the constituent does not contain functional groups that could lead to a cross-linked polymer (Beck, 1999). Also triterpenoid resins (C30), which are mainly produced by broad-leaved trees, generally do not polymerize.

The process of polymerization and resin hardening starts just after resin exudation, and involves free radical mechanisms, which are initiated by exposure to sunlight and air (Langenheim, 2003). Initial polymerization can
occur involving the terminal groups of a side chain in the molecule of terpenoids, mainly diterpenes with a labdanoid structure (unsaturated labdatriene acids and alcohols, and biformaline), leading to the formation of a general polymeric structure (Anderson et al., 1992; Clifford and Hatcher, 1995; Clifford et al., 1997) (Figure 4). As a complex mixture, amber contains also nonpolymerizing compounds such as succinic acid in succinite, or monoterpenes, which are trapped (cross-linked) into the polymer network (Clifford and Hatcher, 1995; Clifford et al., 1997) and function as plasticizers.

Formation of amber occurs through the process of “maturation,” which is defined as the progressive changes of the resin occurring after the hardening and burial into the sediment, due to diagenetic and catagenetic processes. The chemical reactions in maturation of the resin to yield fossil resin include cross-linking, isomerization, and cyclization (Clifford and Hatcher, 1995; Clifford et al., 1997). One typical reaction in polylabdanoid fossil resins is the depletion of exocyclic methylenes (Figure 4) in the C8–C17 double bond of the molecule, and the formation of intramolecular cyclization. The modification of amber structure during its maturation leads to a progressive loss of unsaturated bonds, a decrease in functionalized groups and an increased proportion of aromatized groups (Grimalt et al., 1988).

Although the degree of resin maturation is dependent on the age of the resin, the rate at which this process occurs depends on several geological conditions, including the thermal history of the sample (Anderson et al., 1992) and on the structure and resin composition (Langenheim, 2003). Although a certain direct correlation between resin maturation and the age has also been demonstrated, by means of thermal analysis (Ragazzi et al., 2003), maturity and age cannot always be directly linked, since chemical transformation of resin increases at higher temperatures, and therefore the geothermal variable should be considered. Other physicochemical properties of amber, such as hardness and solvent solubility, may suggest the degree of maturation (Poinar, 1992; Rodgers and Currie, 1999; Langenheim, 2003; Ragazzi et al., 2003).

An alternative approach to define the age of a resin was proposed by Anderson (1997) on the basis of carbon-14 content. Resins that are older than the detection limit of carbon-14, that is, at about 40,000 years, have been considered fossil resins, and their age should be determined based on the stratigraphical data of the embedding sediment. Resins of age between 40,000 and 5,000 years are considered subfossil, while resins between 5,000 and 250 years are ancient resins, and those younger than 250 years are considered as modern or recent. This

**Figure 3** Chemical structure of major conifer resin components. Left column presents the general skeleton, and right column, an example of specific compounds (for further details, see Langenheim, 2003).
terminology should be preferred to that referring to “young amber” or “copal,” which is often misleading with respect to the age of the sample.

Physicochemical characteristics of amber

Most amber pieces are minute ranging from millimeter to centimeter size. Resin pieces may be found preserved in their original shape as solidified in the amber forest (e.g., drops, elongate stalactites, lumps with a flattened side which was attached to the bark or to other surfaces). Fossil resin is sometimes still attached to fossil wood, or even in situ in wood or twigs which can help to identify the amber-bearing trees of a particular deposit. Amber pieces are more or less rounded after being transported and redeposited.

Amber has been extensively investigated under the physicochemical aspect. The relative hardness according to Mohs’ scale is 2–2.5 (corresponding to 199–290 MPa) for most types of amber, indicating that it is slightly harder than gypsum and that it is possible to scratch the surface with calcite or copper. The specific gravity, that is, the ratio of the density of a substance to the density of water, ranges from about 0.96–1.12. For this reason most amber is nearly floating in sea water (specific gravity of the Baltic sea water is very low, about 1.005) and it sinks to the bottom in quiet water, but it can easily be carried along by disturbed water and washed ashore. Because of its low density, amber floats on a salt solution (about 15 g of NaCl/100 ml), while several types of plastic, often used as amber imitation, sink due to their higher density (e.g., the phenolic plastic bakelite, which has a specific gravity of about 1.4). Amber has resinous luster, and might appear translucent or opaque. Its refractive index ranges between 1.539 and 1.545. The color of translucent amber varies from a honey-like yellow and yellow-orange to light and dark red; the latter color is often caused by weathering. Rare specimens of blue (e.g., some Dominican amber) and green (e.g., some Mexican amber) samples have been found. Resins may be opaque because of microscopic bubbles or tiny detritus particles. Sometimes, densely arranged filaments of bacteria and fungi
that grew into the resin in its liquid stage cause opacity of the outer parts of resin pieces (Figure 5). If exposed to ultraviolet light, amber often produces fluorescence of blue, yellow, or green color. When amber is heated, it becomes soft at 150–180°C and decomposes around 280–300°C, emitting fumes with aromatic odor. After touching amber with a hot needle (hot point test), it produces smoke and burnt-resinous odor; this method is empirically used to distinguish between amber and plastic or copal. Exposed to a flame, amber burns easily. Amber is insoluble in water, and partly soluble in organic solvents, such as diethyl ether, acetone, and dichloromethane, as well as in mixtures of turpentine oil and acetone. After applying a drop of solvent to the surface, recent resins (copal) or plastic are readily dissolved and the surface becomes sticky, while amber (polymerized fossil resin) is unaffected. Although resin is macroscopically amorphous, some of its constituents may form microscopic drop- or bubble-like and elongated microstructures. Some of these “pseudoinclusions” may resemble enclosed microorganisms but can be distinguished by their variable size and shape and by the absence of diagnostic surface features (Figure 6).

Chemical analyses and classification of amber
Since early chemical investigations in the nineteenth century, amber has been identified as being composed of carbon, hydrogen, and oxygen. It is not possible to indicate a univocal composition formula of amber since it is an amorphous, noncrystalline material. The composition of different types of amber, as a result of elemental analysis, has been indicated approximately in the following range: carbon 75–87%, hydrogen 8.5–11%, oxygen 5–15%, with traces of sulfur (up to 1.7%) and other elements (Kosmowska-Ceranowicz, 1984; Ragazzi et al., 2003).

Since amber is mostly insoluble in organic solvents, a complete characterization of its chemical composition has been obtained using the total solid sample, by means of specific analytical techniques that have become available, such as infrared spectroscopy (IRS), pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS), and nuclear magnetic resonance (NMR) spectroscopy.
Infrared spectroscopy of powdered samples, since the first application in the study of fossil resins (Beck et al., 1964), has become one of the main techniques for obtaining structural information and identification of amber from different origins (Kosmowska-Ceranowicz, 1999). Infrared spectra allow to define the fingerprinting of any fossil resin, and in particular it has been successful as diagnostic for Baltic amber, observing the presence of the characteristic Baltic shoulder in the 1,150–1,260 cm⁻¹ region of the spectrum, attributed to the absorption of ester groups, in particular esterified succinic acid. It is noteworthy that the free succinic acid levels in Baltic amber range between 50 and 400 ppm (Tonidandel et al., 2009), despite the high total succinic acid content of 1–8%.

Anderson et al. (1992; also Anderson and Crelling, 1995), on the basis of various physicochemical methods, mainly Py-GC/MS, proposed a classification of fossil resins into five main classes:

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>Class I</strong></td>
<td>The macromolecular structures are derived from polymers of primarily labdanoid diterpenes, which include labdatriene carboxylic acids, alcohols, and hydrocarbons</td>
</tr>
<tr>
<td>la</td>
<td>Derived from/based on polymers and copolymers of labdanoid diterpenes with regular [1S, 4aR, 5S, 8aR] configuration, including communic acid, communol, and succinic acid; example: succinite (Baltic amber)</td>
</tr>
<tr>
<td>lb</td>
<td>Derived from/based on polymers and copolymers of labdanoid diterpenes with regular [1S, 4aR, 5S, 8aR] configuration, including communic acid, communol, bifornene, but not succinic acid; example: New Zealand’s fossil kauri resin</td>
</tr>
<tr>
<td>lc</td>
<td>Derived from/based on polymers and copolymers of labdanoid diterpenes with enantio [1S, 4aS, 5R, 8aS] configuration, including ozic acid, ozol, and enantio bifornenes; examples: Mexican and Dominican amber, African resinites</td>
</tr>
<tr>
<td><strong>Class II</strong></td>
<td>Derived from/based on polymers of bicyclic sesquiterpenoid hydrocarbons, especially cadinene, and related isomers; examples: Utah and Indonesian amber</td>
</tr>
<tr>
<td><strong>Class III</strong></td>
<td>Natural (fossil) polystyrene; example: some New Jersey amber, Sieburgite from Germany</td>
</tr>
<tr>
<td><strong>Class IV</strong></td>
<td>Nonpolymeric, incorporating sesquiterpenoids based on the cedrane carbon skeleton; example: Moravian amber from the Czech Republic</td>
</tr>
<tr>
<td><strong>Class V</strong></td>
<td>Nonpolymeric diterpenoid carboxylic acid, especially based on abietane, pinarane, and iso-pinarane carbon skeletons; example: Highgate copalite and other retinites in European brown coals</td>
</tr>
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</table>

The high-resolution NMR spectra of the carbon nuclei in powdered samples provided detailed information on the types of carbon functionality in the fossil resin, over the entire range of resonances, indicating the presence of saturated carbons (resonances 0–100 ppm), unsaturated carbons such as alkenic and aromatic (resonances 100–160 ppm), and carbonyl groups (resonances above 160 ppm) (Lambert and Frye, 1982). Using NMR, comparative analysis of samples from different origin permitted to distinguish four major groupings (Group A through D) of fossil resins (Lambert and Poinar, 2002), in good agreement with Anderson’s et al. classification based on Py-GC/MS.

**Age, global occurrence, and botanical origin of amber**

Amber might be found on every continent but most amber deposits have been discovered in the northern hemisphere, so far. A few ambers are known from South America, Africa, Australia, and New Zealand. Many new Mesozoic and Cenozoic amber localities have been described in the last decade (see Poinar, 1992; Grimaldi, 1996; Krumbiegel and Krumbiegel, 2005, for age and global occurrence of ambers).

A few resins were reported from the Carboniferous of Europe and the USA (Bray and Anderson, 2009). The first conifers which produced significant amounts of resin appeared in the Carnian stage of the late Triassic (about 220 million years ago). Remarkable amounts of small Triassic amber pieces have been found in the petrified forests of Arizona (Litwin and Ash, 1991), in the Molteno Formation of Lesotho (Ansorge, 2007) and the Heiligkreuz-Santa Croce Formation of the Italian Dolomites (Roghi et al., 2006, Figure 7). The latter is the oldest fossiliferous amber containing various microorganisms (Schmidt et al., 2006, see Figure 8). The Jurassic is particularly poor in amber and just few samples have been reported from the late Jurassic age of Portugal, Denmark (Bornholm), Germany, and Thailand.

The oldest Cretaceous ambers come from the Wealden Formation of southern England (Nicholas et al., 1993) and from the Kirkwood Formation of South Africa (Gomez et al., 2002), both being Valanginian in age. The most important fossiliferous Cretaceous ambers are (1) the Middle East ambers from Lebanon, Israel, and Jordan, ranging from the Hauterivian to the Aptian, (2) the Aptian–Albian Alava amber from Peñacerrada in the Basque county of northern Spain, (3) the Siberian amber from the Albian to Santonian of the Taimyr Peninsula, (4) the Albian–Cenomanian amber from the Czech Republic, (5) the Burmese amber from Myanmar which is Albian in age (Grimaldi et al., 2002; Cruickshank and Ko, 2003), (6) the Atlantic coastal plain ambers of the USA which comprise several localities of various Upper Cretaceous ages from Maryland and New Jersey to South Carolina, and (7) the Upper Cretaceous Canadian amber (Cedar Lake in Manitoba and other localities). Further remarkable Cretaceous resins come from Alaska, from several Albian to Coniacian localities in Japan, and from...
the Aptian age of Golling in Austria, but these ambers yield few fossils.

Cenozoic ambers are largely Eocene to Miocene in age, and some deposits already produced tons of resin. The most well-known fossil resin is the Baltic amber which can be found in the Baltic sea area (especially along the coast of Russia and Poland) in Eocene sediments, washed ashore and also as glacial erratic. The sediments bearing the Baltic amber are considered to be 38–47, some up to 55 million years old (Ritzkowski, 1999). Other fossiliferous Eocene ambers come from the Paris Basin in France (50–55 million years old; Nel et al., 1999), from Vastan (Gujarat) in western India (52 million years old; Alimohammadian et al., 2003) and from Fu Shun in the Chinese Liaoning province. The Bitterfeld amber from a coal mine near the city of Bitterfeld in central Germany is uppermost Oligocene in age (24 million years old, see Wimmer et al., 2006) and also produced a plethora of inclusions, mainly arthropods (Knuth et al., 2002). The most fossiliferous Cenozoic New World ambers come from the Dominican Republic and from Chiapas in Mexico (see Poinar, 1992). Recent studies suggest a Miocene age (15–20 million years old) for these Caribbean ambers (Iturralde-Vinent and MacPhee, 1996; Solórzano Kraemer, 2007). Further fossiliferous Miocene ambers were found in western Amazonia in Peru, and in Sicily and Borneo.

Representatives of various gymnosperm and angiosperm families have produced resin in the Earth’s history (see Langenheim, 2003; Krumbiegel and Krumbiegel, 2005, for overview). The Italian Triassic amber was possibly produced by members of the Cheirolepidiaceae, an extinct gymnosperm family. Among others, various representatives of the Araucariaceae (Figures 9 and 10) and Cupressaceae are considered to be resin-bearing trees in the Cretaceous. Angiosperm resins are recorded since the Eocene, for example, Glessite stems from Burseraceae and Siegburgite were produced by Hamamelidaceae. The tropical genus Hymenaea (Leguminosae) produced the Dominican and Mexican ambers. The main resin-bearing trees of the Baltic amber forest probably belonged to conifers of the family Sciadopityaceae, as recently suggested by Wolfe et al. (2009).

**Embedding and preservation of organisms in resin**

Resins are produced by plants as defensive reaction against mechanical damage (injury to the bark, fire, herbivores) and potential invasion by pathogens, such as bacteria and fungi. Also, climatic reasons and ecological disturbance have been discussed to be the reasons for extensive resin production (Henwood, 1993).

The exceptional preservation of organisms included in amber is due to the dehydration environment made possible by the resin and by natural embalming properties due to antiseptic activity of the resin constituents. Components that do not participate in polymerization of the resin, such as some diterpene resin acids (e.g., copalic acid), can remain available for the interactions with biota embedded in resin (Henwood, 1993) and contribute to the preservation even of soft-bodied inclusions. Even cell organelles such as chloroplasts of algae are sometimes well-preserved in amber (Figure 11).
Extraction of ancient DNA from amber has been of particular interest in working with fossils embedded in amber, and in the early 1990s first attempts to establish methods for extraction, amplification, and sequencing of fossil DNA from amber were carried out (e.g., Cano et al., 1994; Cano and Borucki, 1995). However, critical reinvestigations suggest that the DNA obtained in these studies was modern contamination and because even the amplification of DNA from copal failed, Austin et al. (1997) concluded that resin is not predestined for the preservation of complex molecules, although morphological structures are recorded very well.

**Amber, Figure 9** Resinous forest of *Araucaria columnaris* growing at the coast of Maré, New Caledonia (photograph by Alexander R. Schmidt).

**Amber, Figure 10** Extensive resin flows of ~30 cm length induced by fire at the bark of *Araucaria columnaris* in New Caledonia (photograph by Alexander R. Schmidt).

**Amber, Figure 11** Preservation of chloroplasts in the conjugatophyte *Palaeozygnema spiralis* from the Cenomanian resin of Schliersee in the Bavarian Alps. The cells are ~15 μm in diameter (photograph by Alexander R. Schmidt).
Attached to fresh sticky resin (Figure 10), organisms or parts of them are entirely covered by further resin flows. Amber inclusions are therefore usually found at the surfaces of successive resin flows in amber. Invertebrates of appropriate size, especially arthropods (e.g., diptera, hymenoptera, see Figure 12), and small plant remnants fallen onto the resin are therefore predestined for getting stuck and embedded. Arthropods, plant remnants (flowers, leaves, stellate hairs, wood, pollen, spores) and microorganisms are typical amber inclusions and sometimes, also small vertebrates (frogs, lizards) and parts of larger animals (reptile skin, feathers, hairs) are preserved. Some groups of organisms are only recorded as amber fossils, since few other fossilization processes occur in terrestrial environments which may preserve morphological structures even of soft-bodied organisms very well.

Many life forms and representatives of all trophic levels of a biocoenosis may be found in a single piece of resin: bacteria, algae as producers, protozoans, micrometazoans, molluscs, arthropods as consumers, and fungi as decomposers (Perrichot and Girard, 2009). Although many members of a biocoenosis can potentially become embedded in resin, selective embedding has been observed (Schmidt and Dilcher, 2007). Because of their motility, moving or flying arthropods have a higher probability of encountering and becoming attached to the resin. Therefore, the amount of amber inclusions of a species is not correlated with its abundance in the ancient ecosystem.

Most amber inclusions are fossils of terrestrial organisms which lived at or close to the resin-bearing trees. Much resin solidified at the forest floor, not on the bark (Henwood, 1993) and the term litter amber has been proposed by Perrichot (2004) for amber pieces which largely contain soil and litter-dwelling arthropods (Figure 13).

To find aquatic organisms in tree resin may seem to be highly unlikely but the fossil record provides amber-preserved limnetic arthropods (e.g., water beetles, water striders, crustaceans, larvae of caddisflies and mayflies) and microorganisms (e.g., algae, ciliates, testate amoebae, rotifers). Limnetic organisms may get stuck or enclosed when resin comes into contact with water or even flows into water (Schmidt and Dilcher, 2007).

Few amber inclusions of marine organisms (crustacea, diatoms) have been reported from amber forests which grew directly at the coast (Vonk and Schram, 2007; Girard et al., 2008; see Figure 14). These organisms were probably introduced by wind or spray from beach and sea onto the resin flows in the nearby woods.

Although resin has fungicidal properties, some fungi and bacteria are able to grow into liquid resin and can therefore be found in almost every fossil resin. Usually, these filaments grow in random orientation as long as the resin is liquid. Growth stops when the resin solidifies and the filiform inclusions become well-preserved in the resin. Resinicolous fungi such as ascomycetes of the Mycocaliciales are adapted to their special substrates and are also able to grow on fresh resin. Therefore, they may be found in places where herbivore insects induce long-term resin flows (Rikkinen, 1999; Rikkinen and Poinar, 2000).

Resin which dried at the bark is exposed to the air, and processes of weathering and degradation start rapidly. Long-time preservation of resins and its inclusions and

Amber, Figure 12 Hymenoptera (Scolionidae), fossilized in Lower Cenomanian amber of Fours, Charente-Maritime, southwestern France. The inclusion is 1.8 mm in length (courtesy of Vincent Perrichot).

Amber, Figure 13 Marchandia magnifica, a mole cricket from Albian amber from southwestern France. It is 3.8 mm in length (photograph by Vincent Perrichot, courtesy of Elsevier/Academic Press, Cretaceous Research, 23, 307–314, 2002).
formation of amber therefore generally needs redeposition into marine sediments or at least a cover by sediment layers to stop processes of oxidation.

Summary
Amber is fossilized plant resin, which is largely found in the Cretaceous and in the Eocene to Miocene when various representatives of gymnosperms and angiosperms produced considerable amounts of resin. After exudation from plants, the volatile components evaporate and the remaining nonvolatile constituents polymerize which leads to the hardening of the resin. Formation of amber occurs through the process of “maturation,” which is defined as the progressive changes of the resin occurring after the hardening and embedding into the sediment. The chemical reactions in the maturation of the resin to yield fossil resin include cross-linking, isomerization, and cyclization. Several different parameters can play relevant roles in amber composition, such as original environment, age, plant source, diagenetic history, and also alteration processes after amber mining. Inclusions of many life forms may be found in amber, although selective embedding has been observed. The taphocoenosis in a piece of amber may potentially contain organisms of different habitats such as tree bark, soil and litter, freshwater ponds, and littoral.

Bibliography


Cross-references
- Algae (Eukaryotic)
- Bacteria
- Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification
- Biomarkers (Molecular Fossils)
- Diatoms
- Fungi and Lichens
- Geomycology
- Protozoa (Heterotroph, Eukaryotic)

ANAEROBIC OXIDATION OF METHANE WITH SULFATE

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Definition
Anaerobic oxidation of methane (AOM): microbially mediated oxidation of methane to CO₂ by electron acceptors other than oxygen.

Introduction
Methane is the most abundant hydrocarbon in the atmosphere, and an important greenhouse gas (see *Methane, Origin*). A great deal of research has focused on the cause and climatic consequences of the variation in fluxes of methane to the atmosphere, throughout the Earth’s history. Three key functional groups of microbial organisms play a central role in regulating the fluxes of methane on the Earth, namely the methanogens, the aerobic methanotrophic bacteria, and the more recently discovered anaerobic methanotrophic archaea (ANME). It is estimated that AOM is a major sink for methane on the Earth, and of similar relevance as its photooxidation in the atmosphere (Hinrichs and Boetius, 2002; Reeburgh, 2007).

Today, most methane is produced by methanogenesis, i.e., the final step in the fermentation of organic matter taking place in soils, wetlands, landfills, rice fields, freshwater and marine sediments, as well as in the guts of animals. Almost all of the methane produced in ocean sediments is consumed by AOM within the sulfate penetrated seafloor zones. Hence, the ocean does not contribute significantly to the atmospheric methane budget (<2%, Reeburgh, 2007). The first evidence for the removal of methane within anoxic sediments and seawaters came from geochemical observations in the 1970s and 1980s (Reeburgh, 1969, 1976; Barnes and Goldberg, 1976; Martens and Berner, 1977). The involvement of sulfate as terminal electron acceptor was demonstrated by radiotracer measurements of methane oxidation and sulfate reduction in parallel incubations of undisturbed sediment samples (Iversen and Jørgensen, 1985). Field observations and experiments led to the hypothesis of a coupled mechanism, in which both methanotrophic archaea and sulfate-reducing bacteria (SRB) could profit from AOM, despite the generally low thermodynamic energy yield from this reaction (Hoehler et al., 1994). Finally, consortia of such microorganisms were detected in sediments from methane seeps, and the substantial depletion of their specific biomarkers in the stable carbon isotope 13C suggested that methane or a methane-derived compound is used as carbon source by these organisms (Hinrichs et al., 1999; Boetius et al., 2000; Orphan et al., 2001b). Till recently, all known anaerobic methanotrophs were clades related to the methanogenic Euryarchaeota termed ANME. In 2008, bacteria were found capable of oxidizing methane anaerobically with nitrate (Ettwig et al., 2008; 2010). In the past decade, much has been learned about the distribution, activity, and physiology of the ANME, however, not a single member of these groups has been obtained in culture and the biochemical functioning of AOM remains unknown.

The process of AOM
The AOM with sulfate as the final electron acceptor according to

\[\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O} \quad (1)\]

is the major sink of methane in the oceans and hence a significant process in the global carbon cycle and methane budget (Reeburgh, 2007). Aggregated methanotrophic archaea (ANME) and SRB are assumed to act synthrophically (Hoehler et al., 1994; Hansen et al., 1998; Hinrichs et al., 1999; Boetius et al., 2000) where the archaean partner activates and metabolizes methane, leading to an intermediate that is scavenged as electron donor by the sulfate-reducing partner. So far, there is no evidence for the involvement of extracellular intermediates such as hydrogen, formate, or acetate (Nauhaus et al., 2002; Boetius et al., 2008). The process of AOM with sulfate has been maintained in vitro in the laboratory and investigated with respect to substrate-product stoichiometry and dependence on pressure, temperature, and a variety of other environmental factors (Nauhaus et al., 2002, 2005, 2007; Girguis et al., 2003, 2005). The ANME doubling time was reported to be approximately 7 months, and the molar growth yield of AOM was 0.6 g cell dry weight (mol CH₄ oxidized)⁻¹. Corresponding to this estimate, only 1% of the consumed methane is channeled into the ANME biomass...
(Nauhaus et al., 2007). Further steps toward an understanding of the metabolic pathway were the purification of a candidate enzyme (Ni-protein I) that may catalyze methane activation in a reverse terminal methyl-coenzyme M reductase reaction (Krüger et al., 2003) and genome-based observations of nearly all genes involved in methane production present in one specific ANME type (Hallam et al., 2004; Meyerdierks et al., 2009) both supporting the hypothesis that ANME carry out AOM by reverse methanogenesis.

**Anaerobic methanotrophs**

**Discovery of ANME**

Hinrichs et al. (1999) discovered a new archaeal group in sediments from a methane seep in the Eel River basin off California, together with characteristic archaeal lipid biomarkers strongly depleted in $^{13}$C, indicating that methane must be the carbon source (Hinrichs et al., 1999). The dominant archaeal group was defined as ANME-1 and a second major group associated with was identified later as ANME-2 (Orphan et al., 2001a). Boetius et al. (2000) provided the first microscopic evidence for a syntrophic association of ANME archaea and SRB (Boetius et al., 2000). In both studies specific archaeal lipid biomarkers, i.e., archaeol, hydroxyarchaeol, crocetane, and pentamethylicosane (PMI), and bacterial lipid biomarkers common for SRB, i.e., iso- and anteiso-C$_{15}$ fatty acids, were strongly depleted in $^{13}$C. The first direct link between the identity and metabolic activity of ANME was provided by using a combination of ion microprobe mass spectrometry (SIMS) and fluorescence in situ hybridization (FISH) (Orphan et al., 2001b, 2002). These experiments confirmed the extreme $^{13}$C depletion in the aggregate biomass, as predicted from the biomarker extractions. In 2006, a third archaeal ANME group, ANME-3, was discovered at the submarine mud volcano Haakon Mosby in the Barents Sea (Niemann et al., 2006b). Recently, a new clade of ANME-associated archaea (AAA) was identified in a bioreactor inoculated with sediments from a Dutch canal, but their relevance in freshwater and marine environments remains unknown (Raghoebarsing et al., 2006; Knittel and Boetius 2009).

**Diversity and morphology of ANME organisms**

All yet known ANMEs are Euryarchaeota, and they form clusters distantly or closely related to the orders *Methanosarcinales* and *Methanomicrobiales*, which comprise a major part of the cultivated methanogens (Figure 1a).

The phylogenetic distance between the three ANME groups is large with 75–92% sequence similarity. Thus, members of ANME-1 and ANME-2 certainly belong to different orders or families, which have apparently similar physiological properties, i.e., the capability to oxidize methane anaerobically.

ANME-1 cells have a typical rectangular morphology and are autofluorescent under UV light, a feature characteristic for methanogenic archaea containing coenzyme F$_{420}$. ANME-1 archaea most often occur as single cells (Figure 2a) or in chains of two to four cells. In the Black Sea, “mat-type” associations of ANME-1 archaea and sulfate-reducing partners (Figure 2b) or ANME-1 and ANME-2 archaea (Figure 2c) form microbial mats (Michaelis et al., 2002; Knittel et al., 2005; Treude et al., 2007).

ANME-2 archaea belong to the order *Methanosarcinales*, but sequence similarity to cultivated representatives is below 90%. Similarity between subgroups, ANME-2a to ANME-2c, varies with an inter- and intra-group similarity of 75–90% and 90–100%, respectively. Diverse forms of associations between ANME-2 and their partner bacteria have been found (Figure 2d–k). Single ANME-2 cells were also reported (Figure 2d, l; Treude et al., 2007) as aggregated ANME-2 without any partner (Figure 2m, n; Orphan et al., 2002; Treude et al., 2007). In addition to the differences in phylogenetic origin, the two subpopulations ANME-2a and -2c could be distinguished from each other by their aggregate morphology. In general, ANME-2a/Desulfoarcina (DSS) aggregates represent the “mixed-type.” Here, archaea and SRB are completely mixed and the aggregates are not always spherical (Figure 2f–k). Typical ANME-2c/DSS aggregates represent the common “shell-type” with an inner core of ANME-2 which is partially or fully surrounded by an outer shell of SRB (Figure 2o–x). Sometimes SRB grow into the inner ANME-2c core, but they do not seem to mix completely (Figure 2u–w; Knittel et al., 2005). Microscopical analysis of a growing ANME-2 population showed that population growth occurred both as an increase in the number as well as in size of the shell-type consortia (Nauhaus et al., 2007). Starting from a few archaea and SRB cells, consortia seem to develop from smaller sized consortia to big aggregates of up to 100,000 cells (Holler and Knittel, unpublished data). Larger consortia separate evenly into two or unevenly into more aggregates when SRB grow into the archaeal colonies. The average diameter of consortia is 3–5 µm in situ with the smallest detected aggregate consisting of one ANME and one SRB cell and the largest 20 µm (Boetius et al., 2000; Knittel et al., 2005; Løsekann et al., 2007). Even much larger aggregates have been observed in enrichment cultures with >40 µm in diameter (Nauhaus et al., 2007; Holler, unpublished data). After reaching a specific size they appear to burst, releasing single cells into the environment (Figure 2ab).

The ANME-3 clade also belongs to the order *Methanosarcinales* and is closely related to cultivated species of the genus *Methanococcales* with 95% 16S rRNA sequence similarity (Figure 1a). They form shell-type aggregates with a Desulfovoribius population as sulfate-reducing partner, however, only very few bacterial cells are associated (Figure 2y–z, aa).
Anaerobic Oxidation of Methane with Sulfate, Figure 1 (Continued)
Identification of ANME by methyl coenzyme M reductase gene phylogeny

The first genomic and proteomic analyses of sediments and microbial mats naturally enriched in the ANME biomass revealed a nickel protein similar to methyl coenzyme M reductase (MCR) as well as genomic fragments coding for nearly all genes typically associated with methane production. Based on these observations, earlier hypotheses on the role of reverse methanogenesis in archaea (Zehnder and Brock, 1979, 1980; Hoehler et al., 1994) appeared in a new light. Subsequently, molecular studies were conducted targeting the enzyme MCR, which catalyzes the terminal step in biogenic methane production (Shima and Thauer, 2005). Investigations have revealed a remarkably high phylogenetic diversity within mcrA genes among ANME archaea (Hallam et al., 2003; Inagaki et al., 2004, 2006a; Dhillon et al., 2005; Kelley et al., 2005; Alain et al., 2006; Lloyd et al., 2006; Nunoura et al., 2006; Løsekkann et al., 2007) which have been classified into four subgroups (Hallam et al., 2003; Løsekkann et al., 2007), group a–b (ANME-1), group c–d (ANME-2c), group e (ANME-2a), and group f (ANME-3) (Figure 1b). These groups are all distinct from those formed by methanogens. ANME mcrA gene phylogeny appears to be partially phylogenetically congruent to the 16S rRNA gene (Figure 3; Hallam et al., 2003; Nunoura et al., 2006; Løsekkann et al., 2007). Quantification of specific ANME groups based on their mcrA gene abundance is now an alternative method to 16S rRNA based FISH to study distributional patterns of anaerobic methanotrophs in AOM zones (Nunoura et al., 2006).

Identification of ANME by their specific lipid biomarkers

Another molecular method, which has been crucial in the investigation of the natural distribution of ANME populations, is the identification of specific lipid biomarkers (see Biomarkers) and their stable carbon isotope signatures. This method can integrate phylogenetic information with function (13C signatures indicating methane assimilation) and can be used for comparative analyses of community biomass. All three known ANME groups and their partner bacteria incorporate light (13C-depleted) methane-derived carbon into their membrane lipids (Orphan et al., 2002; Niemann et al., 2006b). Using either naturally 13C depleted methane (Nauhaus et al., 2007) or 13C labeled methane as substrate (Blumenberg et al., 2004) for active populations of ANME in environmental samples have helped to identify typical membrane lipid profiles. Crocetane is the only biomarker lipid specific for ANME, i.e., not found in methanogen cultures. Glycol dibiphytanylglycerol tetraethers (GDGT) were also used as indicator of ANME distribution (Schouten et al., 2003), but the ANME GDGTs appear to show some overlap with those of benthic Crenarchaeota, which often share the same niche in the seabed (Knittel et al., 2005). Recently, the biomarker approach was extended to include intact polar lipids (IPLs) which are of higher taxonomic specificity (level of families to orders) and property to select for living biomass (Sturt et al., 2004; Biddle et al., 2006; Rossel et al., 2008). Characteristic IPL molecular fingerprints have been reported for each specific ANME type: the lipids of ANME-1 archaea were dominated by diglycosidic GDGT derivatives, while IPLs of ANME-2 and ANME-3 archaea are dominated by phosphate-based polar derivatives of archaeol and hydroxyarchaeol (Rossel et al., 2008).

Bacterial partners of ANME

ANME-1 and ANME-2 archaea are usually associated with SRB of the SEEP-SRB I branch, subgroup a, of the Desulfosarcina/Desulfococcus (DSS) group (Figure 2; Schreiber et al., 2010). These SRB are physically attached to the ANME-2 archaea, whereas ANME-1 is most often found without close physical association to other cells. The diversity within the SEEP-SRB1 cluster is high, and it is still unknown if the partners of the ANME-2a, -2b, and -2c subgroups belong to the same species. The morphology of the DSS cells varies from cocci (mostly associated with ANME-2c cells, Figure 2p–x) to rods (mostly associated with ANME-2a cells, Figure 2f–j). Other SRB have not yet been identified to be associated with ANME-1 and -2. ANME-3 archaea are most often associated with SRB of the Desulfobulbus (branch (Figure 2), but have also been detected together with SRB of the Desulfosarcina/Desulfococcus group in shallow subsurface sediments of cold seeps from the Hydrate Ridge (Løsekkann et al., unpublished data). Single cells of ANME-1, -2, and -3 have also been found in situ as well as in vitro enrichments, indicating that the physical association may be not obligate, but certainly the typical life mode of the anaerobic methanotrophs in most habitats.

There is also some evidence that the diversity of bacteria associated with ANME may be larger than anticipated. The analysis of ANME-2c consortia captured by whole-cell magnetofISH showed a diversity of bacterial partners of ANME-2 far beyond the Deltaproteobacteria.
Anaerobic Oxidation of Methane with Sulfate, Figure 2  Epifluorescence micrographs of different ANME single cells and aggregates visualized by fluorescence in situ hybridization (FISH) or CARD-FISH. (a) Single ANME-1 cells living in a microbial mat from the Black Sea; (b) mat-type consortium formed by ANME-1 (red) and DSS cells (green); (c) aggregated ANME-1 (green) and ANME-2 cells (blue) in a microbial mat from the Black Sea; (d) single ANME-2a cells in an enrichment culture from Hydrate Ridge sediments; (e–k) mixed-type consortia of ANME-2a (red) and DSS (green) cells observed in different seep sediments; (l) single ANME-2a cells; (m) monospecific ANME-2a aggregate; (n) corresponding DAPI staining in an enrichment culture from Hydrate Ridge sediments; (o–x) shell-type consortia of ANME-2c (red) and DSS (green) cells of different sizes and structure observed in different seep sediments; (y–aa): ANME-3; (y) monospecific aggregation of ANME-3 at Haakon Mosby mud volcano, (z, aa) ANME-3/Desulfobulbus consortia; and (ab): DAPI staining of a large ANME-2c aggregate. Unless otherwise indicated, scale bar 10 m.
Lösekann et al. found evidence for ANME-3 aggregates with unidentified partners showing a mixed-type morphology in sediments from the Arctic mud volcano Haakon Mosby (Lösekann et al., 2007). Since their discovery, the distribution of the ANME organisms has been studied intensively, mainly based on 16S rRNA gene phylogeny. More than 1800 16S rRNA gene sequences are available now from more than 50 different marine methane seeps (Hinrichs et al., 1999; Orphan et al., 2001a; Aloisi et al., 2002; Tourrov et al., 2002; Mills et al., 2003, 2004, 2005; Inagaki et al., 2004; Heij et al., 2005, 2007; Knittel et al., 2005; Lanoil et al., 2005; Stadnitskaia et al., 2005; Arakawa et al., 2006a, b; Fang et al., 2006; Lloyd et al., 2006; Martinez et al., 2006; Niemann et al., 2006a; Nunoura et al., 2006; Reed et al., 2006; Lösekann et al., 2007), vents (Takai and Horikoshi, 1999; Teske et al., 2002; Kelley et al., 2005; Brazelton et al., 2006; Inagaki et al., 2006a), and “sulfate–methane transition zone” (SMTZ) (Thomsen et al., 2001; Niemann et al., 2005; Parkes et al., 2007) differing in e.g., temperature, methane flux, salinity, and pH. Furthermore, the presence of ANME organisms in the water column (Black Sea; Vettori et al., 2003; Schubert et al., 2006a) and in a terrestrial mud volcano in the Carpathian mountains (Romania; Alain et al., 2006) has also been reported. A comparison of these reports shows that globally, the two most abundant and diverse phylogenetic groups of methane-oxidizing archaea are the ANME-1 and ANME-2 clades. The distribution of ANME-2a and ANME-2c is as wide as for ANME-1, but only few sequences have been reported belonging to subgroup ANME-2b. ANME-2b, originally defined on the basis of few sequences as a separate group (Orphan et al., 2001a), now seem to be rather a subgroup of ANME-2a than a monophyletic group (Figure 1a).

Today it is known that ANME are present wherever methane and sulfate co-occur, at a wide range of environmental conditions (Knittel and Boetius, 2009). However,
usually only one specific ANME group dominates a habitat and seems to be responsible for most of the measured AOM (Knittel et al., 2005). High rates of methane oxidation, sulfate reduction, and/or sulfide production can be used as an indicator for high densities of ANME populations (Boetius et al., 2008). Continuous high fluxes of both AOM substrates methane and sulfate at mM concentrations sustain high cell densities of $>10^{10}$ ANME cells cm$^{-3}$ in the environment and can even form some of the densest and largest cell accumulations known to exist in nature (Michaelis et al., 2002).

Seep ecosystems

Cold seeps form where tectonic or gravitational forces advect free gas, methane-rich porewaters and/or mud upward into the sulfate-penetrated surface sediments and sometimes into the hydrosphere (Judd and Hovland, 2007; see Cold Seeps). The increased availability of dissolved methane at higher hydrostatic pressures increases the energy yield of AOM and leads to a natural enrichment of ANME populations. The products of AOM, sulfide and carbonate accumulate in the seabed, forming the typical features of cold seep ecosystems such as carbonate precipitates and high sulfide fluxes. The sulfide is oxidized chemically and microbially with oxygen, nitrate, or iron close to the seafloor surface. One of the most striking visual features of submarine cold seep ecosystems are mats of thiotrophic bacteria covering the seafloor. These mats are usually associated with high AOM rates and dense ANME communities inhabiting the underlying sediments (Sahling et al., 2002; Treude et al., 2003; Joye et al., 2004; Niemann et al., 2006b). Also, a variety of thiotrophic microbe–animal symbioses profit from AOM, such as siboglinid tubeworms and mytilid as well as vesicomyid bivalves (Sibuet and Olu, 1998). Thirotrophic mats and invertebrate symbioses may enhance the growth of ANME populations by rapidly removing the toxic endproduct sulfide, and by replenishing sulfate in the sediments (Treude et al., 2003; Cordes et al., 2005; Niemann et al., 2006b). In active cold seep sediments, AOM rates of 500–5,000 nmol CH$_4$ cm$^{-3}$ day$^{-1}$ (>2 mol m$^{-2}$ year$^{-1}$) are reached, associated with very dense ANME populations of $>10^{10}$ cells cm$^{-3}$ (Boetius et al., 2000; Knittel et al., 2005; Niemann et al., 2006b).

Cold seep ecosystems from Eel River Basin (Hinrichs et al., 1999; Orphan et al., 2001b), Hydrate Ridge (Boetius et al., 2000; Knittel et al., 2003, 2005), Black Sea (Thiel et al., 2001, 2003, 2007; Michaelis et al., 2002; Blumenberg et al., 2005; Reitner et al., 2005a, b; Treude et al., 2005a, 2007), Gulf of Mexico (Orcutt et al., 2005; Lloyd et al., 2006; Martinez et al., 2006), the Tommelen and Gulf of Alaska area in the North Sea (Wegener et al., 2008), and mud volcanoes from the Mediterranean Sea (Omoregie et al., 2008) and the Barents Sea (Niemann et al., 2006b; Lösekann et al., 2007) have been intensively studied. With the exception of cold seeps in the Black Sea (see next) most of the studies indicate a predominance of ANME-2 or ANME-3. A prominent example is the gas hydrate bearing sediment from the Hydrate Ridge. Hot spots of ANME-2 are surface layers just above the gas hydrates with up to $10^{8}$ aggregates cm$^{-3}$. Interestingly, ANME-2 subgroups revealed preferences for either Beggiatoa (ANME-2a) or Calyptogena (ANME-2c) fields (Knittel et al., 2005) indicating that different environmental conditions select for different ANME groups.

Two seep systems are known yet where other ANMEs dominate: (1) sediments overlaying a brine pool methane seep in the Gulf of Mexico are dominated by ANME-1b archaea. Here, the ANME-1b community was found in the sulfate-methane interface, where low methane concentrations of ca. 100–250 µM are present and coexist with sulfate concentrations of ca. 10 mM, and (2) microbial mats from Black Sea cold seeps. These seeps are unique ecosystems and AOM is important both in sediments and in the water column (Pimenov et al., 1997; Wakeham et al., 2003). Tall reef-like structures composed of porous carbonates and microbial mats are found on the seafloor. These mats have been shown to mediate AOM and consist mainly of densely aggregated ANME-1 cells and SRB (Michaelis et al., 2002; Blumenberg et al., 2004, 2005; Knittel et al., 2005; Reitner et al., 2005a, b; Treude et al., 2005a, 2007). Treude et al. (2007) combined radiotracer incubations, beta-microimaging, SIMS, and CARD-FISH to locate hot spots of methanotrophy. Incorporation of $^{13}$C from radiolabeled CH$_4$ indicated a hot spot for methanotrophy close to the mat surface associated with a dominance of ANME-1 archaea (Treude et al., 2007). The mats, however, are very heterogeneous and also provide niches for ANME-2. Black nodules from the top of the reef seem to be dominated by ANME-2 as shown by specific $^{13}$C depleted lipids and FISH (Blumenberg et al., 2004). Reitner et al. found intracellular precipitation of iron sulfide (greigite) by bacteria growing in close association with ANME-2 and suggested iron cycling as an additional pathway involved in AOM (Reitner et al., 2005b).

ANME-3 archaea are commonly found at submarine mud volcanoes (Niemann et al., 2006b; Heijis et al., 2007; Lösekann et al., 2007; Omoregie et al., 2008) but also at other cold seeps (Orphan et al., 2001a; Inagaki et al., 2004; Knittel et al., 2005), hydrothermal vents (Brazelton et al., 2006), and recently in subsurface sediments (Parkes et al., 2007).

Sulfate–methane transition zones (SMTZ)

The most common ANME habitat on the Earth is the so-called SMTZ in the seabed (Thomsen et al., 2001; Ishii et al., 2004; Niemann et al., 2005, 2006a; Treude et al., 2005b; Parkes et al., 2007). SMTZ are found in all seabed horizons where the diffusive transport of methane from below and sulfate from above leads to a zone of AOM. Methane is completely consumed in the SMTZ, which may be found at decimeters to tens of meters below the seafloor (D’Hondt et al., 2004), depending on the depth
of the methane production zone, on the transport velocity of methane and sulfate and on their consumption rates. In diffusive seabed systems, the distribution of ANME is restricted to SMTZ. Population densities of ANME are low with \(<10^6\) cells cm\(^{-3}\) (Niemann et al., 2005) and associated AOM rates are below 10 nmol cm\(^{-3}\) day\(^{-1}\) (\(<0.04\) mol m\(^{-2}\) year\(^{-1}\)). The 16S rRNA gene libraries of ANME populations associated with SMTZ zones are very similar to those of cold seeps. The in situ dominance of ANME groups varies between the habitats. In SMTZ, sediments of Eckernförde Bay (German Baltic) ANME-2 archaea dominated (Treude et al., 2005b) as in the Skagerrak off the Danish coast (Parkes et al., 2007) or in a tidal sand flat of the German Wadden Sea (Ishii et al., 2004) while in the SMTZ of the Tommeliten seep area or at Hydrate Ridge ANME-1 were dominant (Niemann et al., 2005; Orcutt et al., unpublished data). Most likely, the SMTZ ANME archaea represent the seed populations for cold seep communities. It remains unknown whether SMTZ ANME possess special physiological adaptations to their energetically less favorable habitat compared to those at cold seeps.

So far, no ANME-related sequences have been published from the deep subsurface (D’Hondt et al., 2004; Newberry et al., 2004; Biddle et al., 2006; Inagaki et al., 2006b; Sorensen and Teske, 2006; Webster et al., 2006). Even in an extensive study by Inagaki et al. sequencing of several thousands of clones obtained from methane hydrate sites from Peru and Cascadia Margin did not result in the retrieval of ANME sequences (Inagaki et al., 2006b). Instead, archaea of the Marine Benthic Group B (MBGB, synonym Deep Sea Archaeal Group, DSAG) and the Miscellaneous Crenarchaeotal Group (MCG) were consistently the dominant phylotypes. Sediments lacking methane hydrates showed no or few MBGB phylotypes. The study by Biddle et al. supported these findings and suggested, based on stable isotopic compositions and intact archaeal lipids that these archaea assimilate organic carbon other than methane (Biddle et al., 2006).

**Hydrothermal vents**

High methane fluxes are also found at hydrothermal vents of mid ocean ridges (see Hydrothermal Environments, Marine), but these ecosystems may not offer many niches for ANME populations. The seafloor of mid ocean ridges consists of basalts and lacks a sediment cover, hence, the habitats for ANME-related organisms are limited to small anoxic zones within vent chimneys (Schrenk et al., 2004; Kelley et al., 2005; Brazelton et al., 2006). Furthermore, the fluids of most hydrothermal vents are sulfate free, and the seawater, which could provide sulfate, is oxygen rich – and hence toxic for ANME organisms. Temperatures range from \(<40^\circ\text{C}\) to \(90^\circ\text{C}\) at pH 9 to 11. By contrast, sedimentary hydrothermal systems such as the Guaymas basin offer suitable habitats within the surface seafloor similar to the cold seep systems (Teske et al., 2002; Schouten et al., 2003) although AOM accounts for \(<5\%\) of sulfate reduction (Kallmeyer and Boetius, 2004). Another extreme environment in which ANME have been recently found is the CO\(_2\) vented sediments of the Yonaguni Knoll hydrothermal field, southern Okinawa Trough, with an in situ pH \(<5\) (Inagaki et al., 2006a).

**Water column**

The efflux of methane from cold seeps on the continental margin and mud volcanoes on the abyssal plain make the Black Sea the world’s largest surface water reservoir of dissolved methane (Schubert et al., 2006b). Methane concentrations of up to 12 µM and AOM rates in the range of nM day\(^{-1}\) have been measured (Reeburgh et al., 1991). ANME-1 and ANME-2 archaea are suggested to be responsible for pelagic AOM in the Black Sea (Vetriani et al., 2003; Schubert et al., 2006a, b) accounting each for 3–4% of total cells (Durisch-Kaiser et al., 2005). ANME-1 and ANME-2 appear as single cells in anoxic water, compared to the sediment, where they most often form cell aggregates. Similar to the findings for sediments, one ANME-group dominates, i.e., below approximately 600 m water depth ANME-1 archaea and above 600 m ANME-2 archaea are suggested to be responsible for AOM (Schubert et al., 2006a).

**Terrestrial habitats**

The presence of ANME has also been reported for terrestrial habitats. ANME-2a archaea are the organisms responsible for AOM activity in a mud volcano field located in the Carpathian Mountains (Romania). Here, thermal alteration of sedimentary organic compounds leads to the release of methane and higher hydrocarbons into the environment (Alain et al., 2006). Additionally, presence of ANME-1 and -2 archaea in a freshwater lake has been reported (Lake Plußsee; northern Germany (Eller et al., 2005)). Long anoxic periods during the summer together with a high organic load in Lake Plußsee favor anaerobic processes in the sediment. In a distinct layer in the anoxic water body, low numbers (\(<1\%\)) of single ANME archaea were found.

**Methane oxidation coupled to denitrification**

Methane oxidation could theoretically also be coupled to electron acceptors such as NO\(_3^-\), Fe(III), and Mn(IV). Recently, Raghoebarsing et al. (2006) demonstrated AOM coupled to denitrification of nitrate and nitrite in an enrichment culture according to

\[
5\text{CH}_4 + 8\text{NO}_3^- + 8\text{H}^+ \rightarrow 5\text{CO}_2 + 4\text{N}_2 + 14\text{H}_2\text{O}
\]

\(
\Delta G^\circ = -765 \ \text{kJmol}^{-1}\text{CH}_4
\)

\[
3\text{CH}_4 + 8\text{NO}_2^- + 8\text{H}^+ \rightarrow 3\text{CO}_2 + 4\text{N}_2 + 10\text{H}_2\text{O}
\]

\(
\Delta G^\circ = -928 \ \text{kJmol}^{-1}\text{CH}_4
\)
Anoxic sediment from the Twente canal in the Netherlands was used as inoculum to grow these organisms. The canal contained nitrate concentrations of up to 1 mM and the sediment was saturated with methane, which is typical for freshwater habitats receiving agricultural runoff. The bioreactor biomass was dominated by bacteria of the NC10 clade, a deep branching diverse phylogenetic group (Rappe and Giovannoni, 2003; Raghoebarasing et al., 2006). Recently, the genome of the dominant bacteria Candidatus Methylomirabilis oxyfera has been sequenced and surprisingly this apparently anaerobic, denitrifying bacterium encoded, transcribed and expressed the well-established aerobic pathway for methane oxidation, whereas it lacked known genes for dinitrogen production. Isotopic labelling experiments indicated that ‘M. oxyfera’ bypassed the denitrification intermediate nitrous oxide by the conversion of two nitric oxide molecules to dinitrogen and oxygen, which was used to oxidize methane (Ettwig et al., 2010).

Summary

The AOM is a key biogeochemical process on the Earth, and represents a globally important sink for methane. ANME are present in all environments where methane and sulfate overlap. Unfortunately, no member of the ANME groups or of their sulfate-reducing partner bacteria has been cultivated yet, and the biochemical process is not understood. As long as pure biomass of the archaea is missing, culture-independent molecular tools are the most helpful techniques to investigate the functioning of AOM, including metagenomics and proteomics. Protein patterns of different enrichments help in elucidating the most abundant and hence the most relevant proteins for both partners. Questions to the genome of both partners include for example, whether the ANME themselves may possess the genes for sulfate reduction, and what carbon and energy sources the sulfate reducers could utilize. Answering such questions from metagenomic data sets, however, remains difficult due to the relatively high diversity in the environmental enrichments, and the high proportion of open reading frames in the metagenomes which cannot be assigned to a certain functional category. Here, single cell technologies may provide solutions in the future.

Bibliography


ANAEROBIC OXIDATION OF METHANE WITH SULFATE


Cross-references

Anaerobic Transformation Processes, Microbiology
Archaea
Biomarkers (Molecular Fossils)
Biomarkers (Organic, Compound-Specific Isotopes)
Biosignatures in Rocks
Carbon (Organic, Cycling)
Cold Seeps
Fluorescence in Situ Hybridisation (FISH)
Isotopes and Geobiology
Methane Oxidation (Aerobic)
Methane, Origin
Microbial Communities, Structure, and Function
Microbial Mats
Microbiomes, Modern
Nickel, Biology
Sulfate-Reducing Bacteria

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ANAEROBIC TRANSFORMATION PROCESSES, MICROBIOLOGY

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Definition and Introduction
Microbial activities in geochemical processes are most often redox reactions, either between organic matter and inorganic compounds or between different inorganic compounds. In surface soils and lake sediments, organic matter typically derives from primary production by land plants or aquatic plants including algae, provided either directly or after primary digestion by animals. Underground soils and groundwater may receive major amounts of comparably stable organic matter, most often petroleum and its derivatives, through spills and accidents. Microbial oxidation helps to eliminate such contaminants, with concomitant reduction of inorganic electron acceptors. The surprising abundance of bacteria-like structures and even living bacteria in Deep Sea sediments and terrestrial rock material down to several 100 m below surface raised the question of how microbial life can be fuelled in the apparent absence of perceptible external substrate input. The present overview will try to provide access for geoscientists and other nonbiologists to the concepts of microbial life in the anaerobic world in general.

The terms “anoxic” and “anaerobic” are often used simultaneously with more or less the same meaning. Anoxic means “oxygen-free” and refers to habitats, incubation conditions, etc. The term anaerobic contains the Greek root “bios” (life) and refers to microbes, microbial activities, enzymes, etc.

Organic matter as electron donor in geochemical processes
Organic matter as supplied by plant primary production consists mainly of carbohydrates, proteins, fats, and nucleic acids. Digestion by soil animals decreases the carbohydrate and protein fraction, and increases the content of polyannellated phenolic residues (humic compounds, Stevenson, 1994). The complete oxidation of carbohydrates at pH 7.0, using glucose as a representative molecule, yields electrons at $E_0' = –434 \text{ mV}$ on average (Figure 1a). These electrons are transferred inside microbial cells via specific electron carrier systems, most often via NAD$^+$/NADH, at $E_0' = –320 \text{ mV}$. Other carriers operate at lower (e.g., ferredoxins around $–420 \text{ mV}$) or higher (flavins, quinones, cytochromes; Figure 1b) redox potentials.

![Redox potentials of organic substrates, biochemical electron carriers, and natural electron acceptor/donor systems.](image)

**Figure 1** Redox potentials of (a) organic substrates, (b) biochemical electron carriers, and (c) natural electron acceptor/donor systems. Values taken or calculated from Thauer et al. (1977) and Zehnder and Stumm (1988). MQ menaquinone; UQ ubiquinone.
potentials, and finally all electrons may end up at the O$_2$/H$_2$O-couple at $E_0^\circ = +810$ mV (Figure 1c). Electron transport from low to high redox potentials provides chemical energy, which the cell conserves in energy-rich phosphoric acid anhydride linkages in the form of ATP which can be formed in the cell basically by two alternative processes (Thauer et al., 1977): in substrate-level phosphorylation, the transformation of a substrate molecule leads directly to an energy-rich intermediate from which ATP is formed in a stoichiometrically linked reaction. In electron transport phosphorylation, electron transport over a sufficient redox potential difference is coupled to the establishment of a proton (sometimes Na$^+$) gradient across the cytoplasmic membrane which secondarily drives ATP synthesis via a membrane-bound ATP synthase reaction. Major steps in electron transport phosphorylation are the electron transports between NADH and the quinones, between quinones and cytochrome c, and between cytochrome c and oxygen (Figure 1b and c). In total, oxidation of glucose to 6CO$_2$ with oxygen as electron acceptor can yield up to 38 mol ATP per mol glucose (Fuchs, 2006).

Partial digestion of biomass leads to less energy-rich derivatives that are less valuable as electron donors. Incomplete oxidation and polymerization of aromatic and aliphatic plant matter residues forms humic compounds with a comparably long residence time of up to 100 years in soils. An average redox potential of humic compounds can be calculated with hydroquinone as a representative constituent, yielding a value at $-280$ to $-300$ mV. Fermentation of sugars and proteins produces acetate as the most important primary product; its oxidation yields electrons at $-290$ mV on average. Oxidation of aliphatic and aromatic hydrocarbons releases electrons at $-240$ to $-270$ mV. These examples illustrate that the typical constituents of partly digested or stabilized organic matter are less valuable electron donors in oxidation processes. Moreover, especially humic compounds and hydrocarbons are already comparably inert derivatives. Efficient degradation of these compounds requires molecular oxygen as a co-substrate in the initial oxygenase reactions for activation. Since oxygen-independent alternative reactions of activation of hydrocarbons (Widdel et al., 1993) or polyanellated aromatic compounds (humic compounds) are slow compared to the known oxygen-dependent ones, a major amount of organic matter (e.g., peat) is conserved in the absence of oxygen (“latch hypothesis”; Freeman et al., 2001).

The biogeochemical redox chain

Due to the low solubility of oxygen in water (about 300 µM O$_2$ in freshwater at air saturation and 10$^0$C), aerobic oxidation of organic matter depends on a continuous supply of oxygen, either by plant photosynthesis or by convective transport from the atmosphere. Therefore, this oxygen supply is restricted to only few millimeters below the top surface in sediments or soils where only diffusive transport is possible. Under such conditions, other electron acceptors can substitute for oxygen in its function as an electron acceptor, and the sequence of these subsequent redox processes is determined by the standard redox potentials of the individual redox processes (Zehnder and Stumm, 1988; Figure 1c). After oxygen consumption, nitrate is reduced first to nitrite, and afterwards either to N$_2$ via NO and N$_2$O, or to ammonia. In any case, nitrite is an important intermediate which may accumulate in laboratory experiments to toxic concentrations. In nature, nitrate concentrations rarely exceed 50 µM and nitrite never becomes toxic, therefore. As the redox potentials listed in Figure 1 indicate, nitrate reduction to N$_2$ (denitrification) appears to be energetically more feasible than nitrite reduction to ammonia (nitrate ammonification). However, some redox steps in denitrification are rather inefficiently coupled to ATP synthesis, thus wasting a considerable amount of energy and rendering nitrate ammonification the more efficient way of nitrate-dependent energy conservation (Strohm et al., 2007).

Subsequently, manganese(IV) oxides, e.g., MnO$_2$ (Braunstein), may be reduced to Mn$^{2+}$ ions. The distribution of manganese oxides in soils is rather patchy; in lake sediments, it depends primarily on the chemistry of the catchment area. In Lake Constance, the eastern part of the lake receives ample supply of manganese oxides through the Alpine Rhine river whereas the western part of the lake is nearly devoid of manganese. Different from manganese, iron is a very dominant constituent of rocks and sediments, and can make up to 1% of the total dry mass. Iron oxides, similar to manganese oxides, are nearly insoluble at neutral pH, and the redox potentials of Fe(III)-oxide reduction to Fe$^{2+}$-ions depends even on the individual iron(III) mineral involved ($+100$ mV with ferrihydrite, $-287$ mV with hematite; Widdel et al., 1993; Straub et al., 2005). The main problem in reduction of iron and manganese oxides is their apparent insolubility (Cornell and Schwertmann, 1996) and, with this, the necessity for bacteria to transport electrons to an acceptor outside the cell (see below).

Reduction of sulfate to sulfide is the dominant process of organic matter oxidation in marine sediments because seawater contains sulfate at high concentration (28 mM). Freshwaters contain far less sulfate (50–200 µM) rendering this process substantially less important at first sight. Nonetheless, sulfide can be reoxidized, e.g., with Fe(III)-oxides (see below) thus making reduction of sulfur compounds probably far more important in freshwater systems than assumed so far. The key problem in sulfate reduction is the initial reduction of the comparably stable sulfate molecule to sulfite which corresponds to a very low redox potential ($E_0^\circ = -527$ mV) that cannot be fed directly with electrons from organic carbon oxidation. The sulfate reducers overcome this problem by ATP investment into this step, thus shifting the resulting redox potential to $-60$ mV (for details, see Cypionka, Aerobic Metabolism). The ATP required for this initial activation step is recovered in the further reduction of sulfite to sulfide.
Elemental sulfur is reduced by specific sulfur-reducing bacteria, but also by sulfate reducers. Elemental sulfur is easy to reduce and does not require prior activation. The problem of sulfur metabolism is again its low solubility in water which may be circumvented by the intermediate formation of water-soluble polysulfides in the presence of the formed hydrogen sulfide.

The terminal process in organic matter degradation in freshwater environments is the formation of methane, either by reduction of CO$_2$ or by cleavage of acetate (Wolfe, 1992; Thauer, 1998); alternatively, acetate may be formed first by CO$_2$ reduction, and secondly be converted to methane (Drake et al., 2002; K. Kuesel, this series). Since the methanogenic archaeobacteria are restricted to the use of only very few substrates (H$_2$, C$_1$ compounds, and acetate) complex organic matter has to go through fermentations by several groups of specialized bacteria (Schink, 1997) before it finally ends up in a mix of CH$_4$ and CO$_2$, i.e., in complete dismutation of the carbon atoms.

Hydrogen is an important product of microbial fermentations of organic matter. It can be formed as well by geochemical processes and may be a source for microbial life in deep sediments and rocks.

The middle axis in Figure 1(b) positions the key elements of the biochemical electron transport chain between organic matter on the left and the various acceptor systems on the right. This arrangement illustrates the major paths of electron flow in microbial transformation processes. One should emphasize, however, that biological systems are able to shift electrons also against the “normal” electron flow by “reversed electron transport” at the expense of energy supplied by transport of other electrons in the “normal” direction from negative to positive potentials. The scheme illustrates that glucose is comparably a valuable electron donor whereas the electrons derived in degradation of acetate, humic compounds, or hydrocarbons cannot all be transported via NADH, rather, many of them have to enter the transport chain at the level of quinones (either menaquinone or ubiquinone) with the consequence that substantially less ATP is formed. The scheme also illustrates that, e.g., reduction of ferrihydrite can be fed with electrons only at the potential of quinones, and that necessarily the ATP yield of this process is much smaller than, e.g., that of the reduction of nitrate or oxygen. It also becomes evident that phosphate can hardly be reduced to phosphate in a respiratory process, analogous to sulfate or nitrate reduction, but would require substantial amounts of energy rather than yield energy. The origin of traces of phosphine in certain anoxic environments is still unclear; it probably derives from corrosion of iron phosphides in scrap metals (Ding et al., 2005).

**Lithotrophic re oxidations**

The reduced derivatives formed in the processes above during oxidation of organic matter can act again as electron donors in microbial energy metabolism, e.g., if H$_2$S, reduced Fe$^{2+}$, or Mn$^{2+}$ diffuses upwards and meets electron acceptors of higher redox potential, thus again creating a redox couple that can yield energy. Such metabolic activities are called “lithotrophic” (Greek lithos = rock) because in this metabolism inorganic electron donors serve as electron source. A lithotrophic metabolism is most often, but not necessarily, combined with autotrophic fixation of CO$_2$ into cell material, i.e., bacterial growth. Obviously, synthesis of organic matter from inorganic sources has to be fueled by energy (ATP) that is derived, in this case, from inorganic redox processes. Therefore, the yield of organic matter, i.e., of microbial cell mass, in these processes is usually small. As the redox chain in Figure 1(c) illustrates, H$_2$, H$_2$S, and Fe$^{2+}$ are good electron donors whereas nitrite, selenite, and Mn$^{2+}$ are not. Behind every such redox process stand the activities of specialized bacteria and archaea which try to exploit every fraction of energy that can be derived from these redox processes.

An exceptional case is methane oxidation which can proceed either with oxygen as a co-substrate and an electron acceptor, yielding a huge amount of energy or with sulfate as electron acceptor (anaerobic methane oxidation; see Boetius, Anaerobic Oxidation of Methane with Sulfate) yielding extremely little energy. Similar problems apply to higher aliphatic or aromatic hydrocarbons (Spormann and Widdel, 2000).

Oxidation of sulfide with ferric oxides should yield energy as well. Nonetheless, this reaction proceeds spontaneously as a chemical reaction (see below), and so far there is no indication for microbial activity exploiting this redox reaction.

**Energetics**

Bacterial growth requires energy which is derived from substrate conversions in the form of ATP. Irreversible synthesis of the energy-rich acid anhydride linkage of ATP by condensation of ADP with a phosphoryl residue under the conditions prevailing in living cells requires energy ($\Delta G^\circ$ or Gibbs free energy) in the range of 60–70 kJ per mol (Thauer et al., 1977; Schink, 1997). Nonetheless, metabolic energy can also be converted into ATP at smaller increments. The membrane-bound ATPase enzyme mentioned above catalyzes the synthesis of ATP reversibly with a translocation of protons or Na$^+$-ions; the stoichiometry is 3–4 H$^+$ or Na$^+$ ions per ATP. Thus, any reaction that can be coupled to the translocation of protons or Na$^+$-ions across the membrane can be associated with ATP synthesis, down to reactions transferring only 1 H$^+$/Na$^+$, equivalent to an $\Delta G^\circ$ in the range of 15–20 kJ per mol. Of course, reactions can proceed also with lower energy yields, but then they cannot feed ATP synthesis and bacterial growth. Growth, on the other hand, is a very important means to maintain a degradative capacity in a certain environment and to adjust its activity to the local needs (different from so-called “co-metabolic” activities which are not positively correlated with bacterial growth; McCarty, 1988).
Extracellular electron transfer

Electron transfer from or into the electron transport chain (Figure 1b) is easy with substrates that can be taken up into the bacterium in a dissolved form. Non-dissolved substrates have to be oxidized or reduced outside the cell, either at the immediate cell surface or via dissolved electron carriers. The reduction of ferric iron oxides can be accomplished in direct contact with the bacterial cells; perhaps, even small cellular appendages can act as “nanowires” for electron transfer (Reguera et al., 2005). Alternatively, either dissolved humic compounds (Lovley et al., 1996) or low-molecular weight quinoid or phenazine compounds may act as electron carriers (Hernandez et al., 2004). As a further alternative, sulfur-reducing bacteria can indirectly reduce iron via \( \text{H}_2\text{S} \) at low (<0.1 mM) concentration (Straub and Schink, 2004). At low \( \text{H}_2\text{S} \) concentration, \( \text{H}_2\text{S} \) delivers electrons to ferric oxides and is converted to a more oxidized species, perhaps thiosulfate or polysulfide, which is reduced again by the bacterium. In this case, a small sulfur cycle acts as an electron delivery system. One should emphasize that in the latter cases, the bacteria do not actually reduce the ferric oxide. They reduce quinoid or sulfur compounds, and the ferric oxides are reduced indirectly in a subsequent unspecified reaction. Such secondary electron transfer reactions may be important also with respect to other reductive transformations such as nitro group reduction, dehalogenation, etc. (Holliger et al., 1997).

The example of indirect ferric iron reduction via sulfur compounds appears to contradict the generally accepted preference of biochemical redox processes according to the sequence mentioned above: since the redox potential of the iron(III)/Fe\(^{2+}\) couple is higher than that of the sulfate/sulfide system, iron should be reduced before the sulfur compounds. Nonetheless, for bacteria not only the redox potentials but also the bioavailability aspect counts: since the sulfur compounds are easier to access they may be preferred over the insoluble iron oxides.

Phototrophic activities

Photosynthesis as performed by green plants and algae is called oxygenic because molecular oxygen is released from water through the activity of two combined light reactions. This biochemical potential was evolved by cyanobacteria (formerly called blue-green algae) and the evolution of oxygenic photosynthesis has changed the chemistry of our planet around 3.5 billion years ago in a most dramatic way from a reducing to an oxygenated atmosphere. The massive precipitation of iron oxides in the so-called “Banded Iron Formations” has for long times been considered to witness the evolution of molecular oxygen in the Earth’s atmosphere, but the oxygen thus evolved would have been consumed to a large extent over several hundred million years in the oxidation of Fe\(^{2+}\) to ferric oxides. Anoxygenic phototrophic bacteria perform a simpler, older type of photosynthesis from which oxygenic photosynthesis was later derived. The discovery of Fe\(^{2+}\)-oxidation by anoxygenic phototrophs (Widdel et al., 1993) changed also our understanding of the development of Banded Iron Formations; this process became possible also without the involvement of molecular oxygen, and thus formation of Banded Iron Formations could now testify only the evolution of anoxygenic, rather than of oxygenic photosynthesis (Kappler et al., 2005).

Spatial/seasonal oscillations

Many microbial habitats such as the Deep Sea and its sediments, or rocks in the deep underground are highly stable systems which do not change their properties over many thousands or millions of years. On the other hand, microbial life conditions in a top soil may change dramatically with every rainfall, and bacteria inhabiting the sediment surface in a shallow, light-exposed lake may undergo dramatic life changes in diurnal cycles of light and oxygen supply which are probably the most dramatic changes for living systems at all. As shown in Figure 2, oxygen profiles during light exposure versus darkness show dramatic differences with respect to the oxygen concentration and to oxygen penetration depth into the sediment. Other parameters change over longer time scales, e.g., with seasons. Figure 3 shows a section of the littoral sediment of Lake Constance in January when the water level is low.
poor with respect to utilizable energy sources, in order to assess the contributions of life to geochemistry correctly.

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Bibliography


Cross-references

Aerobic Metabolism
Anaerobic Oxidation of Methane with Sulfate
Archaea
Bacteria
Carbon (Organic, Degradation)
Fe(III)-Reducing Prokaryotes
Fermentation
Geobacter
Hydrogen
Iron Sulfide Formation
Methane, Origin
Microbial Communities, Structure, and Function
Nitrogen
Photosynthesis
Shewanella
Sulfate-Reducing Bacteria
Terrestrial Reducing Bacteria

ANAMMOX

Anammox refers to the microbial anaerobic oxidation of ammonium by nitrite (NO$_2^-$), which forms N$_2$. See entry “Nitrogen” for further reading.

ANIMAL BIOCALCIFICATION, EVOLUTION

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Definition

SOM. Soluble organic matrix

IOM. Insoluble organic matrix

GRN. Gene regulatory network

Introduction

One of the major events in the evolution of multicellular animals was the transition from soft-bodied organisms to those that possessed mineralized hard parts for protection and support. This major evolutionary hallmark supported the rapid diversification of animals and their occupation of a diverse range of novel ecological niches at the dawn of the Phanerozoic, between 560 and 530 million years ago, when mineralized skeletons appeared relatively synchronous in a variety of Phyla during the so-called Cambrian Explosion (Knoll, 2003; Conway Morris, 2006). However, it is still unclear exactly what drove this sudden capacity to construct mineralized structures, be it changes in ocean chemistry (Brennan et al., 2004) or the evolution of more diverse ecologies (Cohen, 2005), including predators (Vermeij, 1989). Investigation of biomineralization processes will provide insight into the molecular and genetic mechanisms that accompanied the appearance of mineralized body parts during the early Cambrian, shedding light on the processes that generate the diversity of life we see around us today.

Until recently, studies of biomineralization processes were mainly restricted to describing the (ultra)structure of the minerals and their interaction with the organic material provided by the organism (Lowenstam and Weiner, 1989; Mann and Webb, 1989). However, the generation and elaboration of biominerals is ultimately controlled by genes (Jackson et al., 2006, 2007a, b), which must in turn be expressed in the appropriate tissue and at an appropriate stage of development. Understanding the evolution of such biomineralizing genes and the gene regulatory networks (GRNs) in which they act is, therefore, vital to the understanding of how animals evolved and will also provide clues for the fabrication of biomimetic materials.

Biomineralization

Biomineralization refers to the processes by which organisms form minerals (e.g., calcium carbonate, calcium phosphate, silica, iron oxides) under strict biological control. These processes are, therefore, defined as biologically controlled mineralization, with the skeletons formed being integral, functional parts of the organisms (Lowenstam and Weiner, 1989). There are, however, several other serendipitous processes linked to living organisms (e.g., microbes) or organic substances that result in mineral (mainly calcium carbonate) formation that are not under strict control of organisms. These processes are termed either biologically induced mineralization, where inorganic minerals are precipitated by adventitious precipitation resulting from secondary interactions between various metabolic processes and the surrounding environment (Mann, 2001), or organomineralization, a term used to describe mineralization processes that involve organic molecules or particles, whether linked to living organisms or not (e.g., degrading organic matter) (Trichet and Defarge, 1995).

While both biologically induced and organomineralization play an important role in the formation of carbonate systems in the geological record (e.g., microbialites, stromatolites, ooids) (Reitner and Neuweller, 1995; Arp,
Biologically controlled mineralization almost always has organic macromolecules associated with the process of mineral formation, which can comprise up to 5% of the total mass of a biomineral and fulfill important functions during initial crystal nucleation, crystal growth, and growth termination, as well as contributing to the biomechanical properties of the mature product (Simkiss, 1986; Simkiss and Wilbur, 1989). Following the removal of the mineral itself, these organic macromolecules are often subclassified as being either water soluble organic matrices (SOMs), or water insoluble organic matrices (IOMs). IOMs are largely neutrally charged, often highly polymerized, and act as frame building matrices akin to collagen, chitin, or silk proteins (Yan et al., 2007; Yano et al., 2007). SOMs are often highly acidic, due to negatively charged carboxylate groups, are typically rich in aspartic acid (asp) and/or glutamic acid (glu), and usually have mean molecular weight of 10–30 kDa (Bédouet et al., 2001, 2006).

While organic macromolecules appear ubiquitous and pivotal in biologically controlled biomineralization (with the focus here on biocalcification), an increasing number of studies now show that a transient amorphous (noncrystalline) phase of calcium carbonate (ACC) is frequently involved in the formation of the earliest stages of calcium carbonate biominerals (Aizenberg et al., 2003; Politi et al., 2004; Weiner and Dove, 2003). However, little is known about the ACC as well as the organic macromolecules involved in calcification in most animal taxa.

Several SOM proteins have been sequenced and characterized, mainly in sea urchins, mollusks, and vertebrates (Treccani et al., 2003; Wilt et al., 2003). About 45 soluble proteins were detected by 2D gel electrophoresis in demineralized spicules from the embryo of the sea urchin Strongylocentrotus purpuratus (Killian and Wilt, 1996). Several of these proteins were later cloned and sequenced (e.g., SM27, SM30, SM50) (Zhu et al., 2001) and their role and function in the biomineralization process confirmed by immunohistochemical methods (Ameye et al., 1999; Urry et al., 2000), in situ hybridization (Illies et al., 2002), or by using antisense oligonucleotides directed against spicule matrix proteins (Peled-Kamar et al., 2002). All of the cloned spicule matrix proteins of Strongylocentrotus share as common features a C-type lectin domain and proline rich repeat regions. Furthermore, the first 35 amino acids of SpSM32 and SM50 are identical, probably representing splicing variants of the same transcript, although this has yet to be confirmed (Wilt et al., 2003). The precise functions of any of these proteins are unknown (Wilt et al., 2003) and their evolutionary origin and relationships remain largely unresolved (but see Bottjer et al., 2006). An increasing number of reports are, however, elucidating the functional roles of various biomineralizing proteins. For example, the starmaker protein is known to influence crystal size and lattice formation in otolith biomineralization (Söllner et al., 2003), while the nacre protein perculin (Mann et al., 2000; Weiss et al., 2000) is known to nucleate the growth of aragonitic calcium carbonate crystals (Blank et al., 2003).

Identification and characterization of the genes directly involved in the process of biocalcification, and the ways in which these “structural genes” are regulated, is essential for our understanding of the evolution of metazoan biocalcification. One of the most advanced systems currently used to study the complexity of the GRNs involved in specifying biomineralizing cell types is the sea urchin. The manipulability of urchin embryos and the availability of a high-quality genome sequence have allowed researchers to identify specific transcription factors (genes that activate the expression of target genes) that activate biomineralizing genes and proteins (Ettenson et al., 2003, 2007). Furthermore, recent work has identified the nodes in biomineralizing GRNs that are responsible for specifying cells involved in larval skeletogenesis and adult skeletogenesis (Gao and Davidson, 2008), providing insight into the evolution of the metazoan biphasic lifecycle. Such endeavors are also highly relevant to issues of global climate change and ocean acidification – by assessing how resilient marine calcifying organisms are to fluctuations in ocean chemistry, highly species-rich marine ecosystems such as coral reefs may be effectively managed.

The evolution of biocalcification
In the geological record, major animal phyla began biomineralizing shortly before, or during, the so-called Cambrian Explosion, about 530 million years ago, when a highly diverse group of animals appeared suddenly in the fossil record (Knoll, 2003). As most of these phyla are thought to have diverged well before this biomineralization event (Peterson et al., 2008), various causes for this relatively synchronous adoption of biomineralizing strategies have been postulated and remain an area of continuing debate. These include the evolutionary driving force of predation (Vermeij, 1989) or the result of a response to changes in ocean chemistry with greater than previous levels of calcium, requiring the need for effective cellular calcium-detoxification mechanisms (Degens et al., 1985; Kazmierczak et al., 1985).

The vast majority of newly appearing biomineral products in the Cambrian was based on calcium (either as calcium carbonate or calcium phosphate) (Lowenstam and Margulis, 1980). Precise control of intracellular calcium levels is necessary for the formation of microtubules which are needed by eukaryotic cells. Based on this, Lowenstam and Margulis suggested that calcium regulation and transport systems in the late Precambrian metazoans provided the evolutionary prerequisites for their eventual use in biomineralization. CaCO3 biomineral formation can be useful for the organism because this process allows the elimination of a cell-toxic surplus of Ca2+ through enzymes such as calmodulin and membrane...
bound Ca\textsuperscript{2+}-ATPase, which directly transports calcium to loci where it is used as a physiological control factor (Degens, 1979).

The question of whether the ability to biocalcify evolved several times independently among various animal groups, possibly to produce armor in response to increased pressure from predators (Marshall, 2006), or whether preexisting calcium regulation and transport systems in the late Precambrian metazoans provided the evolutionary prerequisites for their eventual use in biomineralization (Lowenstam and Margulis, 1980), still remains open to debate. If calcium carbonate biomineralization evolved as a physiological response (mutual to many taxa) to increasing calcium levels in the late Precambrian ocean, then an evolutionary link between the proteins and the regulatory elements involved in skeletogenesis can be postulated among metazoan groups. This evolutionary connection could either be due to massive horizontal gene transfer in ancestral Precambrian organisms or as a result of the exaptation of a preexisting genetic makeup into a new role (biocalcification) in many disparate animal lineages (Kirschvink and Hagadorn, 2000). The latter leads to the hypothesis that the last common ancestor of the metazoa (LCAM) provided a “genetic biomineralization toolkit,” a core set of conserved biomineralization genes to regulate and initiate the cellular functions required for the assembly of calcium carbonate biominerals (Ca\textsuperscript{2+}, HCO\textsubscript{3}\textsuperscript{-}). Subsequently, this core gene cassette and associated regulatory elements have been elaborated upon to produce the range of biomineralized structures we observe in the Earth’s history. One major process by which complex biological systems evolve is by taking an existing genetic pattern evolved for a certain function, duplicating it, and adapting it for a new role (e.g., Carroll et al., 2001). The nascent system is gradually debugged and improved through the process of random mutation and natural selection. This powerful hypothesis, in the context of biomineralization, still awaits an expanded program of comparative geobiological research (Knoll, 2003).

However, evidence for conserved biocalcification mechanisms is beginning to emerge. Some homologies between certain molluskan (Weiss et al., 2001) or sea urchin (Ettenson et al., 2003) and vertebrate matrix proteins have already been postulated. Ettenson et al. (2003), for example, found homologies of their sea urchin Sp/LvAlx1 protein, which is an essential component of the gene network that controls downstream genes required for biomineralization, with members of the vertebrate Cart1/Alx3/Alx4 protein family. Members of this gene family have been implicated to be instrumental in the formation of the limb skeleton and the neural crest-derived skeleton of the face and neck in vertebrates. Further, there seems to be some fundamental underlying immunological similarity between macromolecules involved in vertebrate hydroxyapatite and invertebrate aragonite formation (i.e., molluskan nacre), because freshly ground nacre did not result in an immune response when injected into human jaw bones, but in fact stimulated bone regeneration (Marin and Westbroek, 1998; Lopez et al., 1992). Coral skeletons are already used as bone implants with a similar (albeit weaker) effect on bone regeneration (Demers et al., 2002). Although bone, nacre, and coral skeletons are not homologous structures as such, parts of the complex underlying physiological machinery that controls biomineral formation probably are—a hypothesis strongly supported by the above mentioned results. However, these homologous parts may have served a different function than biomineralization in the (nonskeletonized) common ancestor of corals, mollusks, and vertebrates, later being co-opted for producing biominerals when the necessity arose.

If, as outlined above, calcium carbonate biomineralization systems in the major metazoan phyla evolved from the core skeletogenic toolkit of the last common ancestor of animals, then ancestral metazoan systems should be used as an anchor for a paleogenomic approach to reconstruct this toolkit and explore the evolutionary road map of diversification by comparison with more derived animal taxa. Paleogenomics analyzes the genomes of contemporary taxa to reconstruct ancient genomes and genomic features and to infer the function of ancestral genes.

Recent paleogenomic studies go beyond the focus on classical matrix proteins. Expressed sequence tags (ESTs), cDNA libraries, and recently sequenced genomes have been utilized to examine the inheritance of ancestral toolkits that were involved in skeletogenesis. By using a calcifying coralline sponge of a “living fossil,” Astrosclera willeyana, a key component of the ancestral skeletogenic toolkit—alpha-carbonic anhydrase—has been identified (Jackson et al., 2007b). Duplication of this gene is likely to have allowed biocalcification to evolve in many clades of metazoans. In contrast, the recently sequenced genome of the sea urchin Strongylocentrotus purpuratus has revealed that genes essential for the construction of the echinoderm skeleton (the stereotype) are echinoderm-specific and are likely to be the same as those in the earliest echinoderms and their direct ancestors more than 520 million years ago (Livingston et al., 2006). Similarly, in gastropods (snails; Haliois asinina and Lottia scutum) an unexpected level of genetic novelty and complexity in the skeletogenetic toolkit suggests that there are significant molecular differences in the ways in which gastropods synthesize their shells (Jackson et al., 2006). These features, along with the modular design of the molluskan mantle, contribute to the spectacular morphological and mineralogical variety we observe among contemporary mollusk shells (Jackson et al., 2007a).

From the limited data available, we can develop the following scenario as a working hypothesis (Figure 1): the last LCAM possessed a core biomineralization toolkit that at least consisted of an alpha-carbonic anhydrase, providing the core capacity to regulate the reversible hydration of CO\textsubscript{2}, necessary for biocalcification. Other, as yet undiscovered, parts of this toolkit most likely contained genes that regulate Ca\textsuperscript{2+} trafficking, scaffolding genes for organic matrix production, as well as regulatory
elements. During subsequent animal evolution, genes in this core set were duplicated in different lineages, resulting in a relatively simple genetic repertoire in basal metazoans, but in a highly diverse gene array in the various lineages of the Bilateria (Ecdysozoa, Lophotrochozoa, and Deuterostomia). A high degree of lineage-specific innovation on top of a conserved gene-set then allowed for the diversification of the numerous skeletal architectures we observe since the dawn of the Cambrian.

Conclusion
While the structural processes of biomineralization, and in particular biocalcification, are receiving vigorous attention from various international groups, a far more detailed characterization of the core ancestral skeletogenic toolkit as well as lineage specific innovations are needed before we understand how the impressive diversity and beauty of metazoan skeletal structures originated and evolved. As this knowledge is acquired, we can anticipate the construction of new high-performance biomimetic materials and the measurement and prediction of the resilience of marine calcifying organisms to rapidly changing environmental parameters, such as ocean acidification.

Bibliography


Introduction

Skeletal biomineralization (skeletonization) was part of the initial diversification of early animals. The fossil record provides an important clue to the origin of biomineralization in the animal phyla (Kirschvink and Hagadorn, 2000). The earliest occurrence of animal skeletons was in the late Ediacaran, e.g., Cloudina, Sinotubulites, and Namapoikia, although the generic diversity was quite low and the biomineralization of these calcareous skeletons was not well controlled biologically (Grant, 1990). The early Cambrian (542–525 Ma) saw the largest burst of diversified animal skeletons, represented in the fossil record by poriferans, cnidarians, many extant bilaterian phyla, and some problematic taxa (Figure 1) (Bengtson, 2004). In terms of structural and mineralogical diversity of animal skeletons, the early Cambrian represents a quantum jump (Bengtson and Conway Morris, 1992). The abrupt advent of diversified skeletal fossils not only has been taken as a major piece of evidence for the Cambrian radiation but also was envisioned the biomineralization event. Since most of these skeletal fossils are typically small in size, and lots of them represent disarticulated sclerites and are enigmatic with respect to their biological affinities, they are commonly referred to as “small shelly fossils (SSFs)” in the literature (Matthews and Missarzhevsky, 1975; Qian, 1999). But some of the early skeletal fossils, such as the sponge-like archaeocyaths, trilobites, and bivalved arthropods, are rarely described under this nontaxonomic term, while some fossils described under the term of SSFs, such as embryo fossils and cnidarian polyps, may have been originally composed of resistant organic membranes or tubes without biomineralization, and the skeleton-like appearance is mainly due to the secondary phosphatization.

Cross-references

Animal Skeletons, Advent
Calcium Biogeochemistry
Carbonate Environments
Critical Intervals in Earth History
Ediacaran Biota
Microbial Biomineralization
Organomineralization
Reefs
Soda Ocean Hypothesis
Sponges (Porifera) and Sponge Microbes
Trace Fossils: Neoproterozoic

ANIMAL SKELETONS, ADVENT

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Synonyms

Early biomineralization event; Small shelly fossils

Definition

Animal skeleton. Biologically, an animal skeleton is a hard or rigid framework that could provide protection and support in many types of animals. Most of the animal skeletons are mineralized, but some of them may not be mineralized (e.g., some annelid tubes being agglutinated). They could be subdivided into exoskeletons and endoskeletons, excluding hydrokeletons. The “skeletons” of resistant organic biopolymers are not included herein.

Exoskeleton. Exoskeletons are external hard parts. They may enclose the soft tissues and organs of the body, or may be external sclerites.

Endoskeleton. Endoskeletons are internal hard parts (typical of many vertebrates). Most of the endoskeletons are generally surrounded by skin and musculature.

Small shelly fossils (SSF or SSFs). A non-taxonomic term was first used by Matthews and Missarzhevsky in the title of their review paper in 1975 to denote the earliest skeletal fossils, although some of these fossils may not be small and most of them are actually not shells. It has been popularly used in literature somewhat as a catchall term for the early Cambrian skeletal fossils, including spicules, shells, tubes, and diverse disarticulated sclerites.
Mineralogy of early animal skeletons

Animals use a variety of minerals to fabricate their skeletons (Lowenstam and Weiner, 1989). However, there are only three principal classes of minerals being widely used by organisms, including calcium carbonates, calcium phosphates, and silica. Calcium, carbonate, and phosphate ions are abundant in the oceans, and are ready for use in skeletons (Kirschvink and Hagadorn, 2000), despite skeletogenesis being a biologically controlled, complex process. Animals rarely switched their mineralogy after their first skeletogenesis, indicating that, for most lineages, environment does not strongly influence skeleton composition, although the selection of skeletal mineralogy during the first acquisition of skeletons in a clade may be somewhat related to ambient seawater chemistry (Knoll, 2003; Porter, 2007; Zhuravlev and Wood, 2008).

Animal Skeletons, Advent, Figure 1 Distribution of major skeletal animal taxa (biomineralized and agglutinated) from the late Ediacaran to the early Cambrian.
It is in most cases not apparent to identify the original minerals of early fossil skeletons because of diagenetic alterations. Especially, there was a very significant phosphogenic event around the Ediacaran-Cambrian transition (Cook and Shergold, 1984), and thus secondary phosphatization of early skeletal fossils was quite common (Qian and Bengtson, 1989). The original composition of fossil skeletons can be learnt by inference and comparative analysis. The fossil record shows that early animals mainly used calcium carbonates, apatites, and silica to build their skeletons (Figure 1).

Several polymorphs of calcium carbonates are the major skeleton-forming minerals, including calcite (high-Mg calcite, low-Mg calcite), aragonite, and vaterite. Vaterite is an extremely unstable polymorph of CaCO$_3$ and is rarely used as a biomineral. Aragonite is an unstable mineral and it is easily transformed to low-Mg calcite during diagenesis; hence, the original mineralogy of early calcareous fossils is difficult to identify in many cases (Bengtson and Conway Morris, 1992). The earliest animal skeletons during the late Ediacaran-Tommotian interval were composed exclusively of aragonite and high-Mg calcite, being consistent with an aragonite sea (Porter, 2007; Zhuravlev and Wood, 2008). Since the early Attabanian, there occurred many clades of animals using low-Mg calcite to make skeletons, implying the onset of a calcite sea.

Apatite is another major mineral for early animals to make skeletons (Figure 1), and it is diagenetically the most stable among the major skeleton-building minerals (Bengtson and Conway Morris, 1992). Phosphatic skeletons are usually a combination of phosphate and organic material and hence are called organo-phosphatic. Comparative study indicates that phosphatic skeletons in the earliest metazoans appear to have been more widespread than today (Runnegar and Bengtson, 1990). The early phosphatic skeletal metazoans include brachiopods (Linguliformea), cnidarians (hyolithelminths, byroniids, and conulariids), bradoriids, lobopodian, protoconodonts, and conodont-like fossils, tommotiids, etc. The high diversity of phosphatic skeletons is somewhat related to wide distribution of phosphate deposits (phosphogenesis event) in various parts of the world during the early Cambrian (Cook and Shergold, 1984).

Siliceous (opal) skeletons differ in distribution from those made of CaCO$_3$ since silica biomineralization is limited to intracellular precipitation. Therefore, in animals, siliceous skeletons are limited to sponge spicules and other minor occurrences (Bengtson and Conway Morris, 1992). Opal has been used by sponges (including Demospongea and Hexactinellida) to build their skeletons since the late Ediacaran (Gehling and Rigby, 1996). Biogenic opal is unstable and it usually recrystallizes to quartz during diagenesis.

Agglutinated conchs are occasionally found in the Lower Cambrian rocks such as Salterella, Volborthella, and Platysolenites. Both Salterella and Volborthella are metazoans (Yochelson and Kisselev, 2003), but their biological affinities are uncertain. They have a calcareous sheath partially filled with detrital grains, agglutinating into laminations. Platysolenites represents an agglutinating foraminiferan (Glaessner, 1963).

**Morphological variety of early animal skeletons**
The morpho-types of the animal skeletons in the late Ediacaran are relatively few. They are mainly represented by weakly mineralized calcareous tubes (e.g., *Cloudina*, *Sinotubulites*).
Various types of the animal skeletons abruptly occurred in the early Cambrian. Using the theoretical “Skeleton Space” concept, Thomas et al. (2000) made an analysis of Cambrian animal skeletons. Their analysis showed that about 50% of skeletal design elements recognized among living and extinct marine metazoans were exploited in the early Cambrian (Tommotian) and about 80% exploited in the middle Cambrian Burgess Shale animals. The early Cambrian animal skeletons include univalved (Figure 4d, i, n) and bivalved shells (Figure 4e, f), arthropod carapaces (Figure 4g, h), diverse tubes (Figure 4a–c, l), spicules, diverse external sclerites (Figure 3), tooth-like structures (Figure 3c–e), calcareous massive reinforcements (e.g., archeocyathans), echinoderm ossicles, etc.

**Late Ediacaran skeletal metazoans**

Prior to the Cambrian, the evidence for animal life was almost the soft-bodied Ediacaran fauna and the advent of skeletal animals had been considered to be in the lowermost Cambrian until Germs (1972) described the first Precambrian skeletal fossil *Cloudina* from the Nama Group in Namibia. Since then, biomineralized fossil assemblages have been widely found in the latest Ediacaran over the world, although the biodiversity is very low. Up to now, there are only several skeletal fossil genera being discovered from the Upper Ediacaran rocks, including *Cloudina*, *Sinotubulites*, *Namapoikia*, *Namacalathus*, and *Paleophragmodictya*. Most genera became extinct at the base of the Cambrian, suggesting that a mass extinction occurred at the latest Ediacaran (Amthor et al., 2003).

The worldwide distributed *Cloudina* is a calcareous conical fossil consisting of funnel-like, apically flaring cones nested with one another (Figure 2a, b). The zoological affinities of *Cloudina* are uncertain, but occasional evidence for budding supports either a cnidarian interpretation
(Grant, 1990) or an annelid relation (Hua et al., 2005). The Cloudina tubes contain the earliest evidence of predatory borings (Figure 2b). The enigmatic Sinotubulites (Figure 2c, d) is a cylindrical fossil, and its tube is composed of several thin layers and characterized by “tube-in-tube” structure (Chen et al., 2007). It has been only documented from the Dengying Formation in South China and co-occurred with Cloudina. The irregular wrinkle ornamentation of outer layer shows the Sinotubulites tubes being plastic, implying that the tube wall was dominantly organic with aragonitic mineralization (Chen et al., 2007).

Namapoikia is a late Ediacaran, fully biomineralized metazoan from the Nama Group, Namibia, and it is up to 1 m in diameter and bears a complex and robust calcareous skeleton (Wood et al., 2002). It resembles chaetitid sponges or simple colonial cnidarians. Namacalathus is another calcified animal from the late Ediacaran, and it is goblet shaped up to 2 cm across (Grotzinger et al., 2000). It is more likely to be a cnidarian than a sponge. Both Namapoikia and Namacalathus co-occur with Cloudina.

Hexactinellid sponges have also been found in the late Ediacaran, including the body fossil Paleophragmodictya (with a reticulating net of spicules) (Gehling and Rigby, 1996) as well as clusters of siliceous spicules (Brasier et al., 1997).

Early Cambrian skeletal metazoans
The great watershed in skeletal biomineralization was the early Cambrian, where the diversity of skeletal animals increases nearly exponentially over the interval. Compared with the limited diversity of skeletal fossils in the Ediacaran, the Lower Cambrian is marked by an extraordinary burst of skeletal fossils. Most skeletal metazoans (extant and extinct) appeared for the first time during the early Cambrian (Figure 1). Among these skeletal fossils, many “skeletons” were not integrated and consisted of numerous discrete sclerites, e.g., cambroclaves, tommotiids, halkieriids, chancelloriids, etc. (Figure 3).

Although the advent of animal skeletons in the early Cambrian is quite abrupt or explosive in relation to the long geologic history, the first appearances of different clades in the early Cambrian are sequential (Li et al., 2007). Anabaratids, hyoliths, maikhanellids (mollusks),

Animal Skeletons, Advent, Figure 4 Examples of early Cambrian tube-dwelling and shell-bearing animals. (a) Torellella, a tube-like fossil of unknown affinity; (b) Anabarites, a tube-like fossil of cnidarian. (c) Conotheca, a hyolith; (d) Aldanella, a possible gastropod; (e) A ventral valve of Eohardrotreta, acrotretid brachiopods; (f) A ventral valve of Palaeolobus, lingulid brachiopods; (g) Dabashanella, a phosphatocopid; (h) Kunmingella, a bradorid; (i) Canopoconus, a possible mollusk; (j) Hexangulacunularia, a conularid (Cnidaria); (k) Lathamella, a stem-group brachiopod; (l) Cupitheca, a tube-like fossil of unknown affinity; (m) Watsonella, a rostroconch (Mollusca); (n) Archaeospira, a gastropod. All from the Lower Cambrian of South China (b, c, i, n: After Qian and Bengtson, 1989). Scale bars = 100 μm.
and protoconodonts (chaetognaths) first occurred in the late Meishucunian, coeloscleritophorans, tommotids in the middle Meishucunian, while crown group brachiopods, skeletal arthropods, and lobopods first occurred in the early Qiongzhusian (Figure 1). The skeletal fossil record shows that the clades occurring in the pre-trilobitic interval (Meishucunian) include many extant phyla such as Porifera, Cnidaria, Annelida, Mollusca, Chaetognatha, Brachiopoda (Lingulida), and a number of extinct taxa such as Hyolitha, stem-group brachiopods, tommotids, coeloscleritophorans, cambroclaves, coleoloids, paracarinichitids, etc. The pre-trilobitic skeletal fossils were dominated by helcionellids (Mollusca), hyoliths, other problematic sclerites, cap-shaped fossils, and tube-like fossils. They are distinctively different from the "Cambrian Evolutionary Fauna," and were christened the "Tommotian Fauna" (Sepkoski, 1992).

In the Qiongzhusian (Atdabanian) stage, besides many extinct clades, nearly all extant skeletal invertebrate phyla occurred (except Byrozoa with first occurrence in the early Ordovician). The dominant and characteristic skeletal fossils of this interval include arthropods, brachiopods, and echinoderms, representing an initial stage of the "Cambrian Evolutionary Fauna."

### Summary

The earliest skeletal fossils came from the late Ediacaran rocks, but the early Cambrian witnessed the largest burst of animal skeletons. The abrupt advent of diverse skeletal fossils is an important part of the Cambrian explosion, and biomineralization of animal hard parts provides many advantages for metazoan diversification. Although the trigger for this early biomineralization event has been a subject of extensive debate in literature, it should be an integrating result of extrinsic and intrinsic (biological) factors. The fossil record also exhibits that, along with the rapid diversification of metazoa in the early Cambrian, the metazoans progressively exploited more ecological space. Most of the pre-trilobitic skeletal faunas came from the shallow-water deposits, but during the Qiongzhusian, there already existed a distinct differentiation of shallow water and deep water habitats: a strong linkage between the occurrences of the skeletal fossils and sedimentary environments.

### Bibliography


Cross-references
Animal Biocalcification, Evolution
Breakup of Rodinia
Ediacaran Biota
Gondwanaland, Formation
Origins of the Metazoa
Trace Fossils: Neoproterozoic

ARCHAEA

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Synonyms
Archaeabacteria (term abandoned)

Definition
The Archaea are single-celled or filamentous prokaryotes that constitute the third phylogenetic domain of life, besides the Bacteria and the Eukarya. The word “Archaea” (singular archaeum, archaean) is derived from the Greek word for “the old ones”.

History
The discovery of the Archaea dates back to 1976 when Carl Woese, at his laboratory at Illinois University, compared prokaryotic small subunit ribosomal RNA sequences using oligonucleotide catalogs (Woese, 2007). Woese recognized Methanobacterium thermautotrophicum as the first member of a fundamentally distinct group of prokaryotes that clustered away from all other bacteria. Consequently, Woese and Fox (1977) established the concept of two separate prokaryotic “urkingdoms,” Eubacteria and Archaeabacteria. Later, the term “Archaeabacteria” was changed to “Archaea” to emphasize the fundamental differences between both groups. Based on these discoveries, Woese and his coworkers proposed the now-accepted division of life into the three domains of Archaea, Bacteria, and Eukarya (Figure 1; Woese et al., 1990).

Specific traits
Like the Bacteria, most Archaea show a cell size between 0.5 and 2 µm and lack a cell nucleus, internal membranes, and organelles. However, a number of traits distinguish the Archaea from members of the other domains of life:

1. The genetic machinery of Archaea is different from Bacteria and Eukarya. The distinctive archaean “signature” contains more than 1,000 archaean protein-encoding genes. These signature genes account for a significant portion (9–15%) of archaean genomic DNA (Graham et al., 2000). The archaean RNA polymerase, an enzyme producing RNA chains from DNA gene templates, consists of eight (methanogens

![Archaea, Figure 1 The phylogenetic tree of life. (Redrawn after Madigan and Martinko, 2006.)](image)
and halophiles) or ten (hyperthermophiles) individual polypeptides. These polymerases are quite different from those of the Bacteria (four polypeptides), but somewhat similar to those of the Eukarya (10–12 polypeptides; Madigan and Martinko, 2006).

2. Unlike bacteria, the cell walls of the Archaea contain no peptidoglycan (a polymer consisting of sugars and amino acids, also known as murein). Rather, archaeal cell walls exhibit a variety of compositions (Kandler and König, 1998). They may consist of the murein analog pseudomurein, polysaccharides, proteins, or glycoproteins (Madigan and Martinko, 2006).

3. Archaea possess glycerol-based phospholipids as building blocks of their cell membranes, as do bacteria and eukaryotes. However, some features of the archaeal membrane lipids set them apart, namely (a) the stereochemistry of the glycerol moiety (L instead of D), (b) the linkage between the alkyl chains and the glycerol molecules (ether instead of ester bonds), and (c) the design of the alkyl chains (isoprene-based instead of acyl-based; DeRosa and Gambacorta, 1988). In many archaea, these chains are long enough (C_{25}) to span the whole membrane, forming a monolayer instead of the lipid bilayer typical for other organisms (Koga et al., 1993). These isoprenoid lipids, or degradation products thereof, are excellent tracers for archaea-derived organic matter inputs and biogeochemical processes in modern and ancient sediments (Hopmans et al., 2000; Kuypers et al., 2001; Peckmann and Thiel, 2004; see entry Biomarkers).

Phylogeny
Most Archaea, and all cultured species, belong to two major, well-established subdivisions, the Euryarchaeota, which include methanogens and extreme halophiles, and the Crenarchaeota, which encompass predominantly hyperthermophiles and anaerobic respirers (Figure 1). Further lineages, namely Korarchaeota (Barns et al., 1996), Nanoarchaeota (Huber and Rachel, 2007), and the Ancient Archaeal Group (Takai and Horikoshi, 1999), have been proposed based on 16S rDNA amplification from environmental samples and/or co-cultivation in mixed laboratory enrichments (Gribaldo and Brochier-Armanet, 2006; Schleper, 2007). However, most of these newly proposed groups still await detailed characterization.

It is as yet unclear at which time in Earth history the domain Archaea arose (Gribaldo and Brochier-Armanet, 2006). Isotopic signatures indicate the presence of methane-cycling archaea 2.8–2.6 billion years ago. Other molecular estimates placed the divergence between Euryarchaeota and Crenarchaeota even 4.1 billion years ago, but this was inferred by using the plant/animal divergence as a calibration point (Battistuzzi et al., 2004). Unequivocal archaeal biosignatures in Precambrian rocks are therefore urgently needed to provide reliable calibration points for the molecular dating of Archaea and prokaryotes in general (Gribaldo and Brochier-Armanet, 2006).

Ecology
Until recently, it had been thought that archaea are largely restricted to particular ecological niches. Indeed, many archaea are extremophiles (see entry Extreme Environments), with growth optima at the most extreme pH values, ranging from pH 1 (Sulfolobus acidocaldarius) to pH 12 (Natronococcus). Moreover, numerous species are hyperthermophiles, with growth optima above 80°C. The current record for growth temperatures (122°C) is held by a methanogen, Methanopyrus kandleri (Takai et al., 2008). Likewise, many halophilic archaea can tolerate extremely high salt concentrations, even at the saturation limit for NaCl (5.5 M; Barns and Nierzwicki-Bauer, 1997; see entry Halobacteria – Halophiles). However, ongoing research employing culture-independent molecular techniques and biomarker studies revealed that archaea are by no means restricted to extreme environments. Rather, they are globally distributed and occur in virtually all “normal” settings as well, including soils and freshwater environments (Schleper, 2007), marine planktonic communities (>20% of the total rRNA, >14% of the total cell counts in sea water; Giovannoni and Rappé, 2000), and marine sediments (Lipp et al., 2008). Archaea are also found as partners in symbioses, e.g., with other archaea (Huber and Rachel, 2007), bacteria (Boetius et al., 2000; Stams and Plugge, 2009), ciliates (van Hoek et al., 2002), sponges (Margot et al., 2002), ruminants (Wright et al., 2006), and even in human intestines (Eckburg et al., 2005; see entry Symbiosis).

Metabolism
Some examples for biochemical pathways used by the Archaea are given in Table 1. The Archaea exhibit a wide range of metabolic capacities, however, all members are chemotrophic. Phototrophy, in a strict sense, does not occur among the Archaea, although some halophilic archaea produce pigments, bacteriorhodopsin-like, light driven proton, or ion pumps that allow them to utilize sunlight for biosynthesis, but in a way very different from phototrophic organisms (Madigan and Martinko, 2006). Chemoheterotrophic archaea utilize organic substances, particularly metabolic products of other microbes formed during organic matter degradation (Barns and Nierzwicki-Bauer, 1997). These substrates usually consist of simple C_{1} and C_{2} compounds, such as methanol or acetic acid. Methane and CO_{2} are typical metabolic products of archaeal chemoheterotrophy. Chemolithotrophic archaea fix inorganic carbon, with hydrogen gas derived from geochemical processes or microbial metabolism being widely used as an energy source (Pedersen, 1997; Berg et al., 2010).

Some archaea perform iron reduction using Fe(III) as an electron acceptor and hydrogen gas or organic compounds as an electron donor (Bond and Lovley, 2002;
Kashefi et al., 2002; see entry Fe(III)-Reducing Prokaryotes). Other members of the Archaea drive pathways of the nitrogen cycle, such as ammonia oxidation, nitrate respiration and denitrification, and assimilatory pathways like N2 fixation and nitrate assimilation (Cabello et al., 2004; see entry Nitrogen). In addition, sulfur is a central component for reactions performed by numerous thermophilic archaea. Elemental sulfur (S0), thiosulfate (S2O32−), sulfite (SO3−), or sulfate (SO42−) may be used as electron acceptors, and elemental sulfur (S0) or H2S as electron donors, depending on species and environmental conditions (Barns and Nierzwicki-Bauer, 1997; see entry Sulfur Cycle).

Methanogenic archaea (methanogens) are anaerobic euryarchaeotes that produce methane as a metabolic end product of various reactions (Table 1; see entry Methane, Origin for details). For several reasons, these archaea are of particular interest from a geobiological perspective.

(a) Chemoorganotrophic methanogens thriving on simple metabolic products of other (micro)organisms such as methanol or acetate (acetoclastic methanogenesis, Table 1) are key players in the degradation of organic matter in sediments (see entry Carbon, Organic, Degradation). (b) Chemolithotrophic methanogens, utilizing only hydrogen gas and CO2 may thrive in dark and extremely organic-lean environments. (c) Many methanogens are thermophiles (growth temperatures >60°C) or hyperthermophiles (growth temperatures >80°C) with some species being even capable of withstanding temperatures above 100°C (Huber et al., 1990; see entry Extreme Environments). Archaea are therefore predestined to pioneering life in extreme settings such as the Deep Biosphere (Hallbeck and Pedersen, 2008), Hot Springs, and Hydrothermal Environments (see these entries). Third, the product of archaeal methanogenesis, “biogenic” methane, may accumulate in sedimentary reservoirs either as free natural gas or, under certain low-temperature/high-pressure conditions, frozen as solid gas hydrates (see entry Cold Seeps). Sedimentary methane reservoirs play a crucial role as an energy resource for man, but when released into the atmosphere, methane may act as a strong driving force of global change (Mascarelli, 2009). In as much as methane is an effective greenhouse gas being more than 20 times as potent as CO2 per molecule, it presently contributes more than 20% to the current global greenhouse effect (IPCC, 2007). Several dramatic events in Earth history, such as the Paleocene/Eocene thermal maximum, have been attributed to sudden increases of atmospheric methane concentrations, possibly induced by rapid methane release due to gas hydrate dissolution (Zachos et al., 2008).

Methanotrophic archaea (ANME, anaerobic methane oxidizers, Figure 2), organisms that reversed the methanogenic metabolism, have recently been specified as members of sedimentary microbial communities in anoxic sediments. Living together in a syntrophic consortium with sulfate reducing bacteria, ANME oxidize methane by sulfate or nitrate (Raghoebarsing et al., 2006; Knittel and Boetius, 2009; see entry Anaerobic Oxidation of Methane with Sulfate).

### Biomineralization

In methane-rich environments, ANME are key players in the formation of calcium carbonate (CaCO3) deposits, the so-called seep carbonates or methane-derived...
carbonates (Figure 3; Peckmann and Thiel, 2004; Reitner et al., 2005; see entry Cold Seeps). This biologically induced mineralization process is driven by an increase in Alkalinity resulting from the anaerobic oxidation of methane. The anaerobic oxidation of methane may also be involved in dolomite (Ca, Mg(CO$_3$)$_2$) precipitation in marine sediments (Moore et al., 2004). Likewise, methanogens have been linked to dolomite formation in shallow groundwater aquifers (Roberts et al., 2004). Recent laboratory experiments identified aceticlastic and autotrophic methanogenesis associated with dolomite formation (Kenward et al., 2009; see entry Dolomite, Microbial).

Summary
The Archaea are a group of unicellular prokaryotes representing the third domain of life, in addition to Bacteria and Eukarya. The Archaea differ from the latter by aspects of their genomic machinery, cell wall composition, and membrane lipid structure. Although the timing of their emergence has not been determined, it is believed that the Archaea have been key players within the carbon, nitrogen, and sulfur cycles for the major part of Earth history. Regarding their ecophysiology, individual archaeal groups display unique capacities, such as methanogenesis, extreme salt tolerance, and survival at temperatures even higher than 100°C. However, environmental surveys revealed the Archaea to be abundant and metabolic versatile not only in extreme environments, but also in “normal” habitats such as soil, oceans, and freshwater. Archaea involved in methan cycling have also been identified as drivers of biologically induced mineralization resulting in (carbonate) rock formation.

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Cross-references

- Alkalinity
- Anaerobic Oxidation of Methane with Sulfate
- Anaerobic Transformation Processes, Microbiology
- Bacteria
- Biomarkers (Molecular Fossils)
- Biomarkers (Organic, Compound-Specific Isotopes)
- Carbon (Organic, Degradation)
- Chemolithotrophy
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Definition

Arsenic, element 33, is a metalloid in row VB of the periodic table. The main oxidation states are arsine (−3) elemental As(0), arsenite (+3), and arsenate (+5). Arsenate, As(V), and arsenite, As(III), are most common, with HAsO₄²⁻ and H₂AsO₄⁻ predominating under oxic conditions and H₃AsO³⁻ and H₂AsO₃²⁻ prevalent in anoxic environments. Arsenic is found in minerals such as arsenosulfides (e.g., arsenopyrite, realgar), iron hydroxides and aluminosilicates (e.g., clays), fossil fuels, geothermal sources, and weathered volcanic and evaporitic deposits. Anthropogenic inputs may also be considerable as organoarsenicals such as roxarsone (3-nitro-4-hydroxy benzene arsonic acid) and monosodium and disodium arsenates (e.g., calcium arsenate), and organoarsenicals (e.g., roxarsone). Thus organisms that metabolize arsenic can impact the speciation, mobility, and toxicity of arsenic in the environment (Oremland and Stolz, 2005).

Bibliography


Cross-references

Biomining (Mineral Bioleaching, Mineral Biooxidation)
Geomycology
Ores, Microbial Precipitation and Oxidation
Shewanella
Sulfide Mineral Oxidation

ASTEROID AND COMET IMPACTS

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Definition

The impact of an extraterrestrial object (either an asteroid or comet) with the Earth’s surface.
Introduction

There are now over 170 asteroid and comet impact craters that have been identified on the surface of the Earth (Table 1). These craters represent a small subset of the total impacts that have occurred during the history of life. Today, impact craters probably represent something on the order of 50,000 km² of Earth’s surface. Although this is a small habitat area, impacts are the only extraterrestrial mechanism capable of causing localized ecological disturbance. Because impacts are a mechanism capable of delivering a pulse of energy into ecosystems, from a geobiological point of view they present an important addition to the overall picture of how ecosystems are disturbed and how they recover through time. Comparison to other processes involving ecological disturbance and recovery including volcanism, glaciation/deglaciation, storm damage, fire, landslides, and coastal erosion allows for a more complete picture of the role of catastrophic change in shaping the geobiology of the Earth.

Effects of impacts on the local environment

The kinetic energy associated with the collision of an asteroid or comet with the Earth is enormous. The collision of a rock with a 10-km diameter could release the equivalent energy of 10 million tonnes of TNT. An event on such a scale is expected to occur, on average, once in every 100 million years, and may be capable of causing global-scale disruption and extinction. Such an event is implicated in the Cretaceous–Paleogene extinctions. It is perhaps not surprising, therefore, that the biological destructiveness of impact events has overwhelmingly influenced our view of these objects and their role in ecological and evolutionary processes. However, it is important to realize that the great majority of impacts only cause effects at the local scale, although they may distribute material from the point of impact over wide areas. This contribution will focus on the local geobiological effects of impacts.

The impact of an asteroid and comet with the surface of the Earth has two profound effects that are relevant for life. First, the impact delivers energy into the target area with important geological consequences and second, it rearranges the hydrologic cycle, with implications for the availability of liquid water. Temperatures generated in the impact cratering process can exceed several thousand degrees centigrade (Melosh, 1989). Most biologically important macronutrients volatilize at temperatures much lower than those associated with temperatures generated in the crater during impact (e.g., nitrates, 200°C; organic phosphorus, 350°C; inorganic phosphorus, 750°C; sulphur, 450°C; potassium, 570°C; and sodium, 880°C). In some parts of the crater, the substrate will not only be sterilized (made devoid of life), but will also be rendered oligotrophic (i.e., lacking in nutrients). The process and/or the extent of recolonization on melted and shocked substrates might be very different to that taking place on unprocessed substrate either inside or outside the crater and it might be very different from that associated with other agents of local ecological disturbance.

Recovery from impacts

Following the impact, there will be a well-defined sequence of biological recovery within the crater (Cockell and Lee, 2002). As there has been no large scale impact event in recorded human history, there has been no opportunity to observe these changes directly unlike post-volcanic recovery. However, observations on the present-day colonization of impact craters, which are in various states of preservation, can yield insights into the likely sequence of events (Cockell and Lee, 2002) and particularly the long-term influence of impacts on the geobiology of a region. Some general phases can be identified that are likely to occur. Following impact, the thermal pulse delivered by the impactor will heat the surrounding rock. High-temperature hydrothermal fluids generated in the modified thermal environment (Osiniski et al., 2001) may, in the early stages following impact, provide a habitat for thermophilic and hyperthermophilic (heat-loving) microorganisms. For example, in the Haughton impact structure, a 24-km diameter structure on Devon Island in the Canadian High Arctic, the hydrothermal system may have lasted for at least 10,000 years. The crater cavity formed during the impact acts as a hydrologic depression and will provide a new habitat for aquatic life (either freshwater on land or marine if the impact occurred in the ocean and excavated a new salt-water filled environment).

Following impact, intracratere lacustrine ecosystems become established, depending upon the size of the structure. Even today, many ancient impact craters support lakes. The biota associated with impact crater lakes has now been studied in several cases. For example, the low biomass and poor diversity of the 3.4-km-diameter New Quebec Crater in Canada and the 18-km-diameter El’gygytgyn crater in Siberia (Gronlund et al., 1990; Cremer and Wagner, 2003), contrast with the rich, abundant bacterial and algal ecosystem associated with the 1-km-diameter Tswaing impact crater in South Africa (Ashton and Schoeman, 1983; Schoeman and Ashton, 1982) (Figure 1). The Tswaing crater is situated in South African bushveld and receives organic input from the rich

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**Asteroid and Comet Impacts, Table 1** Information on the craters discussed in this article

<table>
<thead>
<tr>
<th>Name of crater</th>
<th>Country</th>
<th>Size of crater (km)</th>
<th>Age of crater (Myr)</th>
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<tr>
<td>Chesapeake</td>
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<td>El’gygytgyn</td>
<td>Russia</td>
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<td>3.5 ± 0.5</td>
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<td>Sweden</td>
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<td>Mjølnir</td>
<td>Norway</td>
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</tr>
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</tr>
<tr>
<td>Tswaing</td>
<td>South Africa</td>
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<td>0.22 ± 0.05</td>
</tr>
</tbody>
</table>
vegetation that grows within the impact crater cavity. The area is characterized by erratic rainfall (between 400 and 750 mm a year) and summer temperatures that vary between a mean minimum and maximum of 14.2 and 35.2°C, respectively. The New Quebec crater is in a cold environment; temperatures are relatively constant at approximately 2.5–3°C down to the bottom (267 m) of the lake. Although the biota that takes advantage of an intra-crater lake will be influenced by the local climatic regimen, these examples show how impact excavation provides benefits for life by forming a cavity, in which water collects.

Once the crater begins to cool, exposed melt rocks can provide a substrate for primary succession (Cockell et al., 2001). The drainage of the lake by the breach of the crater wall will expose impact lithologies which then provide a substrate on which colonization can occur. Thus the sequence of geobiological events that occur after impact will be influenced by the general characteristics of impact craters (e.g., presence of a thermal pulse, an impact cavity) and the specifics of any given crater (e.g., duration of thermal pulse, local climate, size and depth of intra-crater lake, substrate into which impact occurs, and the scale of the impact).

Ultimately a crater will become buried or eroded to point where the internal ecology may be indistinguishable from the exterior ecology.

As three-quarters of the Earth is ocean, most impacts occur in water and one initial effect is the large-scale movement of water bodies. Investigations of strata in a corehole 30 km northeast of the Mjølnir crater, formed in the late Jurassic by the impact of an object into the 300–400 m deep paleo-Barents Sea, suggest that the impact event mixed water masses and caused a large introduction of nutrients into the Sea (Smelror et al., 2002). High concentrations of marine algae suggest that the increase in the availability of ammonia, nitrates, phosphates, and iron contributed to a postimpact bloom. The backwash of a tsunami, caused by the impact, is postulated to be the reason for the high concentrations of freshwater algae in the strata. The mixing of the water, caused directly by impact, would also have been exacerbated by the collapse of the crater after formation.

**Geomicrobiological influence of impacts**

In the sense that impacts merely create a “hole in the ground,” their aquatic ecosystems may be indistinguishable from any topographic low in a similar location. However, it is evident that asteroid and comet impacts can have an important influence on the target lithology that is quite specific to impact and can alter the habitat for rock-dwelling microorganisms, making impact craters an important site for geomicrobiological investigations (Cockell et al., 2002). The temperatures and pressures of impact commonly increase the fracture space within rocks, which provide a greater abundance of habitats for endoliths, i.e., organisms which inhabit cracks within rocks. In some instances, the increase in permeable space within the target material in combination with an increase in the translucence of the rock in the visible part of the spectrum can yield new habitats suitable for phototrophs. Impact-shocked Precambrian gneiss in the Haughton crater has been shown to preferentially offer cryptendolithic habitats for photosynthetic cyanobacterial colonists (Figure 2). The rock is not otherwise a suitable habitat for them (Cockell et al., 2002). This finding constituted empirical evidence for the formation of a microbial habitat specifically by an asteroid or comet impact.

The fate of highly porous substrates versus low porosity substrates during impact is different (Kieffer, 1971). In the former case more of the impact energy must be taken up in pore collapse. Thus, an examination of the effects of asteroid and comet impacts on the colonization of porous sedimentary target lithologies can provide a model with which to understand more generally the effects of impacts on the colonization potential of rocks (Cockell et al., 2005). In a study of impact-shocked sandstones from the
Haughton impact structure, Canadian High Arctic, it was shown that shock pressures from 0 to \( \sim 10 \) GPa caused the pore structure to collapse, thus rendering the material less amenable to microbial colonization. However, as shock pressures are increased to \( \sim 50 \) GPa the material becomes successively better for colonization on account of the formation of vesicular regions in the rock from rock fracturing and melting, improving conditions for colonization. At higher shock pressures colonization potential is again decreased as a solid glass is formed which is impermeable, although potentially still accessible to rock-boring microorganisms.

Thus, in summary, we can say that in general impact events may have the effect of turning lithic habitats on their head. Habitats that were previously impermeable become better sites for colonization as the rocks are fractured. Habitats that were good sites for colonization are rendered less permeable because of pore collapse, although, as the sandstones of Haughton illustrate, the effects on any given lithology tend to be complex.

Within and around impact craters new minerals can be generated in hydrothermal systems that later become habitats for life. Gypsum deposits laid down in the Haughton crater have become a habitat for cyanobacteria which inhabit the inter-sheet spaces between the sulphate crystals (Parnell et al., 2004). The extent to which these organisms are active is uncertain, but they must certainly be halotolerant.

During impact, the target lithology, especially if it is stratified and diverse, will be fragmented and mixed, leading to a heterogeneous substrate in which diverse geochemical sources of nutrients are brought into close proximity. A study of the weathering of a tsunami resurge deposit in the Lockne impact crater in Sweden (formed \( \sim 455 \) million years ago) showed how lichens preferentially first colonized the soft chlorite clasts incorporated into the impactite (Cockell et al., 2007). The dissolution of the calcium carbonate clasts by fungi and other organisms creates cavities in the rock, enhancing weathering and causing the rock to exfoliate in sheets. Previous studies on the chemical weathering of impact-shocked rocks from the Ries crater, Germany, showed that the deformation features introduced into quartz were sites of preferential weathering (Leroux, 2005). Diverse impact-generated lithologies can be used as a model to understand microbial weathering of other mixed sedimentary lithologies such as greywackes and microbe–mineral interactions in extremely disturbed geological settings in general.

Most of the geobiological studies conducted to date pertain to the surface environment. The first drill core retrieved from an impact crater with robust microbiological contamination control came from the Chesapeake Bay Impact Crater and was retrieved during the ICDP (International Continental Drilling Project)-USGS (United States Geological Survey) deep drilling project in 2005 (Gohn et al., 2008). A core of length 1.8 km was retrieved. The sequence included a 600 m sequence of postimpact sediments laid down since the impact in the late Eocene and 1.2 km of postimpact tsunami and “fall-back” deposits laid down in the crater cavity immediately after impact. This work has shown that the microbial diversity and abundance of microorganisms correlates to the lithologies in the crater cavity, showing that impacts can significantly disrupt the characteristics of the deep biosphere.

**Impact events and astrobiology**

The study of the geomicrobiology of impact craters provides useful clues to the opportunities for life on the Archean Earth since the emergence of life \( \sim 3.5 \) Gya ago, when the impact flux were higher than today. The formation of hydrothermal systems and the fracturing of rocks are two such opportunities that impact craters might have offered to early life. Insofar as asteroid and comet
impacts are universal (in that no solar system-forming process is known that is completely free from remnant material), habitats created in craters yield insights into the possible habitats for life on other planets, such as Mars. In particular, the likelihood that impacts melt permafrost, or generate deep fracturing into regions of a planet where liquid water is available, suggests that they offer intriguing possibilities as sites to examine the potential habitability of Mars, past and present.

Summary

Asteroid and comet impacts can cause global environmental perturbations through gross changes in atmospheric chemistry. However, much more commonly they alter geobiology at local scales. Through local disruptions of hydrology and geology, they generate new habitats and substrates, the most conspicuous being intracrater lakes. The energy delivered during impacts can also change habitats at the micron scale through changes in target lithology creating entirely novel geomicrobiological environments which, through pressure and temperature effects, are distinguishable from volcanic and other disturbed environments. The geobiological study of asteroid and comet impacts completes our picture of the role of catastrophic disturbance in influencing life on Earth alongside terrestrially mediated mechanisms of environmental change.

Bibliography


Cross-references

Astrobiology

Biological Volcanic Rock Weathering

Cyanobacteria

Deep Biosphere of Sediments

Endoliths

Extreme Environments

Hydrothermal Environments, Terrestrial

Mass Extinctions, Phanerozoic

Meteoritics

ASTROBIOLOGY

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Synonyms

Bioastronomy; Exobiology

Definition

Astrobiology is the interdisciplinary science that studies the origin, evolution, distribution, and future of life in the Universe. It is a highly interdisciplinary field that engages many scientific disciplines, including biology, biogeochemistry, paleontology, earth and atmospheric sciences, planetology, and astrophysics among others.

Historical perspective

Astrobiology as a term was first coined in the 1940s to encompass early scientific ideas about how to explore for extraterrestrial life (Dick and Strick, 2004). During the 1960s, astrobiological research was focused more
narrowly than today, with an emphasis on the origin of life, and programs funding these activities developed under the umbrella term "exobiology." This early period of activity culminated in the Viking mission to Mars, which carried life detection experiments designed to explore for life in Martian surface materials (Klein et al., 1976). With the failure of the Viking experiments to detect unambiguous evidence for life on Mars, interest in the search for life elsewhere in the solar system waned. Then in 1995, Dr. Wesley Huntress, who was the National Aeronautics and Space Administration’s (NASA) Associate Administrator for Space Science at the time, reintroduced astrobiology to refer to a broad range of scientific activities that had begun to coalesce around questions of the potential for habitable environments and life beyond Earth. The following year, growth of the field of astrobiology was accelerated by the announcement by a team of scientists at NASA Johnson Space Center of possible signs of life in Martian meteorite, ALH84001 (McKay et al., 1996) (Figure 1). With this came a renewed interest in the search for Martian life. Over the next few years, NASA expanded research to develop more reliable approaches to extraterrestrial life detection, which helped to revitalize the field of astrobiology.

In 1998, NASA helped promote the growth of astrobiology by creating the NASA Astrobiology Institute (NAI), a virtual research organization comprised of interdisciplinary teams of scientists from NASA Research Centers and universities around the United States. An important goal of the NAI has been to promote the growth of astrobiology through the sponsorship of collaborative, transdisciplinary research that could provide a scientific and technology development framework for future space missions. Following the establishment of the NAI, the growing community of scientists converged on a strategic plan that would help guide astrobiological research, including a scientific roadmap, with well-defined goals and objectives, and technology developments that would be needed to enable missions (Des Marais et al., 2008). Since its inception, the astrobiology roadmap has been revised and updated every few years to reexamine scientific priorities in the light of new scientific discoveries. The NAI has also embraced partnerships with other astrobiology institutions in Spain, Australia, and Great Britain. This includes international cosponsorship of yearly workshops designed to promote collaborative research and train the next generation of astrobiologists. Maturing of the field is also apparent in the development of two international journals for Astrobiology, international conferences each year and the establishment of an Astrobiology Society.

In 2008, the National Research Council reviewed the growth of the science of astrobiology (NRC, 2007a), summarizing the important collaborative efforts and scientific discoveries that had occurred since the inception of the NAI in 1998. Studies of the deep geological record of Earth have helped refine our knowledge of the early evolution of life on Earth, based on the fossil record, while providing new constraints on the nature and evolution of early environments. Studies of prebiotic organic chemical systems have revealed important new insights into the steps that may have preceded the emergence of terrestrial life, as well as the environments where life may have emerged (Russell et al., 1993; Deamer et al., 2002; Ricardo et al., 2004; Ferris, 2005).

Environmental limits for life
Biological studies have also significantly expanded our knowledge of the environmental limits of life on Earth (see Rothschild and Mancinelli, 2001), while the application of new tools of environmental molecular microbiology have revealed the presence of an extraordinarily diverse microbial biosphere, with an equally impressive array of metabolic strategies for extracting energy from Earth’s environments (e.g., Staley and Reysenbach, 2001). Life has been shown to occupy a broad range of temperature (−15°C up to 122°C; see Kashefi and Lovely, 2003; also, Figure 2 from Takai et al., 2008) and nearly the full range of pH from acidic to alkaline (pH ~1.0 to 13.0), over a range of salinities from freshwater to hypersaline brines and water activities >0.6. We have also learned of the importance of the deep subsurface biosphere, which may account for possibly half or more of the Earth’s biomass (Gold, 1998). The discovery of lithoautotrophs that can use the simple chemical compounds, such as hydrogen and methane (CH₄), released...
during the low-temperature inorganic weathering of rocks or sulfide (H₂S) formed by hydrothermal processes has opened up opportunities for the survival of microbial life in the deep subsurface, independent of photosynthesis (Stevens, 1997). These discoveries and others in the fields of biology and paleontology have helped broaden the search space for habitable environments and life elsewhere in our Solar System and beyond.

Defining life

Earth has the distinction of being the only place in the Universe where life is known to exist. Thus, terrestrial life provides a logical starting point for evaluating the prospects for the existence of life elsewhere and identifying strategies for astrobiological exploration. For exploration, we need to identify basic attributes of living systems that can be used to inform exploration strategies to search for life beyond the Earth. Ideally, we would like to follow universal criteria that are not tied to the particular circumstances found on Earth. But, universal definitions of life have proven elusive and challenging to apply (Cleland and Chyba, 2002). As a practical approach to exploration, the search for life begins with what is known of terrestrial life, following a terracentric definition that combines features like a water-based carbon chemistry, and self-replication, with cellular systems that evolve through processes of Darwinian natural selection to adapt to environmental changes. Although such attributes are regarded as essential properties of life, many inorganic systems display similar features, such that a single property, while necessary, is not regarded as sufficient to define life. Methods of life detection for astrobiological exploration of other planetary environments are still in their infancy but presently combine several instrumental techniques that can independently measure life’s attributes, as a basis for detection. An example is the Pasteur instrument, which combines the detection of amino acids in soils and ices, with measurements of the chirality (right- versus left-handedness) in amino acid building blocks of proteins (Skelley et al., 2005). Terrestrial life selects left-handed amino acids when making proteins, and this selectivity has been suggested to be a biosignature that could be used to explore for life elsewhere (Figure 3).

Exploring for life in the Solar System

In exploring for life in the Solar System, NASA has adopted a simple approach that emphasizes the search for those environmental factors that are known requirements of terrestrial life. At the top of the list is liquid water, which is required by all known life-forms. In their exploration of the Solar System and beyond, NASA has pursued an exploration strategy designed to “Follow the Water.”
Liquid water plays an essential role in living systems as a medium of biochemical transport and exchange during metabolic reactions. The unique solvent properties of liquid water are primarily derived from the dipolar charge distribution of water molecules and the ease with which hydrogen bonds may be formed and broken in solution. These properties also determine many of the unique physical properties of water, including the broad temperature range, over which it remains liquid and the decrease in density that occurs upon freezing. This has the interesting consequence that the crystalline structure of water expands and density decreases during freezing, so that the ice floats at the surface instead of sinking to the bottom. Thus, oceans freeze from the top down, thereby enhancing long-term habitability. Terrestrial examples that illustrate the importance of this for habitability include perennially ice-covered lakes of the “dry valleys” of Antarctica. High salinity (which depresses the freezing point), combined with the self-insulating properties of the frozen surface of the lake, promotes the persistence of habitable zones of liquid water environments, even in the winter when air temperatures have fallen far below the freezing point (Andersen et al., 1995). On Mars, one consequence of this is that ice-covered lakes could have persisted on Mars long after the planet began to lose its atmosphere, thus rendering liquid water at the surface unstable (Wharton et al., 1995; McKay and Davis, 1991). Recent climatic variations on Mars mediated by changes in the obliquity and recorded in polar deposits, suggests the possibility that transient habitable conditions could have developed at the Martian surface very recently in the planet’s history (Figure 4).

It is also widely appreciated that living systems require more than liquid water. Habitable environments are also sources of the biological elements (C, H, N, O, P, S), inclusive of about two-dozen transition metals that are critical for enzyme function (Crabb and Moore, 2009), and sources of energy for sustaining cellular metabolism, growth, and replication (Hoehler et al., 2007). Thus, the exploration for potentially habitable zones in the Solar System and beyond has been focused on the search for environments where liquid water coexists with sources of biogenic elements and energy (Figure 5).

Recent progress in astrobiology

In parallel with the studies of life on Earth, missions to other bodies in our Solar System have revealed many examples of potentially habitable environments, with evidence for the past, or present existence of liquid water, biologically important elements, and potential energy sources. The exploration of Mars has identified...
widespread geological evidence for climatic changes that could have affected ancient surface water systems, while also strengthening the case for liquid water in the subsurface today (NRC, 2007b). NASA missions have also “followed the water” to the outer Solar System, with the discovery of brines beneath the surfaces of three of the icy Galilean satellites of Jupiter (Europa, Ganymede, and Callisto; Kivelson et al., 2000; Greenberg, 2008), as well as Enceladus, one of the moons of Saturn (Kieffer et al., 2006). In addition, Saturn’s moon, Titan, has also been shown to harbor water ice as well as lakes of liquid methane (Mitri et al., 2007) (Figure 6).

The discovery of lakes of methane on Titan has led to a debate over the potential for life to originate within highly reducing, hydrocarbon-rich environments, where liquid water may be replaced by alternative solvents, such as liquid methane or ammonia, as alternative solvents for life processes (e.g., Benner et al., 2004) (Figures 7 and 8).

Finally, discoveries in observational astronomy have shown that planets and planetary systems to be common features of other stars in the nearby Milky Way Galaxy. Since the first confirmed discovery in 1992, more than 450 extrasolar planets have been identified, and the number continues to grow each year (Schneider, 2010). Orbiting planets have been detected by a variety of methods, but primarily by measuring small perturbations in the position of the parent star due to the gravitational tug and pull of an orbiting planet(s), or as a result of slight decreases in the stars brightness as an orbiting planetary body passes in front of the star. More rarely, extrasolar planets have also been discovered using direct imaging methods (Figure 9).
Summary

The emerging field of astrobiology is taking a highly interdisciplinary approach to the basic question: “Are we alone in the Universe?” An answer to this question is being pursued on a large number of fronts both within and beyond our Solar System. For example, studies of biology on Earth has continued to expand the environmental limits for life while revealing a dizzying array of potential energy sources. This has significantly expanded the potential number of habitable zones in the solar system, opening up new opportunities for astrobiological exploration in the solar system. Ongoing exploration of the planet Mars, long regarded as most Earth-like in its potential habitability, has provided abundant evidence for past water on the planet’s surface, along with potential building blocks for life and sources of energy to support living systems. Although the present surface of Mars appears to be uninhabitable due to an absence of liquid water, life could still exist in the subsurface (Warner and Farmer, 2010), or its remains be preserved in ancient aqueous sediments exposed at the surface (Farmer, 2000; Farmer and Des Marais, 1999). Future exploration will continue to pursue the search for fossil or extant biosignatures of microbial life on Mars, in the hope of making a transformative and revolutionary discovery of life. But exploration is also pursuing the question of microbial life in potentially habitable icy moons of the outer Solar System, specifically three of the Galilean moons of Jupiter (Europa, Ganymede, and Callisto), as well as Saturn’s moon, Enceladus, where internal zones of liquid water are maintained not by solar radiation but by the internal tidal heating resulting from gravitational flexing of the interior of these moons. In addition, Jupiter’s moon, Titan, has provided access to potentially habitable environments that are fundamentally different from Earth. On Titan, we have discovered alternative environments potentially capable of supporting non-terran forms of life, sustained by alternative solvents (oceans of liquid methane and ammonia) and energy sources. Finally, the discovery of numerous planets and planetary systems orbiting other stars in the nearby galaxy has widened the search for habitable environments beyond the confines of our Solar System. These and other important discoveries have shaped the way astrobiologists evaluate potential for the origin, evolution, and persistence of life elsewhere, contributing to the development of a robust conceptual framework for planning missions and developing the technologies needed to explore for extraterrestrial life (e.g., Mix et al., 2006; Plaxco and Gross, 2006; Sullivan and Baross, 2007).

Astrobiology, Figure 8 Enceladus, a moon of Saturn, imaged during flybys of the Cassini orbiter, has been shown to actively erupt plumes of water ice, vapor, and organic compounds from the moon’s interior (Kiefer et al., 2006). These eruptions occur along fracture systems located near the south pole. (Image credit: NASA.)

Astrobiology, Figure 9 Formalhaut b is a multiple Jupiter-mass planet that was directly imaged in its orbit around the star Formalhaut, located about 25 light years away from Earth in the constellation Piscus Austrinus (Kalas et al., 2008).
Bibliography


Cross-references

Asteroid and Comet Impacts  
Biomarkers (Molecular Fossils)  
Biosignatures in Rocks  
Cosmic Molecular Clouds  
Deep Biosphere of Sediments  
Deep Biosphere of the Oceanic Deep Sea  
Extreme Environments  
Hydrothermal Environments, Marine  
Meteorites  
Origin of Life  
Raman Microscopy (Confocal)
BACTERIA

Michael Hoppert
University of Göttingen, Göttingen, Germany

Synonyms
Eubacteria (term abandoned)

Definition
Prokaryotes that constitute, besides Archaea and Eukarya, a domain of life. According to (old Greek) bakterion “small rod.”

History
During the nineteenth century, several fundamental discoveries defined the beginning of modern microbiology (cf. Schlegel, 1999). Louis Pasteur (1822–1895) found that growth of bacteria in nutrient broths is not due to spontaneous generation and that fermentation is caused by the growth of microorganisms. Ferdinand Cohn (1828–1898) could state that bacteria must belong to a phylogenetic group separated from other unicellular plants or animals, due to their size, shape, the mode of cell division, and their metabolic properties. Robert Koch (1842–1910) developed the concept of infectious diseases. The final proof that bacteria must be distinct from plants and animals could be shown not until the distinct compartmentation of bacterial cells was noticed by electron microscopy, in the 1960s. Especially, the absence of a nuclear membrane was a clear indicator for some fundamental differences in cellular processes. Hence, the term “prokaryote” for bacteria (including Archaea) was introduced. The separation of Archaea from Bacteria (as phylogenetic groups) was achieved by Woese and Fox (1977).

Besides the missing nuclear membrane as a common cytological feature, Bacteria and Archaea are structurally and physiologically distinct. The Bacteria are the phylogenetically most diverse domain, with a rapidly growing number of phyla. Currently, more than 80 phyla could be identified (Pace, 2009). About two-third of these phyla are just represented by rDNA sequences from environmental samples. Twenty-five phyla are represented by known species. Among them, twelve phyla contain the majority of currently known and well-characterized species. The well-known representatives of phyla, however, stand for an incomplete and subjective view on the bacterial domain. Human pathogenic species are, for example, overrepresented, because of their importance for human medicine. The significance of several groups relevant for environmental processes, such as Planctomycetes or the Roseobacter clade has just been addressed recently. General knowledge on the physiology of these groups is still limited. Most in-depth analyses on microbial physiology and molecular biology have been performed with selected species from a small number of phyla, such as Escherichia coli, Salmonella, and Pseudomonas species (Proteobacteria) or Bacillus (Firmicutes). Some organisms like Ralstonia (an aerobic hydrogen oxidizing Proteobacterium) or Rhodobacter (a photosynthetic bacterium) are model organisms for environmentally relevant processes.

The phylogenetic tree (Figure 1), based upon 16S-rDNA data shows an overview of these major, well-characterized lineages (Cole et al., 2009; Madigan et al., 2009). In-depth analysis based upon whole genomes may allow, in future, a more accurate positioning of genes and organisms in phylogenetic trees (Ciccarelli et al., 2006; Wu and Eisen, 2008). Though the major phyla have been confirmed, some repositioning may be necessary. Some reports imply that Firmicutes are descendants of...
the stem group, other than expected from rDNA analysis (Ciccarelli et al., 2006).

In the following chapter, a short survey on the hitherto well-investigated groups will illustrate the metabolic diversity of the organisms. Terms denoting defined phyla are capitalized.

Diversity in phylogenetic lineages

Important metabolic key processes are unique to bacteria and have just been overtaken by endosymbiosis to eukaryotes, such as chlorophyll-dependent photosynthesis and mitochondrial respiration. Thus, metabolism in every eukaryote is based upon bacterial metabolism. Eukaryotes, however, received just a small range of the highly various metabolic features from bacteria. Virtually, every reduced organic compound and numerous reduced inorganic molecules can be oxidized in a bacterial metabolism that may be either respiratory, that is, generating a membrane potential, driven by an electron flow toward an oxidized acceptor (oxygen, nitrate, and others) or fermentative (generation of ATP independent of a membrane potential). These metabolic features are unsuitable to discriminate between large groups because they are mostly not specific for only one phylum (Tables 1–4; Schlegel and Jannasch, 2006; Zinder and Dworkin, 2006). Some strategies and bacterial life-styles are, however, suitable to discriminate between certain subgroups within a phylum. In the following, some key features that may be confined to the respective groups are specified. The groups are assigned to phyla according to the “classical” phylogenetic scheme, obtained by 16S-rDNA sequence analysis (Cole et al., 2008; cf. Madigan et al., 2009).

Ancient phyla

Several representatives of the ancient bacterial groups clustering deep in the phylogenetic tree are hyperthermophilic, some of them are chemolithotrophs. The hyperthermophilic genus *Aquifex* is an obligately hydrogen oxidizing, microaerophilic, chemolithotrophic (see *Chemolithotrophy*) bacterium (Huber and Eder, 2006). The fixation of carbon dioxide does not depend on the Calvin cycle (ribulose bisphosphate carboxylase as key enzyme), like in further derived phyla and green plants, but, instead, on a reversed citrate cycle. The organisms are not capable to grow on organic molecules, that is, they are obligate chemolithotrophs utilizing molecular hydrogen or reduced sulfur compounds as electron donors, at optimum growth temperatures of 85°C. Thus, the environmental conditions for the ecological niche of *Aquifex* are equivalent to the reducing conditions of the earth’s early atmosphere. The related genera *Hydrogenobacter* and *Thermocrinis* share most of *Aquifex’* properties, but are aerobic and also utilize organic compounds.

Several important cellular features that are found in most bacterial phyla are already present in this ancient lineage: the cytoplasmic membrane is surrounded by another membrane of lipid bilayer type (outer membrane). Thus, the membranes enclose an additional compartment, the periplasmic space. The periplasmic space contains the rigid murein (peptidoglycan) sacculus. Another feature found in all, including the most ancient bacterial phyla is the bacterial flagellum (Figure 2a). Though analogous
Motility structures are also found in Archaea (and in Eukarya), they are phylogenetically completely unrelated. For other types of appendages (bacterial pili, Figure 2a), homologues in Archaea could be described (Szabo et al., 2007).

Thermotoga and related genera also comprise chemoorganotrophic hyperthermophilic representatives. Comparative genomics revealed that more than 20% of the Thermotoga genome shares strong homologies with genes from various hyperthermophilic archaeal species, accounting for an extensive inter-domain horizontal gene transfer (Nelson et al., 1999).

Chloroflexi and Chlorobi (Bacteria) chlorophyll-dependent photosynthesis (see Photosynthesis) is unique for bacterial organisms (Table 2; Overmann and Garcia-Pichel, 2006). Ancestors of green nonsulfur bacteria (Chloroflexi) were very likely the first photosynthetic organisms of the bacterial domain (Oyaizu et al., 1987). The key genus Chloroflexus is a thermophilic representative of the group. Besides photoautotrophic growth, Chloroflexus is also able to use organic compounds. Carbon dioxide fixation is still Rubisco independent and is carried out by transfer of hydrogen carbonate to activated acetate and propionate.
(acetyl-coenzyme A, propionyl-coenzyme A) in the hydroxypropionate pathway. Green sulfur bacteria (Chlorobi) represent a separate lineage of anoxygenic photosynthetic bacteria. Similar to photosynthetic Proteobacteria, the organisms use reduced sulfur compounds (H₂S, S₀) as well as organic compounds as electron donors. The organisms use a reverse citric acid cycle for carbon dioxide fixation. A remarkable structural feature, the chlorosome, consisting of densely stacked self-assembled antenna bacteriochlorophylls, is a simple but very effective light harvesting complex. This arrangement transfers light energy (excitons) effectively to the reaction centre in the cytoplasmic membrane. The organisms are, hence, adapted to very low light conditions. Chlorosome genes were transferred, by horizontal gene transfer, to some phototrophic lineages, including some genera of the *Chloroflexus* group (cf. Hanada and Pierson, 2006).

**Spirochetes**

Spirochetes are rather structurally than metabolically remarkable (Charon et al., 1992). The spirochete endoflagellum is of the standard bacterial type, but is located in the periplasmic space. In all other bacterial groups, the flagellum (if present) traverses all layers of the envelope. The endoflagellum is helically wound around the flexible protoplast and gives the cell its unique, helically coiled appearance. The movement of the flagellum drives the cell body in a drill-like manner. By this way, movement of the cell in highly viscous media like tissue is facilitated. In fact, numerous symbiotic and pathogenic organisms belong to this group (*Teponema, Borrelia, Leptospira*).

**Deinococcus/Thermus**

Another separate lineage of extremophilic bacteria is represented by the key genera *Deinococcus* and *Thermus*. The organisms mainly oxidize carbohydrates and proteins. *Thermus* species are thermophilic and grow between 50°C and 80°C. The remarkable members of the *Deinococcus* group are adapted to desiccation and ionizing or ultraviolet radiation. The composition and thickness of the cell wall, DNA compaction, and DNA repair systems are special protective features (Da Costa et al., 2006).

**Flavobacteria/Cytophaga**

The Flavobacteria/Cytophaga phylum comprises diverse, mostly heterotrophic, anaerobic or aerobic bacteria (Bernardet and Nakagawa, 2006). Within this group, the members of the genus *Bacteroides* represent are the dominant bacteria in the human large intestine. The second key genus *Flavobacterium* is mostly found in freshwater and marine habitats. The Acidobacteria with the genera *Acidobacterium, Geothrix*, and *Halophaga* are heterotrophic soil inhabitants. It is known from phylogenetic analysis of environmental DNA that uncultured members of the genera are widespread in other terrestrial and aquatic habitats and may thus play an important ecological role. Several members of *Cytophaga* and related genera are important degraders of cellulose and other polysaccharides. Cells move by gliding, a surface motility mechanism that is also observable in other bacterial lineages.

**Table 4: Groups of fermentative bacteria**

<table>
<thead>
<tr>
<th>Fermentative process</th>
<th>Typical species</th>
<th>Lineage</th>
<th>Preferred substrates</th>
<th>Fermentation products (minor products)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol fermentation</td>
<td><em>Zymomonas mobilis</em></td>
<td>Alpha-Proteobacteria</td>
<td>Glucose</td>
<td>Ethanol (CO₂)</td>
</tr>
<tr>
<td>Lactate fermentation – homofermentative</td>
<td><em>Lactobacillus casei</em></td>
<td>Firmicutes</td>
<td>Glucose, lactose</td>
<td>Lactate</td>
</tr>
<tr>
<td>Lactate fermentation – heterofermentative</td>
<td><em>Leuconostoc mesenteroides</em></td>
<td>Firmicutes</td>
<td>Glucose, lactose</td>
<td>Lactate (ethanol, CO₂)</td>
</tr>
<tr>
<td>Butyrate fermentation</td>
<td><em>Clostridium butyricum</em></td>
<td>Firmicutes</td>
<td>Glucose</td>
<td>Butyrate (acetate, H₂, CO₂)</td>
</tr>
<tr>
<td></td>
<td><em>Clostridium acetobutylicum</em></td>
<td>Firmicutes</td>
<td>Glucose</td>
<td>Butyrate (acetate, butanol, 2-propanol)</td>
</tr>
<tr>
<td>Homooacetate fermentation</td>
<td><em>Clostridium kluyveri</em></td>
<td>Firmicutes</td>
<td>Ethanol+ acetate</td>
<td>Butyrate (caproate, H₂)</td>
</tr>
<tr>
<td>Propionate and succinate</td>
<td><em>Clostridium acetoclasticum</em></td>
<td>Firmicutes</td>
<td>Fructose</td>
<td>Acetate</td>
</tr>
<tr>
<td>fermentation</td>
<td><em>Propionibacterium pentosaceum</em></td>
<td>Firmicutes</td>
<td>Sugars, Lactate</td>
<td>Propionate (succinate)</td>
</tr>
<tr>
<td>Mixed acid and butanediol</td>
<td><em>Veillonella alcalescens</em></td>
<td>Firmicutes</td>
<td>Lactate</td>
<td>Propionate (acetate, H₂, CO₂)</td>
</tr>
<tr>
<td>fermentation</td>
<td><em>Bacteroides ruminicola</em></td>
<td>Firmicutes</td>
<td>Sugars</td>
<td>Propionate (acetate, succinate, H₂, CO₂)</td>
</tr>
<tr>
<td>Mixed acid and butanediol</td>
<td><em>Escherichia coli</em></td>
<td>Gamma-Proteobacteria</td>
<td>Glucose</td>
<td>Lactate, ethanol acetate (formate, succinate, H₂, CO₂)</td>
</tr>
<tr>
<td>fermentation</td>
<td><em>Enterobacter aerogenes</em></td>
<td>Firmicutes</td>
<td>Glutamate</td>
<td>Butyrate (acetate, CO₂, NH₃)</td>
</tr>
<tr>
<td>Amino acid fermentation</td>
<td><em>Clostridium tetanomorphum</em></td>
<td>Firmicutes</td>
<td>Lysine</td>
<td>Butyrate (acetate, NH₃)</td>
</tr>
<tr>
<td></td>
<td><em>Clostridium sticklandii</em></td>
<td>Firmicutes</td>
<td>Orotate</td>
<td>Acetate (CO₂, NH₃)</td>
</tr>
<tr>
<td></td>
<td><em>Clostridium oroticum</em></td>
<td>Firmicutes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The gliding motility of *Cytophaga* and relatives, however, is based on a unique, not yet understood, cell envelope-associated mechanism.

**Planctomyces/Verrucomicrobia**

The *Planctomyces/Verrucomicrobia* lineages comprise morphologically uncommon groups of uncertain phylogeny. Planctomyces develop protein stalks as attachment structures and divide by budding (instead of binary fission, the common way of bacterial and archaeal cell division). Peptidoglycan is missing. Planctomyces and the related phylum of Verrucomicrobia also show a highly compartmentalized cytoplasm (Lee et al., 2009). Endocytosis-like processes, a typical feature of eukaryotes, could also be observed (Lonhienne et al., 2010).

The organisms are mostly found in freshwater habitats. The lobed appendages increase the cell surface and facilitate the uptake of substrate molecules in an oligotrophic life-style.

**Cyanobacteria**

Cyanobacteria and prochlorophytes differ from all other phototrophic bacteria in fundamental ways (Overmann...
and Garcia-Pichel, 2006). The oxygenic photosynthesis, conducted by cyanobacteria, requires photosystems acting over a wide redox span. To split the water molecules, the reduction potential of chlorophyll in photosystem II must be more positive than that of the O$_2$/H$_2$O couple. Excited electrons are transferred, via several redox carriers, to a second photosystem (PS I), capable of NAD(P)H regeneration. The reducing power is particularly needed for carbon dioxide fixation (for fixation of 1 mol CO$_2$, 2 mol NADPH is required), carried out by the Calvin cycle. A unique feature for Cyanobacteria are phycobilins as accessory (light harvesting) pigments, an adaptation to low light conditions. Like plastids, cyanobacteria contain intracytoplasmic thylakoid membranes as additional compartments for the membrane-bound photosystems.

Cyanobacteria are the morphologically most diverse group of bacteria, with some organizational levels analogous to eukaryotic algae (Figure 2c; Waterbury, 2006). Many species are filamentous. They are present in marine and freshwater, as well as in terrestrial habitats (Figure 2e, d). Some live in symbiotic relationship to higher plants (owing to the cyanobacterial nitrogen fixing activity). Several filamentous species carry out nitrogen fixation in specialized cells (heterocysts), lacking the oxygen evolving photosystem II.

Since cyanobacteria are responsible for oxygenation of the early atmosphere, the group has left the possibly deepest impact ever on earth’s ecosystems. They are still of global importance for carbon cycling. Marine species are responsible for an estimated 20–40% of carbon dioxide fixation in oceans (e.g., Campbell and Vaulot, 1993). Since recent Cyanobacteria and the endosymbiotic plastids of green plants have common ancestors, most of the photosynthetic processes are basically due to the activity of Cyanobacteria and their ancestors.

**Firmicutes/Actinobacteria**

Next related to Cyanobacteria are the Firmicutes/Actinobacteria phyla (Stackebrandt and Schumann, 2006; Madigan et al., 2009). A common feature of all species within these groups is the lack of an outer membrane. Most members of the groups are chemoheterotroph and exhibit a thick peptidoglycan layer, resulting in a positive reaction of the Gram stain. Thus, the organisms are also referred to as “Gram-positive.” The thick cell wall may be also considered as an adaptation to the high osmotic stresses in terrestrial ecosystems (i.e., soil). The genera *Bacillus* and *Clostridium*, but especially the filamentous Actinobacteria (the genus *Streptomycetes*, in particular) are important groups in soil habitats. Interestingly, also a small group of photosynthetic genera belongs to the Firmicutes. *Heliobacterium* and related genera are anaerobic, phototrophic soil bacteria, with a minimal photosynthetic apparatus and incapable of carbon dioxide fixation (Asao and Madigan, 2010). *Frankia* genera are important nitrogen-fixing symbionts in alder roots. The production of different antibiotic agents is another important feature, especially in Actinobacteria and *Bacillus*. Another unique feature within this lineage is the formation of endospores (Figure 2e).

Key genera of endospore formers are *Bacillus* and *Clostridium*. Whereas * Bacilli* are aerobic respiratory organisms, clostridia lack a respiratory chain and are obligate anaerobic, fermenting organisms. They are important for anaerobic degradation of organic compounds, mostly producing organic acids (Table 4). Several clostridia as well as related acetogenic bacteria (*e.g.*, *Acetobacterium*, *Acetogenium*) grow anaerobically either by using carbon dioxide as the terminal electron acceptor in anaerobic respiration with hydrogen as substrate (i.e., chemolithoautotrophically), or by fermenting carbohydrates. In either case, the acetyl-CoA pathway is used to reduce carbon dioxide to acetate. In the course of carbon dioxide reduction to acetate, a sodium ion gradient (instead of a proton gradient) across the membrane is generated that drives ATP synthesis.

Lactic acid bacteria obtain energy solely by fermentative processes. They typically have limited biosynthetic abilities, and need external sources of amino acids, vitamins, purines, and pyrimidines. This life-style is an adaptation to commensalism with other organisms: lactic acid bacteria mainly live on (decaying) plant leaves and fruit, the skin surface and the intestinal mucosa of mammals. Due to their ability to produce high amounts of lactic acid from carbohydrates (including lactose), their habitats are difficult to colonize by competing microorganisms. This also excludes pathogens from mucosal tissue. Another beneficial effect is the conservation of food (especially dairy products) by lactic acid fermentation.

Interestingly, some members of the Gram-positive lineage lost their peptidoglycan layer. These cell wall-less bacteria (key genera *Mycoplasma*, *Spiroplasma*) live as parasites on animal and plant tissue. The lack of a cell wall facilitates the flow of substrates from the host to the bacterium.

**Proteobacteria**

The most diverse bacterial lineage, the Proteobacteria, is divided into five subdivisions (alpha, beta, gamma, delta, and epsilon). Within most subdivisions, all metabolic key processes, ranging from chemo- and photoautotrophy to chemoheterotrophy, respiration, and fermentation (Tables 1–4), are common (Kersters et al., 2006; Madigan et al., 2009). Here, just the ecologically most important life-styles are pointed out. At least in three subdivisions, photoautotrophs are present (Overmann and Garcia-Pichel, 2006). All of them carry out anoxygenic photosynthesis with one photosystem; different types of intracytoplasmic membranes (lamellar, spheroidal) can be found (Tucker et al., 2010). Electron donors are reduced sulfur compounds (such as H$_2$S) in purple sulfur bacteria (Figure 2f) and mostly organic compounds (H$_2$S also in low concentrations) in purple non-sulfur bacteria. Purple non-sulfur bacteria are photoheterotrophic
organisms that utilize external carbon sources, if available. Most of them also fix carbon dioxide by the Calvin cycle.

Chemolithoautotrophy occurs in all proteobacterial subdivisions. Interestingly, ammonia and nitrite oxidation is mainly confined to the Proteobacteria. In four subdivisions (and one additional, recently discovered lineage, related to Proteobacteria) obligate chemolithotrophic nitrifying bacteria, oxidizing either ammonia to nitrite or nitrite to nitrate are present. Like phototrophs, also nitrifying bacteria contain internal membranes. Oxidation of sulfur and iron is represented by several groups in four proteobacterial subdivisions. Sulfur-oxidizing genera have been found in four proteobacterial subdivisions. Some genera are acidophilic (e.g., *Acidithiobacillus, Thiomonas*). Among sulfur oxidizing chemolithotrophs (thiotrophic bacteria), exceptionally large morphotypes were described (*Beggiaota, Thioploca, Thiomargarita*). Several sulfur oxidizers, especially from the gamma-subgroup, live in symbiosis with marine mollusks (Dubilier et al., 2008).

Up to now, the aerobic methanotrophy is mainly confined to Proteobacteria, though recently, a methanotrophic verrucomicrobial bacterium could be found (Chistoserdova et al., 2009). Methylotrophic bacteria oxidize a variety of C1 compounds (methanol, methylamine, formate). All methanotrophs possess methane monooxygenase as key enzyme for hydroxylation of the chemically inert methane to methanol. Some methanotrophic bacteria are also symbionts in marine animals.

Several genera are well-known model organisms especially for medical microbiology, such as pseudomonads and enterobacteria. Both groups are chemoorganotrophic, respiratory bacteria, enteric bacteria are facultative anaerobes. In global cycles, however, these organisms are largely insignificant. Especially enteric bacteria (within the gamma-subdivision), as commensals and pathogens of diverse higher organisms are well adapted to their hosts.

Sulfate and sulfur reduction is not only restricted to Proteobacteria. *Archaeoglobus*, as an example, is an archaeon, *Thermodesulfobacterium* is a deeply branching genus close to the root of the bacterial phylogenetic tree. Most of the sulfate- and sulfur-reducers are, however, proteobacteria that belong to the delta-subdivision. They are obligate anaerobes using mainly hydrogen, fatty and other organic acids or alcohols as energy sources. Sulfate or elemental sulfur serve as the terminal electron acceptors in an anaerobic respiration with hydrogen sulfide as product. The organic compounds are either oxidized to acetate or to carbon dioxide. These organisms are geochemically important, and are widespread in anaerobic sulfate-rich environments (Barton and Fauque, 2009).

Nitrogen fixation is a feature of multiple bacterial and archaeal genera, only a few groups are important symbiotic organisms. The alpha-proteobacterial Rhizobia conduct specific symbiosis with legume plants by formation of root nodules.

Several proteobacterial groups exhibit differentiated life cycles: filamentous beta-Proteobacteria like *Sphaerotilus* form bundles of sheathed filaments in eutrophic freshwater habitats. Under unfavorable conditions, flagellated swarmer cells are liberated from the open tip of a sheath. Sheaths of *Leptothrix* are involved in the precipitation of Fe3+ and oxidation of Mn4+. In iron-rich waters, mass developments of iron oxide-covered sheaths form large flocculent layers, which are also involved in the formation of bog iron deposits (Crerar et al., 1979).

Prosthecate bacteria are primarily found among the alpha-proteobacterial subgroup. Members of this group have many different types of extensions (prosthecae) that contain cytoplasm and are bounded by the cell wall. Similar extensions are formed by the genera *Hyphomicrobium* and *Rhodomicrobium*. Daughter cells arise as growing buds from the hyphal tips. In *Caulobacter*, a stalk attaches the cell to a surface, whereas at the distal cell pole, a flagellated swarmer cell is formed. After division, the swarmer attaches to a new place and will form a new stalk at the formerly flagellated pole (Angert, 2005). The myxobacteria have a complex life cycle, which culminates in the production of fruiting bodies containing resting structures called myxospores. The formation of these structures requires the cooperation and coordinated differentiation of large numbers of cells. Aggregates of cells form through a chemotactic response (Zusman et al., 2007).

**Summary**

The brief overview points out that bacteria are taxonomically as well as metabolically more diverse than Archaea. Though the phylogenetic relationships among the bacterial phyla is still a matter of debate, several lines of evolutionary developments can be stated.

Chlorophyll-dependent photosynthesis must have been developed early in bacterial evolution, though some lineages may also have acquired genes of the photosynthetic apparatus by horizontal gene transfer. Fixation of carbon dioxide is not conducted via the Calvin cycle in stem groups, but a common feature in Cyanobacteria and Proteobacteria.

Except from Firmicutes/Actinobacteria and Planctomycetes, bacterial cell envelopes consist of two double-layer membranes. It is a matter of debate, if the Firmicutes are a lineage closest to the root of the bacterial phylogenetic tree or if the membrane got lost as a secondary event. The volume enclosed by both membranes, the periplasmic space, is, especially in Proteobacteria, of significance for cellular transport, DNA transfer, and cellular motility. Intracytoplasmic membranes are widespread in Proteobacteria as well as Cyanobacteria, but have not yet been detected in the basal lineages or in Archaea.

Several major phyla contain symbiotic and pathogenic organisms, especially Firmicutes and Proteobacteria.
(as far as we know, no Archaeon is pathogenic in a strict sense). Especially within the bacterial “crown group,” complex host–symbiont/pathogen interaction has been developed. Mitochondria have very likely an alpha-proteobacterial ancestor (Gray et al., 1999).

Though biofilms, as an elementary type of multicellular organization, are already found in Archaea, some true multicellular stages in bacteria exhibit elaborate cellular differentiations and complex life cycles. In aquatic habitats, the organisms sometimes switch from planktonic to sessile stages. Several groups of bacteria exhibit diverse adaptations to terrestrial habitats: Firmicutes develop extremely durable and heat-resistant endospores, streptomycetes grow and develop like zygomycete or ascomycete fungal mycelia with branched hyphae and conidia-like exosporos. A similar convergence with life cycles to a eukaryote is carried out by myxobacteria. Their life cycle has some striking analogies to cellular slime molds and a eukaryote is carried out by myxobacteria. Their life cycle is initiated at curved regions of the cytoplasmic membrane, then forms both budded and fully detached spherical vesicles. Endocytosis-like protein uptake in the bacterium Gemmata obscuriglobus. Proceedings of the National Academy of Sciences USA, 107, 12883–12888. Madigan M. T., Dunlap, P. V., and Clark, D. P., 2009. Brock Biology of Microorganisms, 12th edn. San Francisco: Pearson/ Benjamin Cummings.

In brief, the development of photosynthesis, the colonization of terrestrial (soil habitats), and the interaction with eukaryotic hosts, are besides the diverse metabolic capabilities, the major achievements of bacteria.

Bibliography


Cross-references
Aerobic Metabolism
Anaerobic Transformation Processes, Microbiology
Bacterioplankton
Cyanobacteria
Fe(II)-Oxidizing Prokaryotes
Fe(III)-Reducing Prokaryotes
Fluorescence In Situ Hybridisation (FISH)
Gallionella
Halobacteria – Halophiles
Histology
Hydrogen
Leptothrix
Metallogenium
Metals, Acquisition by Marine Bacteria
Microbial Communities, Structure, and Function
Nan(n)obacteria
Piezophilic Bacteria
Shewanella
Sulfate-Reducing Bacteria
Symbiosis
Thiomargarita
Thiotrophic Bacteria

BACTERIOPLANKTON

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Synonyms
Aquatic bacteria

Definition
Bacterial component of the plankton that drifts in the water column of both seawater and freshwater ecosystems.

Introduction
The name bacterioplankton comes from the association of the Greek word πλαγκτός “planktós,” meaning “wanderer” or “drifter,” and bacterium, a word coined in the nineteenth century by Christian Gottfried Ehrenberg (Thurman, 1997). In contrast to land, microbes drive the ecology of the aquatic environments both as producers and consumers of fixed carbon. Their colossal biomass vastly outreaches the ones of all other members of the oceanic and freshwaters biota. Considering its size, the Ocean encompasses more bacteria than the count of known stars (estimated to 10^21) in the Universe. Though its role in aquatic ecosystems was for long eluded, the bacterioplankton carries out the largest fraction of the biological activity and occupies a range of ecological niches.

Enumeration and description
Since the beginning of microbiology, counting the number of bacteria that occur in various aquatic environments has been an exciting subject of investigation. Because of the size and the mobility of the bacterioplankton, early microbiologists had difficulties to simply enumerate them with a conventional microscope. With the development of appropriate techniques (e.g., size selection on membrane filters and DNA/RNA specific fluorochromes stainings), as well as advances in microscopy, bacterioplanktonic cells could be observed and counted (Hobbie et al., 1977). This method indicated that the bacterioplankton were on an average ~10^6 cells per milliliter of water. Recent developments in flow cytometry have confirmed and sharpened these counts (Amann et al., 1990). In the water column, the bacterioplankton is also exposed to grazing activity by larger protists and viral infections, but irrespective to variations in primary productivity, these two causes of mortality seem to maintain a relatively constant abundance.

Though it is not always mentioned in the current literature, a clear distinction should be made between the fraction of the bacterioplankton that is attached to drifting particles of organic matters (also called “marine snow” in the marine environment) and the fraction that is free-living in the water column. A common trait for free-living bacterioplankton is their small size, or small cell volume, ranging from 0.1 to 0.22 μm^3, which is about ten times lower than that of the most attached bacterioplankton in aquatic habitats or in soils (Fenchel et al., 1998) and the most cultured cells.

Bacterioplankton biological activity
Based on both direct observations and indirect measurements of bacterial growth rates in whole seawater (e.g., frequency of dividing cells (Hagström et al., 1979), radioactive thymidine or leucine incorporation (Fuhrman and Azam, 1982; Chin-Leo and Kirchman, 1988)) as well as when bacterial grazers (protozans) were removed (Vacelet, 1972), generation times (i.e., the time required to double population) of most bacterioplanktons range from 0.049 to 0.080 h^-1.

Because a significant proportion of the bacterioplankton (99%) of the total counted cells fail to grow on nutrient-rich agar plates, it has been suggested that more than 99% of the cells were either starving cells, dormant (Stevenson and Schmidt, 1998) or even non-viable cells (Kjelleberg et al., 1987). Different in situ measurements of amino acids or glucose uptake by microautoradiography (Hoppe, 1976) indicate that from 5% to >60% of the cells are metabolically active in different aquatic environments (Tabor and Neihof, 1984; Fuhrman and Ferguson, 1986; Smith and del Giorgio, 2003). Whether or not most bacteria in aquatic environment are dormant remains controversial, most scientists will agree however that the bacterial community, as a whole and over
long period of time, plays a paramount role in global biochemical processes.

The blue-green algae, or Cyanobacteria, are the “plants” of the bacterioplankton and carry out photosynthesis, which supports the entire ocean food web. In the open ocean, these phototrophic bacteria are responsible for about 80% of the total oceanic primary production, which represents about half of the planet’s primary production (ranging from 35 to 65 Gt of carbon per year) (del Giorgio and Duarte, 2002). Moreover, a new type of phototrophy has recently been discovered among marine bacterioplankton that carry bacteriorhodopsin molecules, which scavenge light to create an energetic gradient of protons (Beja et al., 2001). Since ~13% of the bacterioplankton cells in the photic zone may carry out proteorhodopsin-based phototrophy (Sabelis et al., 2005), this metabolism surely has a global significance and may be the foremost future investigation field in aquatic microbiology.

However in numbers, marine bacterioplankton are considered mostly heterotrophs, or more precisely saprotrophs, and obtain energy by consuming dissolved organic matter (DOM) released by larger aquatic animals (Smith et al., 1992) or by phytoplankton exudation (Larsson and Hågström, 1979). This activity, known as the “microbial loop” (Azam et al., 1983), is crucial to the aquatic trophic foodwebs because essential inorganic nutrients are recycled through bacterial metabolism into the water column.

Heterotrophic and phototrophic bacterioplankton are continuously grazed by protozoans (i.e., mostly flagellates and ciliates), which are later consumed by larger zooplankton, which are subsequently consumed by fishes. Phages (i.e., viruses infecting bacteria) also affect DOM turnover by lysing cells, which releases organic matter available to surrounding bacterioplankton cells (Proctor and Fuhrman, 1990).

A large fraction of the carbon flow in the aquatic ecosystem is processed by heterotrophic bacterioplankton that respire particulate and dissolved organic carbon (DOC) (del Giorgio and Duarte, 2002). Especially with the rapid increase in global atmospheric CO2 due to modern human activities, this bacterial process is recognized as a key component in global climate regulation (Falkowski et al., 2000).

### Bacterioplankton diversity and global distribution

While growth on agar plates, microscopic observations were intensively used to delineate several types of bacteria, their capacity of differentiation showed quickly to be too restrictive and new methods were required to distinguish between two species with similar cell and colony morphologies. Moreover, a general concern grew among microbiologists when they realized that many bacteria counted under the microscope could not be cultivated on agar plate or in liquid media. For instance, marine microbiologists estimated that only 1% of the cells counted under the microscope formed colonies on agar plates, and named this issue the “Great plate count anomaly” (Jannasch and Jones, 1959; Kogure et al., 1979).

In the early 1980s, a breakthrough in bacterial taxonomy was achieved by Woese et al., who utilized ribosomal ribonucleic acids (rRNA) to catalogue related bacterial species (Fox et al., 1980; Woese et al., 1985, 1987). Applying this approach to aquatic environmental samples often recovers members from about 18 bacterial clades, mostly represented within the alpha-Proteobacteria and gamma-Proteobacteria phyla. Moreover, members from the Cyanobacteria, Actinobacteria, Bacteroidetes, Planctobacteria, Fibrobacter, Chloroflexi and beta-Proteobacteria are often retrieved from environmental rRNA genes clone libraries. Interestingly, though some members of the global bacterioplankton community (e.g., the so-called SAR11 clade) are recurrently found in environmental samples, and therefore show a ubiquitous distribution, endemism of rRNA genes is the most common trait of the bacterioplankton, and their diversity follows a latitudinal gradient as observed in the other macro-organisms (Pommier et al., 2007).

The extensive use of the SSU rRNA as phylogenetic marker has actually reached the level where variations in the bacterioplankton composition can be linked to observed changes both in natural (Acinas et al., 1999; Cottrell and Kirchman, 2000; Hollibaugh et al., 2002) and reconstructed environments (i.e., meso- or micro-cosms) (Riemann et al., 2000). The current view of marine bacterioplankton phylogeny is however being questioned together with the relevance of the SSU rRNA gene to delineate bacterial species.

### Conclusions

Though it was for long considered a mass of dormant cells, bacterioplankton has proven to be a key actor in aquatic ecosystems throughout the world. As for many ecosystems, the bacterioplankton drives the whole biological activity of the water foodweb and occupies a huge variety of niches throughout the water column. Further research aims to show the importance of local bacterioplanktonic processes involved in global biogeochemistry.

### Summary

The bacterial component of the plankton that drifts in the water column of all aquatic ecosystems is named Bacterioplankton. Though it was for long considered solely dormant, this part of the biota is now recognized as a determinant actor on the global recycling of nutrients and at the basis of all aquatic foodwebs. With a million cells per millimeter of water, they represent the most successful organisms, as well as the most diverse biome on our planet. Modern approaches combining molecular biology to epi-fluorescence microscopy have sharpened our knowledge on the abundances, the diversity and the ecology of the bacterioplankton.
Bibliography


BANDED IRON FORMATIONS

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Synonyms
BIFs

Definition
Sedimentary deposits of alternating iron-rich (~20–40% Fe) and iron-poor, siliceous (~40–50% SiO2) mineral layers that primarily precipitated throughout much of the late Archean (2.7–2.5 Ga) and Paleoproterozoic (2.5–1.8 Ga), but then remerged in the Neoproterozoic (~0.8 Ga).

BIF characterization, mineralogy, and distribution
BIFs are globally distributed, with some of the larger formations being those of the Hamersley range in Australia and the Transvaal Supergroup in South Africa (Figures 1 and 2). Other major BIFs include Krivoy Rog Supergroup, Ukraine (2.2 Ga); Labrador Trough, Canada; Lake Superior Region, USA; Gunflint and Biwabik, North America (2.2–2.0 Ga), Carajás Formation (2.6 Ga) and Urucum Region, Brazil (0.8 Ga).

The mineralogy of the least metamorphosed BIFs consists of chert, magnetite, hematite, carbonates ( siderite and dolomite–ankerite), greenalite, stilpnomelane, and riebeckite (Klein, 2005): the presence of both ferric and ferrous minerals gives BIF an average oxidation state of Fe2.3+ (Klein and Beukes, 1992). It is generally agreed that none of the minerals in BIF are primary, but that instead, the minerals reflect both diagenetic and metamorphic overprinting. For instance, the primary iron minerals were most likely ferric hydroxide (Fe(OH)3), greenalite ([Fe3+Si2O5(OH)4]), and siderite (FeCO3) (Klein, 2005).

Based on their depositional environment, BIFs have been categorized as Algoma or Superior type. Algoma type BIFs are generally small in lateral extent and display a volcanic association (Gross, 1965, 1980; Huston and Logan, 2004), while Superior type BIFs are found in shelf environments, are vast, and are without a dominant volcanic influence. The “Superior” type BIFs, including those in the Hamersley Group, Western Australia and the Transvaal Supergroup, South Africa, are hundreds of meters thick, over 105 km2 in areal extent, and contain >1013 t of iron (Trendall, 2002). They are characteristically laminated, with alternating Fe-rich and Si-rich layers (Figures 2 and 3). Banding can be observed on a wide range of scales, from coarse macrobands (meters in thickness) to mesobands (centimeter-thick units) to millimeter and sub-millimeter layers (Trendall and Blockely, 1970). Among the latter is the wide variety of varve-like repetitive laminae, known as microbands.

BIFs have also been categorized according to their Fe oxide composition, as hematite or magnetite BIFs (Figure 3) (James, 1954). The former refers to BIFs facies consisting principally or entirely of interbedded chert and (primary) hematite, which are in some places oolitic (e.g., the Gunflint range). The latter describes Fe oxide layers of magnetite that may grade imperceptibly into silicate rock in some areas, be mixed with carbonate, or sprinkled with hematite or chert lenses (James, 1954).

A third categorization of BIF is based on the granular versus nongranular nature of the silica layers. Granules, seen in the 2.0 Gyr Gunflint BIF are approximately 0.5 mm in diameter, irregular, and can be within a chert matrix. They can have a greenalite or magnetite core. These granules, however, do not show the concentric layering seen in hematitic oolites. Non-granular BIFs are typically thin-bedded or laminated and may chiefly be composed of minnesotaite with magnetite and carbonate in some layers (James, 1954).

Banded iron formations and the study of the early Earth
The height of BIF deposition was 2.7–2.4 Ga (Trendall, 2002; Klein, 2005), a timeframe which overlaps with the currently estimated age for the rise of oxygen on the Earth ~2.5 Ga (Anbar et al., 2007). As it is agreed that the
Banded Iron Formations, Figure 1 Appearance of BIFs in geological time and marked as Algoma and Superior types.

Banded Iron Formations, Figure 2 BIF outcrop, Gamohaan Hill, South Africa (a), and White Mfolozi Gorge, Kwazulu Natal, South Africa, Jasper and Magnetite, 3.0 billion-years-old (b). Pictures by Nicole Posth.
source of iron for these deposits was Fe(II), the Fe(III) minerals in BIF indicate that an oxidizing mechanism was present. Once oxygen began its rise due to the advent of cyanobacteria, it is plausible that an oxic mechanism formed BIF, perhaps even in pockets or oxygen oases. In the case of BIF dated prior to 2.7 Ga, however, an anoxic oxidizing mechanism is required to explain the formation of these vast deposits. The latest BIFs (0.8 Ga), which seem to represent a revival later in the geologic record, may be archives of major climate changes related to the Snowball Earth (Kirschvink, 1992; Kopp et al., 2005). Due to the intricacies of the depositional mechanisms, BIFs have been studied for decades regarding their potential as archives of the early Earth environment, but may also prove to be a detailed record of the early Earth biosphere.

**BIF deposition**

**Depositional setting**

Early studies of BIF mineral association (James, 1954), as well as comparative BIF studies (Trendall, 1968; Beukes, 1973; Gole and Klein, 1981; Simonson, 1985; to name a few) postulated that these sedimentary deposits likely formed on the margins of cratons in semi-restricted, marine basins with minimal wave action (for a comprehensive review, Klein, 2005). They also laid a foundation for both theoretical and experimental geochemical modeling based on atmospheric (oxygen and carbon dioxide) and oceanic (iron and silica) concentrations, which are largely linked to mineralogical, petrologic, and isotopic analyses and have been invoked to describe the BIF deposition mechanism (Drever, 1974; Cairns-Smith, 1978; Han, 1978; Brateman et al., 1983; Baur et al., 1985; Francois, 1986; Garrels, 1987; Morris, 1993).

There is ample evidence that the dominant source of Fe (II) into the Archean ocean was hydrothermal (e.g., Jacobsen and Pimentel-Klose, 1988; Bau and Möller, 1993), but it has also recently been suggested that a significant fraction of the Fe in Archean seawater came from the continents (Alexander et al., 2008). The proximity of the Fe(II) source to the site of deposition, however, is still unclear. The estimated Fe(II) concentration for these basins ranges from 0.02 to 0.5 mM (Holland, 1973; Morris, 1993). This Fe(II) may have been delivered from the deep ocean to the outer continental shelf by upwelling currents from a mid-ocean ridge system (e.g., Holland, 1973). Accordingly, BIFs would sediment from below the wave base (free of wave- and storm-induced currents) onto partially submerged platforms of the continental shelves. Alternatively, Fe(II) could have been directly supplied from plumes in a seamount type system (Isley, 1995), which would curtail the difficulties introduced by the high upwelling rates required in the continental shelf model. After deposition, these sediments would undergo diagenesis, with some BIF sediment possibly even being redistributed to deeper waters via turbidity currents (Krapež et al., 2003).

There is significant evidence that Archean oceans also contained elevated concentrations of dissolved silica, possibly even to saturation with respect to amorphous silica (~2.20 mM; Siever, 1992; Maliva et al., 2005). Under such silica-rich conditions, the direct precipitation of amorphous silica could have taken place directly from the water column onto the sea basin floor (Krapež et al., 2003). Moreover, silica could have reacted with dissolved Fe(II) and precipitated as ferrous or ferric-silicates (Konhauser et al., 2007).

**Theories of Precambrian BIF deposition**

BIF mineralogy dictates that some form of Fe(II) oxidation was necessary for their formation, yet which mechanism(s) dominated is still highly uncertain (Figure 4). While dissolved Fe(II) readily oxidizes in the presence of oxygen at circumneutral pH, to the rise of oxygen on Earth occurred approximately 2.5 Ga (Kopp et al., 2005; Anbar et al., 2007). An alternative, O$_2$-independent
mechanism is therefore necessary to explain the deposition of the earliest BIF. Both abiotic and biotic mechanisms of BIF deposition have been put forth. The potential role of microorganisms in BIF was first proposed 4 decades ago (Cloud, 1968) and in part addressed the difficulties in explaining these deposits based on geochemical processes alone (Brown et al., 1995). Yet, BIFs contain very little or no organic carbon (0.5% [w/w], Beukes and Klein, 1992) which makes it difficult to constrain the responsible microbial process simply based on geochemical and petrographic studies. Any fossil information about the presence of life in the Archean has therefore been garnered from contemporaneous formations. For this reason, isotopic, biomarker, and ecophysiological studies with modern analogues have been implemented in order to supplement the geochemical BIF data and test the plausibility of the depositional models and the role of microorganisms.

### Oxygenic photosynthesis model

The traditional model of BIF deposition was first postulated by Cloud (1968). Herein, Fe(II) is abiotically oxidized by photosynthetically produced O$_2$, allowing for the indirect biogenic precipitation of ferric hydroxide as shown in Equation 1 (Figure 4a). Under an anoxic atmosphere, this O$_2$ could have been confined to local “oxygen oases” associated with cyanobacterial blooms in coastal settings (Cloud, 1965, 1973). Cloud (1965, 1973) further proposed that such primitive O$_2$-producing photosynthetic bacteria, which lacked suitably advanced oxygen-mediating enzymes, required ferrous iron to detoxify oxygen. If so, these microorganisms would have flourished specifically when Fe(II) and nutrients were made episodically available. Once oxygen was present, aerobic Fe(II) oxidizers could also have contributed to biogenic Fe minerals precipitation (Holm, 1989).

$$4\text{Fe}^{2+} + \text{O}_2 + 4\text{H}_2\text{O} \rightarrow 2\text{Fe}_2\text{O}_3 + 8\text{H}^+ \quad (1)$$

The oldest evidence to support the presence of cyanobacteria during the time of BIF deposition came from the 3.45 billion-year-old Apex cherts, Warrawoona, Australia (Schopf, 1993). These findings, however, have since been challenged by Brasier et al. (2002) as being secondary artefacts formed by Fischer–Tropsch type reactions associated with seafloor hydrothermal systems. In fact, Buick (1988) maintained that the chert matrix of the “oldest microfossils” is a secondary hydrothermal deposit of younger age that cross-cuts the primary bedding. The use of kerogen as a marker of past life in these formations has also been challenged by experiments demonstrating the abiotic pathways of kerogen synthesis; simple organic hydrocarbons of abiological origins (e.g., formaldehyde) readily condense onto silica–carbonate inorganic filaments, and subsequently polymerize under gentle heating to yield kerogenous products (García-Ruiz et al., 2003). In light of these studies, stronger evidence for the presence of cyanobacteria comes from the stromatolitic assemblages of the 2.7 Gyr Tumbiana Formation, Western Australia. The presence of oxygenic photosynthesis in these formations is based on their likely habitat in sulfate-deficient evaporative lakes (Buick, 1992). This is further supported by the earliest undisputed fossil assemblages of the 2.6 Gyr Campbell Group, South Africa, that include cyanobacterial genera which helped form stromatolithic reefs in shallow subtidal to intertidal settings (Altermann and Schopf, 1995).

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**Banded Iron Formations, Figure 4** Models of banded iron formation deposition: The traditional model of BIF deposition involves the production of oxygen by cyanobacteria, which is released into the water column to chemically react with hydrothermal dissolved Fe(II) (a). The two proposed mechanisms of deposition in an anoxic ocean water column are the abiotic Fe(II) photooxidation by UV light, which has recently been discounted (Konhauser et al., 2007) (b), and direct microbial Fe(II) oxidation via anoxygenic Fe(II)-oxidizing phototrophy (c).
Biomarkers, organic compounds derived from more complex precursors which still resemble the original biological molecule after a long burial history, may be used to identify a specific group of organisms in ancient rock. For example, 2α-methylhopanes are derivatives of prominent lipids in cyanobacteria (methylbacteriohopanepolyols). Abundant amounts were extracted from bitumens of the 2.6 Gyr Marra Mamba Iron Formation and the 2.5 Gyr Mt. McRae Shale of the Hamersley Group, Western Australia (Brocks et al., 1999; Summons et al., 1999). As these compounds were first solely found in cyanobacteria, their presence was first interpreted as diagnostic of oxygenic photosynthesis, however, they have recently been detected in anoxygenic phototrophs (Rashby et al., 2007), which weakens the use of this biomarker as conclusive proof of oxygenic photosynthesis in ancient rock.

Specific steranes are more indicative of this metabolism. These are 28- to 30-carbon isomers and are unique alteration products of sterols known from extant eukaryotic cell membranes. The only prokaryotes known to synthesize sterols have biosynthetic pathways leading to different structural isomers. Importantly, O₂ is required for the biosynthesis of sterols, hence, their extraction from Archean rock suggests that at least some dissolved oxygen (~0.002 ml O₂ L⁻¹) was present at the time of their production (Runnegar, 1991). To date, there is no experimental evidence to support the alternative theory that steranes may have been produced via an anaerobic pathway utilizing hydrogen peroxide or organic peroxides (Fischer and Pearson, 2007), which make them a specific biomarker of oxygenic photosynthesis. They are found, for example, in the 2.7 billion-years-old shales of the Jeerinah Formation, Hamersley Group (Brocks et al., 1999). Their presence in these shales is particularly intriguing as other redox-sensitive proxies suggest that oxygen arose much later in earth history (Rye and Holland, 1998; Rasmussen and Buick, 1999; Farquhar et al., 2000). Only the presence of localized oxygen oases can explain these conflicting results.

UV photooxidation model
Prior to the rise of atmospheric oxygen and the development of a protective ozone layer, the Earth’s surface was also subjected to high levels of ultraviolet radiation. This led to a theory of BIF deposition in which UV radiation drives an abiotic mechanism of Fe²⁺ oxidation and Fe (III) mineral precipitation. Specifically, under conditions of anoxia and high dissolved Fe(II) concentrations, dissolved ferrous iron species, such as Fe²⁺ or Fe(OH)²⁺, can absorb radiation in the 200–400 nm range. Dissolved ferric iron is formed (Equation 2), which hydrolyzes to form ferric hydroxide at circumneutral pH (Cairns-Smith, 1978; Braterman et al., 1983) (Figure 4b).

\[
2\text{Fe}^{2+} + 2\text{H}^+ + \text{hv} \rightarrow 2\text{Fe}^{3+} + \text{H}_2 \uparrow \quad (2)
\]

These experiments, however, were recently carried out in complex solution simulating disequilibrium Precambrian ocean water chemistry in which Fe(II)-rich hydrothermal waters react with ambient Si-saturated seawater also containing high HCO₃⁻ concentrations (Konhauser et al., 2007). It was shown that the oxidation effects of either UVA or UVC were negligible in fluids with high dissolved Fe(II), Si, and HCO₃⁻, compared to the precipitation of ferrous-iron-silicates and ferrous carbonate. This suggests that under such conditions Fe²⁺ would have been largely deposited as Fe(II) minerals instead of being oxidized by UV radiation. The fact that Fe (III) minerals are present in BIF therefore suggests that in the absence of O₂ the alternative anoxic depositional mechanism, Fe(II) oxidation by anoxygenic phototrophs, drove Fe oxide deposition.

Anoxygenic Fe(II)-oxidizing phototroph model
Anoxygenic photosynthesis was first suggested as a BIF deposition mechanism by Garrels et al. (1973) and Hartman (1984). They proposed that light, not O₂, may have coupled the carbon and iron cycles via photosynthesis that used Fe(II) rather than H₂O as an electron donor and produced Fe(III) rather than O₂ (Figure 2c). It was only 2 decades later that the plausibility of this theory was validated by the discovery of a modern Fe(II)-oxidizing phototroph (Widdel et al., 1993). Seven extant strains of anoxygenic Fe(II)-oxidizing phototrophs are now known to use light energy to catalyze the oxidation of Fe(II) and the corresponding reduction of CO₂, which yields biomass and ferric hydroxide as shown in Equation 3.

\[
4\text{Fe}^{2+} + \text{HCO}_3^- + 10\text{H}_2\text{O} + \text{hv} \rightarrow 4\text{Fe}^{3+} + (\text{CH}_2\text{O}) + 7\text{H}^+ 
\]

In the context of BIFs, anoxygenic Fe(II)-oxidizing photosynthesis (photoferrotrophy) is the only direct biological process that can precipitate Fe(III) minerals in the absence of oxygen.

While no direct evidence exists for the presence of these strains on the Archean Earth, various lines of evidence support the plausibility of anoxygenic phototrophs in BIF deposition. Ecophysiological studies performed with modern anoxygenic Fe(II)- oxidizing phototrophs test the plausibility of this deposition mechanism. It was shown that these strains inhabit a wide range of growth environments; being globally distributed and present in fresh and marine waters (Ehrenreich and Widdel, 1994; Heising et al., 1999; Straub et al., 1999; Croal et al., 2004; Jiao et al., 2005). These organisms do not have a unique form, all being rod-shaped (Figure 5), yet morphological variety is apparent, one example being the presence of vacuoles in one strain, *Thiodictyon* sp. strain F4 (Croal et al., 2004). These strains also utilize a variety of substrates, such as acetate, FeS and H₂, which suggests that they are versatile enough to be an integral group in both
ancient and modern environments. Modeling of the photosynthetic Fe(II) oxidation rates determined in these studies suggest that such microorganisms could have accounted for all of the Fe(III) initially deposited in primary BIF sediment (Kappler et al., 2005). Importantly, while the Fe(II) oxidation rate of these strains is dependent on light intensity, anoxygenic phototrophs can oxidize Fe(II) in light regimes befitting the photic zone of ocean water (of a few hundred meters depth) (Kappler et al., 2005). Also, Fe(II) oxidation is not hindered by high concentrations of dissolved silica (tested at 2 mM Archean ocean concentration) (Siever, 1992; Konhauser et al., 2007).

While modern studies show that cyanobacteria are indeed important in stromatolite building through the trapping and binding of carbonate grains in their filaments and extracellular polymers, several anaerobic pathways, such as sulfate reduction (e.g., Visscher et al., 1998; Paerl et al., 2001), are also integral in carbonate cementation and localized alkalinity generation. Indeed, sequence analysis of small subunit rRNA genes amplified with PCR from genomic DNA showed that anoxygenic phototrophs represent a considerable fraction of biomass in the modern stromatolite communities of Hamelin Pool, Shark Bay, Australia (Papineau et al., 2005). Recently, Bosak et al. (2007) experimentally demonstrated that the anoxygenic phototroph, *Rhodopseudomonas palustris*, stimulates the precipitation of calcite in saturated solutions and builds stromatolite-like structures.

While 2α-methylhopanes were first considered diagnostic of oxygenic photosynthesis, they may have a structural function and are not related to cell metabolism. Indeed, several facultative and obligate anaerobes possess the genes for hopanoid biosynthesis, and *Geobacter sulfurreducens* produces a wide variety of complex hopanoids structurally related to 2α-methylhopane under strictly anoxic conditions in pure culture (Härtner et al., 2005; Fischer and Pearson, 2007). Moreover, it was recently demonstrated that an anoxygenic Fe(II)-oxidizing phototroph, *Rhodopseudomonas palustris*, generates substantial quantities of 2-methylhopanoids in the absence of oxygen (Rashby et al., 2007), which renders their presence in ancient rocks alone as an unsatisfactory marker for a specific metabolism.

Pigment biomarkers may offer further evidence of a microbial role in BIF. Okenane, the fossil hydrocarbon biomarker of the carotenoid pigment precursor, okenone, was recently found in the 1.64 billion-year-old Barney Creek Formation in northern Australia, a marine, sub-wavebase base succession. This pigment is exclusively found in purple sulfur bacteria and in recent sediments under euxinic conditions (Brocks, 2005).

**Isotopic evidence and the BIF depositional mechanism**

The Fe isotope composition of iron minerals in BIFs is markedly different from the homogenous values seen in
igneous rocks and many modern marine sediments ($\delta^{56}$Fe 0.00 ± 0.05%). Specifically, the hematite, magnetite, Fe-carbonate, and pyrite Fe isotope compositions reflect the various processes contributing to BIF formation; equilibrium fractionation of minerals, a variation in the fluid isotope composition from which the minerals precipitated, as well as microbial processes (Johnson et al., 2003; Johnson and Beard, 2005).

In an effort to elucidate the role of microorganisms in the formation of BIF, the Fe isotope fractionation carried out by Fe metabolizing microorganisms was analyzed in controlled laboratory studies of both pure and enrichment cultures. These show that ferric hydroxides are enriched in the heavy isotope relative to Fe(II) for anoxygenic Fe(II)-oxidizing phototrophy (1.5 ± 0.2‰) (Croal et al., 2004), microbially catalyzed aerobic Fe(II) oxidation at low pH (Balci et al., 2006), aerobic chemical oxidation (Bullen et al., 2001), and UV Fe(II) oxidation (Straton et al., 2006), suggesting that all major processes produce similar Fe isotope fractionations.

The isotopic fractionations reported from laboratory experiments correlate well in part with the values recovered from Archean to early Proterozoic banded rocks. The isotopic fractionations reported from laboratory experiments correlate well in part with the values recovered from Archean to early Proterozoic banded rocks of the Transvaal Supergroup, South Africa. $^{56}$Fe/$^{54}$Fe values in hematite are as high as +0.75 to +1.0‰ (Johnson et al., 2003) when compared to the Fe from hydrothermal vents (±0‰). As UV photooxidation was recently ruled out as a significant BIF deposition mechanism (Konhauser et al., 2007), these positive to near-zero $\delta^{56}$Fe values are even consistent with (although not proof of) phototrophic Fe(II) oxidation in the early Archean oceans. However, the similarity in values from anoxicogenic phototrophs with those of Fe(III) oxides formed by chemical oxidation and microbially catalyzed aerobic Fe(II) oxidation make it difficult to distinguish the key biotic and abiotic processes (Bullen et al., 2001; Balci et al., 2006). Iron isotope fractionation during oxidation itself is obviously independent of the oxidation mechanism (chemical or biological) because of the very rapid isotopic exchange between aqueous Fe$^{2+}$ and Fe$^{3+}$, allowing isotopic equilibrium to be attained between reduced and oxidized aqueous species. Moreover, in these ancient formations interpretation of the isotopic signature is complicated by the complexity of the depositional environments, the diagenetic processes which have altered the Fe mineralogy, including the infiltration of external fluids during diagenesis and/or low temperature metamorphism which may have erased the primary isotope record (Hoeß, 1997; Johnson et al., 2003). Indeed, the large variation of $\delta^{56}$Fe (−2.5 to 1.0‰ relative to the bulk Earth) in late Archean and early Paleoproterozoic BIFs from the Transvaal Supergroup, South Africa incorporates the entire range of values measured on the Earth.

The carbon isotopic record of BIFs is constrained by the low occurrence of organic carbon (0.5% [w/w], Beukes and Klein, 1992) in these structures. Carbon isotope signatures observed in contemporaneous formations may offer information about organisms which were present at the time of deposition, but whose record was erased, for example, by postdeposition diagenetic processes. In such ancient formations, negative carbon isotope signatures are often interpreted as an indicator of life. Organisms transform inorganic carbon (e.g., CO$_2$ or HCO$_3^-$) via autotrophic pathways into organic carbon, preferentially incorporating the lighter isotope, $^{12}$C, into the organic phase and producing residual CO$_2$ enriched in the heavier isotope, $^{13}$C. Cyanobacteria, for example, display a $\delta^{13}$C range of −4 to −35‰ (Schiulowski, 2000). For example, early photosynthesis has been linked to mat-forming communities in microfossiliferous units within Archean formations from Western Australia and South Africa (e.g., Altermann and Kazmierczak, 2003; Tice and Lowe, 2004) by carbon isotopic values ranging from −20‰ to −35‰, as well as the −31.0‰ ± 4.7 values from organic carbon in early Proterozoic cherts (Strauss et al., 1992).

Key to this approach is the consideration of overlapping degrees of fractionation produced by various carbon fixation pathways (reductive acetate-CoA, reductive citric acid, Calvin cycle, and hydroxypropionate). This makes it impossible to determine whether these putative biological residues were remnants of chemolithoautotrophs, evidence for early photosynthesis, or abiological C-isotopic fractionations, such as those associated with Fischer–Tropsch type processes ($\Delta^{13}$C between −50‰ and −100‰).

**Theories of iron and silica mineral layering**

One of the characteristic features of BIF are the alternating Fe-rich (hematite, magnetite, and siderite) and the silicate/carbonate (chert, jasper, dolomite, and ankerite) layers that form bands varying in thickness from the microscale (μm in thickness) to meter-thick units (Trendall, 1968; Klein and Beukes, 1992; Klein, 2005). Some deposits, such as in the Dales Gorge Member, Hamersley Group, Western Australia, show laterally contiguous layers up to a 100 km in distance. This suggests that some unifying trigger of iron and silicate mineral precipitation exists over large areas with some regularity, most likely one which largely subdues the precipitation of one mineral while stimulating that of the other. Furthermore, given the range of band thickness from the micro- to macro-scale, as well as the complex mineralogy, it is most likely that layering in BIF is determined by a multilayered mechanism; one producing primary layering and a second mechanism acting post deposition via diagenesis.

Following this observation, many models attribute the primary genesis of micro and mesobands to seasonal stratification, or to yearly or decadal climatic cycles. Episodic hydrothermal pulsation and upwelling of Fe(II)-rich waters into depositional basins already saturated with dissolved silica have been invoked to explain these bands; silification is driven by seasonal evaporation of basin waters (Holland, 1973; Garrels, 1987; Jacobsen and Pimentel-Klose, 1988; Siever, 1992). This mechanism, however, does not explain the laterally contiguous finescale banding, nor does it resolve why the iron and silica
do not co-precipitate but rather form distinct mesobands (Trendall, 1968; Garrels, 1987; Morris, 1993). Furthermore, in light of increasing evidence for an active microbial component in the Fe cycle (Johnson et al., 2003), the potential importance of microorganisms in BIF deposition models has been suggested. Indeed, a mechanism of alternating Fe and silicate mineral layer deposition with a microbial driver triggered by temperature has been recently presented (Posth et al., 2008).

Diagenesis in BIFs: oxidation and the reduction of Fe(III) minerals
The average oxidation state of Fe$^{2.4+}$ can be described by the simultaneous deposition of Fe(II) and Fe(III). Yet, this mixed oxidation state can also be explained by partial biotic or abiogenetic re-reduction of precipitated Fe(III) to Fe(II). Experimental studies testing such diagenetic mineral transformations in the presence and in the absence of biomass have not yet been reported. Nonetheless, the presence of magnetite as (1) disseminated grains within but obscuring sedimentary laminae, (2) laminated beds that clearly truncate sedimentary layering, (3) layer-discordant veins, and (4) cleavage fills, suggests such a secondary origin (Ewers and Morris, 1981; Krapež et al., 2003).

The diagenetic or microbial modification of Fe(III) after sedimentation to produce siderite (FeCO$_3$) and magnetite (Fe$_3$O$_4$) requires a reductant. Even if a surface water oxic zone was generated by cyanobacterial activity (Summons et al., 1999), deep waters remained anoxic (e.g., Canfield, 1998). The dominant anaerobic respiratory processes needed to generate the amount of magnetite as reported in BIF-type macrobands formed diagenetically through biological Fe(III) reduction, i.e., the magnetite is not primary. Based on a predicted rate of Fe(III) deposition annually (1 mm year$^{-1}$), they then quantified the electrons that were needed to generate the amount of magnetite as reported in BIF (one third of Fe minerals; Morris, 1993). The scarcity of O$_2$ limited both nitrate and sulfate. This is supported by both negligible sulfur isotopic fractionations between late Archean and early Paleoproterozoic sulfide and sulfate minerals (Strauss, 2003), as well as the absence of pyrite in BIF, except in association with interlayered shaley units (Ewers and Morris, 1981). The significance of manganese pathways is also refuted by the low relative concentrations of Mn(II) released in hydrothermal effluent, as well as the lack of phototrophic or nitrate-independent Mn(II) oxidizing bacteria in modern environments. In contrast, abundant ferric hydroxide deposited as BIF may point to the importance of a microbial process which coupled the oxidation of organic carbon, dead planktonic bacteria, to the reduction of ferric iron producing such reduced iron mineral phases.

Evidence of BIF diagenesis via ancient microbial Fe(III) reduction comes from Fe isotopic ratios in Fe-bearing minerals from Archean sedimentary rocks that closely mimic those observed during modern dissimilatory Fe(III) reduction. For instance, analyses of carbon- and magnetite-rich rocks, from the 2.9 Gyr Rietkui Formation, Witwatersrand Supergroup, South Africa, revealed $\delta^{56}$Fe values as low as $-2.3\%$ (Yamaguchi et al., 2005). These negative fractionations are very similar to what might be expected from multiple stages of Fe(III) reduction (Johnson et al., 2004), where each single-step bacterial Fe(III) reduction leads to isotopically light ($\delta^{56}$Fe < 1.2\%) aqueous Fe(II) relative to the initial ferric hydroxide substrate (Icopini et al., 2004). The importance of Fe(III) reduction as a means to explain the iron fractionations has, however, been challenged by Rouxel et al. (2005). They suggested that highly variable, but negative values in pyrite from black shales (0.5 to $-3.5\%$) between 2.8 and 2.3 Ga more likely reflect the initial deposition of Fe oxides (e.g., BIFs) which preferentially removed isotopically heavy $^{56}$Fe, driving the ocean waters to the negative $\delta^{56}$Fe values recorded in pyrite. This interpretation is consistent with the notion that partial biological and abiological processes oxidized the dissolved Fe(II) brought into the shallow waters, but is problematic in light of recent studies showing significant isotopic variations for minerals within close proximity and thus, time of deposition (Johnson et al., 2008). This variation is perhaps most easily explained if the Fe isotopes reflect diagenetic pathways and not the bulk seawater (Johnson et al., 2008).

BIF diagenesis and the Archean marine Fe cycle
BIFs have recently been studied to develop a model of the Archean marine Fe cycle. In doing so, Konhauser et al. (2005) made two assumptions. First, they assumed that the bulk of the Fe(II) component in Fe-rich BIF-type macrobands formed diagenetically through biological Fe(III) reduction, i.e., the magnetite is not primary. Based on a predicted rate of Fe(III) deposition annually (1 mm year$^{-1}$), they then quantified the electrons that were needed to generate the amount of magnetite as reported in BIF (one third of Fe minerals; Morris, 1993). Second, they quantified the amount of photosynthetic Fe(II)-oxidizer biomass that may have been generated in the photic zone of the water column (based on Kappler et al., 2005), in order to estimate the amount of Fe recycled prior to burial. The results demonstrated that under ideal growth conditions, as much as 70% of the biologically formed Fe(III) could have been recycled back into the water column via fermentation and organic carbon oxidation coupled to microbial Fe(III) reduction. It was also suggested that some of the biomass may have been ultimately consumed via methanogenesis, i.e., coupling the oxidation of acetate or H$_2$ to methane formation. The hypothesis is to some extent corroborated with the analyses of kerogens (extracted from rocks 2.8 and 2.6 Gyr) with highly negative $\delta^{13}$C signatures (between $-40\%$ and $-60\%$) that possibly formed as the result of methanogenic $^{12}$C-rich gas production, the incorporation of the methane into the biomass of methanotrophic bacteria and inevitably the preservation of $^{12}$C-enriched organic matter (Hayes, 1983).

Summary
Banded iron formations (BIFs) have been studied for decades in regard to their importance as an economically
viable source of iron ore, but their significance also extends to their potential as archives of the early Earth environment.

In spite of this effort, the mechanism of their deposition and in particular whether microorganisms drove the precipitation of BIF minerals, is still uncertain. Increasing evidence of a predominantly anoxic Earth until ~2.5–2.4 Ga forces the investigation of O₂-independent mechanisms for BIF deposition. For this reason, recent studies have explored the long-standing proposition that Archean BIFs may have been formed, and diagenetically modified, by anaerobic microbial metabolisms.

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**Cross-references**

Algae (Eukaryotic)

Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification

Biomarkers (Molecular Fossils)

Biosignatures in Rocks

Critical Intervals in Earth History

Cyanobacteria

Fe(II)-Oxidizing Prokaryotes

Fe(II)-Reducing Prokaryotes

Gallionella

Geobacter

Hydrothermal Environments, Marine

Isotope Fractionation (Metal)

Isotopes and Geobiology
Microbialites, Stromatolites, and Thrombolites
Nanocrystals, Microbially Induced
Ores, Microbially Precipitation and Oxidation
Photosynthesis
Salinity History of the Earth’s Ocean
Sediment Diagenesis – Biologically Controlled
Snowball Earth
Stromatolites

BASALT (GLASS, ENDOLITHS)

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Synonyms
Endolithic microorganisms in basaltic glass; Microbial life in glassy basalt

Definition
Basalt endoliths. Microorganisms that colonize and live inside fractures, vesicles, and other cavities in basaltic rocks.

Basalt. The most common volcanic rock formed by eruptions of magma of basaltic composition.

Seafloor basalt. Basalt that is exposed at the seafloor, most commonly in or close to the ocean spreading ridge axes where the crust is young, or at intraplate seamounts.

Subseafloor basalt. Basalt that has been buried by younger lava flows and/or deep-sea sediments, as the crust aged and moved away from the spreading ridges.

Basaltic glass and endolithic microorganisms
Introduction
Basalt is continuously formed during volcanic eruptions along the global mid-ocean ridge system, and constitutes the upper 0.5–1 km thick layer of the ocean crust which covers more than 50% of the Earth’s surface. When the basaltic magma is extruded into the cold seawater, the 1–2 cm thick outermost surface layer is cooled so rapidly that its chemical species do not have enough time to be arranged into minerals, but freeze to an amorphous glass rind. These glassy margins become heavily fractured due to cooling-related contraction, and the fracture networks provide an immense surface area for rock-water interactions and colonization by microorganisms. As the basaltic glass dissolves and alters more quickly than the minerals (pyroxene, plagioclase) in the crystalline interior of the lava, the glassy proportion plays an important role in the alteration of the ocean crust and the chemical exchange with the ocean water. The alteration is not only controlled by physiochemical factors but also by microbial processes, implied by the presence of endolithic microorganisms (see Endoliths) within the glassy margins. The microbial activity may, on the other hand, be controlled by the geological environment, which includes a variety of complex fluid temperature and chemistry conditions ranging from seafloor weathering to various hydrothermal systems.

Basaltic glass alteration
Low-temperature alteration of basaltic glass results in the formation of concentrically zoned rims of a yellow-brown, hydrous residual alteration product, frequently called palagonite, along the outer surfaces, and internal vesicles and fractures of the glassy margins of lava flows. The altered glass is amorphous (gel-palagonite) to poorly crystalline with clayish character (fibropalagonite) (e.g., Stroncik and Schmincke, 2001), and is usually highly enriched in oxidized Fe and Ti, and depleted in Si and alkaline and alkaline earth elements compared to the fresh, parental glass. The term palagonite was used for the first time by von Waltershausen in 1845 for the brown groundmass of a tuff from Palagonia, Sicily, and was then thought to be a new mineral phase. Although palagonite was later shown to be a heterogeneous material, the term has commonly been used in the literature. However, several authors now recommend referring to this material as altered glass. During the last 2 decades, various field and laboratory studies have shown that the alteration rate and the chemical and crystalline characteristics of the altered glass strongly depend on the physiochemical and hydrological conditions (e.g., Crovisier et al., 2003). The understanding of how the endolithic microorganisms in the glassy margins of basaltic lava flows influence and interact with the alteration process is, however, still poor.

Evidence of microbial life in glassy basalt
The first in situ observations of endolithic microorganisms in basaltic glass was reported from a scanning electron microscopy (SEM) study of recent (<1 million years old (Ma)) volcanic glass deposits from Iceland (Thorseth et al., 1992). Microbial biofilms together with irregular alteration fronts at the fresh-altered glass boundary lead to the suggestion that the degradation of the volcanic glass could be biocatalyzed by the development of more aggressive microenvironments in the near vicinity of the microbial cells. This was subsequently supported by experimental alteration studies, where development of corrosion marks in the glass surface only was observed in combination with biofilm formation and not in sterile controls (Thorseth et al., 1995a; Staudigel et al., 1995, 1998). Similar alteration textures resembling microbial cell morphologies observed in glassy margins of 6 Ma ocean basalt, sampled 432 m below the seafloor at the Costa Rica Rift during the Ocean Drilling Program (ODP) Leg 148, were then stained using DNA-specific dyes to confirm the presence of DNA within these structures (Thorseth et al., 1995b; Giovannoni et al., 1996; Furnes et al., 1996). Further fluorescence in situ
Microbial life in seafloor basalt

Several electron microscopy studies of seafloor lavas from the Arctic Mohns and Knipovich Ridges and the Antarctic Discordance area of the Southeast Indian Ridge (SEIR) have demonstrated that a diverse and numerous endolithic microbial community of various coccolid, rod-shaped, filamentous, and stalked cells colonize the upper oceanic crust at the spreading ridges. In addition, detection of comparable alteration textures and geochemical and isotopic biosignatures (see *Biosignatures in Rocks*) in lava margins of ancient oceanic basalt implies that microbial life and associated biodegradation in the ocean crust occurred very early in the Earth’s history (Furnes et al., 2004; Staudigel et al., 2006).

**Phylogenetic and metabolic diversity**

The endolithic microbial communities in the Arctic seafloor basalts are shown to be almost exclusively from the domain *Bacteria*, and only small proportions are from the *Archaea* domain (Einen et al., 2008). The most frequently retrieved bacterial DNA sequences from these basalts affiliate with the *Gamma-proteobacteria*, *Alpha-proteobacteria*, *Chloroflexi*, *Firmicutes*, and *Actinobacteria*, while the archaeal sequences were restricted to the marine Group 1 *Crenarchaeota* (Lysnes et al., 2004a). The phylogenetic diversity of basalt endoliths is found to be distinct and different from those observed in the surrounding deep seawater.

In contrast to deep-sea sediments where biogenic debris from the photic zone provides an important energy and carbon source for the inhabiting microbial communities, the basaltic basement is rich in reduced inorganic compounds that together with dissolved inorganic carbon may support growth of lithoautotrophic (see *Chemolithotrophy*) microorganisms (Bach and Edwards, 2003). Mid-ocean ridge basalts typically have iron contents of 7–9 wt%, of which approximately 80% is in the divalent state. As the basaltic glass alters to palagonite, Fe(II) is predominantly oxidized to Fe(III). The alteration of the glass may thus provide both potential energy and electron acceptors for the endolithic microbial communities. Observations of branching, twisted filamentous structures resembling stalks of the neutrophilic, iron-oxidizing bacteria (see *Fe(II)-Oxidizing Prokaryotes*) *Gallionella*...
ferruginea (see Gallionella) and the newly characterized Mariprofundus ferrooxidans (Emerson et al., 2007) at the glass-palagonite interface (Figure 1f) indeed indicate that microbial oxidation of Fe(II) from the dissolving glass is associated with the weathering of basalt (Thorseth et al., 2001). Culturing of novel Fe-oxidizing lithoautotrophs from inactive sulfide chimneys (Edwards et al., 2003) furthermore suggests that other, previous unknown Fe-oxidizing species could occur in basalt as well. In addition, preferential accumulation of manganese in some cell encrustations can be explained by the activity of Mn-oxidizing bacteria (Thorseth et al., 2001, 2003; Kruber et al., 2008a, b). Isolation of numbers of diverse heterotrophic Mn(II)-oxidizing bacteria from the surfaces of recent basalt flows from the Loihi Seamount strongly support this (Templeton et al., 2005). Results from DNA analyses and enrichments have furthermore shown that Fe-reducing bacteria (see Fe(III)-Reducing Prokaryotes) affiliating with Shewanella frigidimarina (see Shewanella) as well as other unknown Fe-reducers also are associated with the weathering of glassy seafloor basalt (Lysnes et al., 2004a). Microbial catalyzed cycling of Fe (and Mn) may thus be important in low-temperature alteration of oceanic basalt. Although seafloor basalts are in direct contact with the ambient, oxygenated seawater, the presence of microaerophilic microorganisms such as G. ferruginea and M. ferrooxidans demonstrates that redox-gradients are formed in the seawater-filled fractures.
during an early stage of alteration and microbial colonization of glassy margins. The detection of Fe-reducers furthermore indicates that anaerobic microenvironments locally develop in the fracture network of the glassy margins. Neither sulfate reducing bacteria (SRB) nor secondary sulfide phases, which are commonly reported from marine sediment, have however, been detected.

Production of methane from hydrogen and carbon dioxide during enrichments indicates in addition that lithotrophic methanogens are present in glassy seafloor basalt, although DNA sequences from basalt and enrichments have not matched DNA sequences from any known methanogens (Lysnes et al., 2004a). The hydrogen source for methanogenesis in the basaltic glass is however, unclear. Degasging from deep magma chambers or production by high-temperature water-rock interactions associated with volcanic eruptions and serpentinization of ultramafic rocks (Holloway and O’Day, 2000; Kelley et al., 2002) are all unlikely processes at the actual sampling sites. Simulations of geochemical reactions between water and ultramafic rocks indicate, however, hydrogen production also at low temperatures (Palandri and Reed, 2004). Hydrogen production during low-temperature alteration of basalt has, however, been a matter of debate (e.g., Andersen et al., 1998). Hydrogen could, on the other hand, be produced by microbial processes.

Microbial impact on the alteration: biomineralization and microbial mediated dissolution

The microbial catalysed oxidation and reduction of iron and manganese, and nucleation and precipitation of alteration products on the microbial cells and their extracellular material, are likely to partially control the mobility of elements from the dissolving glass and reacting seawater. In addition, as the microbial growth and fossilization are main controls on the porosity and texture of the alteration rims, these processes are also expected to affect the fluid exchange rate and thus, the dissolution rate in the fractures. Together with the relatively large endolithic biomass, this suggests that the potential microbial impact on the weathering of seafloor basalt and the chemical exchange with the seawater is significant. Observation of pit marks in the glass beneath the attached microbes (Figure 2a) furthermore suggests that the dissolution of the glass is to some extend controlled by microbial processes (Thorseth et al., 2001). It is, however, not known if the pitting is actively caused by local enhanced microbial dissolution due to the utilization of energy or nutrients present in the glass, or to the passive accumulations of metabolic waste products and the development of corrosive microenvironments at the interface between the microbial cells and the glass. Results from recent laboratory experiments have shown that organotrophs (Daughney et al., 2004) as well as lithoautotrophs (Edwards et al., 2004) have the potential to increase the dissolution rate and the alteration of basalt at the seafloor.

In addition to putative microbial mediated pitting, pit marks of similar form and size to microbes that are filled by hemispherical alteration structures and not by characteristic hollow cell encrustations (Figure 2b) suggest that abiotic processes may produce comparable dissolution textures to microbial processes. Such abiogenic corrosion marks most frequently occur in narrow fractures, which suggest that they are related to dissolution at low water-rock ratios (Kruber et al., 2008a).

Microbial life in subsurface basalt

Drill cores: textural, geochemical, isotope, and biomolecular biosignatures

The first reported evidence of endolithic microbes in basalt from the Costa Rica Rift subsequently stimulated to additional studies of basaltic glass in subsurface rocks from various locations and ages. Most of the studies report of similar textural, geochemical, isotope, and biomolecular evidences for microbial activity in the glass, as in the first report (Furnes and Staudigel, 1999; Fisk et al., 1998, 2003; Furnes et al., 1999, 2001a, b, 2003; Banerjee and Muehlenbachs, 2003; Thorseth et al., 2003; Benzerara et al., 2007). The phylogenetic and metabolic diversity of subseafloor endolithic microbial communities inhabiting oceanic basalts have, however, only been described in the study by Lysnes et al. (2004b), and with more limited data in the study by Fisk et al. (2003).

While abiotic alteration of isotropic basaltic glass is expected to produce regular zoned alteration rims with
smooth interfaces between the fresh and altered glasses, the commonly observed irregular alteration fronts have been interpreted to be a result of enhanced dissolution or corrosion mediated by endolithic microbial cells. The most frequently putative biogenic alteration textures reported are (1) irregular patches or zones of individual or coalesced spheres with diameter around 1 µm (also characterized as a granular texture type), and (2) channels or tubular forms 1 µm to few µm wide and up to more than 100 µm in length (Figure 3). After the first reports of DNA-specific staining of altered glass from the Costa Rica Rift, similar textures in samples from other locations have also been shown to contain nucleic acids by fluorescent dyes (Furnes et al., 2001a; Banerjee and Muehlenbachs, 2003; Fisk et al., 2003), as well as elevated amounts of carbon, nitrogen, and phosphorus.

Using alteration textures as discriminating criteria, the extent and importance of biogenic versus abiotic alteration in the oceanic basement has been estimated. Thus, bioalteration has been observed in glasses from 0 to 170 Ma crust at 0–1.5 km depths of burial of lava flows and deep-sea sediments (Fisk et al., 1998; Furnes and Staudigel, 1999; Furnes et al., 2001b; Staudigel et al., 2006). The degree of this bioalteration is highly variable in the upper 300 m of the crust (20–90%), but tends to decrease with depth. The granular texture is found to dominate at all depths. The tubular texture only represents a small fraction of the total bioalteration, and is more common at deeper levels (Furnes and Staudigel, 1999; Furnes et al., 2001b; Staudigel et al., 2006).

The stable carbon isotope composition (δ¹³C) of disseminated carbonate provides additional evidence for endolithic microbial activity in altered oceanic basalts. Most investigated samples show δ¹³C values that are significantly lower than magmatic carbon (5 to 7), indicating microbial utilization and oxidation of organic carbon which leads to ¹²C-enriched CO₂ and carbonate minerals (Furnes et al., 2001a). Positive δ¹³C values of some samples from the Atlantic Ocean have, on the other hand, been explained by lithoautotrophic utilization of H₂ and CO₂ and production of methane. During this process ¹²C-enriched methane is lost, and the remaining carbonate is enriched in ¹³C. This could result from more extensive serpentinization and abiotic formation of H₂ at the slow-spreading Atlantic ridge compared to other faster spreading ridges.

Despite the evidence for ongoing microbial activity in subseafood basalt, few direct observations of evident microbial cell structures, which are easily observed in seafloor basalt, have been reported. Possible candidates are hollow and solid filamentous structures detected in basalt tuff recovered from the Ontong Java Plateau during the ODP Leg 192 (Banerjee and Muehlenbachs, 2003), and hollow spherical structures in pillow basalt recovered from the AAD area of the SEIR during ODP Leg 187 (Thorseth et al., 2003). However, these structures were all supposed to represent fossilized cells.

Petrographic inspection of samples drilled from 14 to 28 Ma ocean crust in the AAD area during the ODP Leg 187 indicated mostly low-temperature, oxygenated alteration conditions. Comparison of 2.5 Ma seafloor basalt from the same area with the much older subseafood basalt indicated that a significant proportion of the glass alteration developed prior to burial. However, diffuse and highly irregular fresh-altered glass interfaces were only observed in the subseafood samples and thus most likely developed after burial. These diffuse alteration fronts are apparently caused by dissolution and alteration of the glass into minute globules (0.05–0.2 µm in diameter), and no evident microbial cell structures could be observed to be associated. Some hollow, Mn-containing spherical structures that were observed within zeolite-filled fractures in one sample could represent fossilized equivalents of Mn-rich encrusted, apparently active microbes, observed in 2.5 Ma seafloor basalt (Figure 1e). However, the presence of few cell-like structures in the subsurface glassy basalt suggests that most of the numerous encrusted and fossilized cells developed during the surface stage are totally destroyed or masked by alteration and mineralization during burial. The fossilized cells within zeolite-filled
fractures in subseafloor basalt, on the other hand, possibly indicate that microbial activity continued in the fractures after burial, for as long as circulation continued.

Pit marks and development of irregular alteration fronts both with and without the presence of microbes, and ambient fractures where smooth alteration interfaces are combined with numerous microorganisms attached to the fresh glass, as observed in seafloor basalt, together with irregular alteration fronts without detectable cells in subseafloor basalt, imply that great care should be taken before using alteration textures as the only criterion for microbial activity. Clearly, much more detailed knowledge is needed to fully understand the mechanisms that control both biotic and abiotic pit mark formation in basaltic glasses.

Drill cores: genetic and metabolic diversity
Drilling of 14–28 Ma ocean crust during ODP Leg 187 was partly aimed at studying the genetic and functional diversity of the subsurface microbial community (Christie et al., 2001; Pedersen et al., 2004; Lysnes et al., 2004b). Despite the sparse detection of only fossilized cells by SEM (Thorseth et al., 2003), the identification of a microbial community in these samples by biomolecular techniques that differ from that of surface seawater used as drilling fluid, document the presence of living cells in this old, sediment buried ocean floor basalt, and demonstrate that water-rock interactions also support a diverse endolithic microbial community in old ocean crust. This microbial community is probably present in fluid transporting fractures developed during tectonic events after burial. The nondetection of microbial DNA in a significant proportion of subseafloor samples could thus reflect not only a lower biomass in subseafloor basalts, but also a low number of open fractures in the samples analyzed, compared to seafloor lavas where DNA was retrieved from nearly all investigated samples (Lysnes et al., 2004a, b).

A major fraction of the endolithic microbial community in the subseafloor basalt from the ADD area was shown to be from the domain Bacteria, similar to the Arctic seafloor basalt. The five phylogenetic classes Gamma-proteobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, and Firmicutes that were present in the seafloor basalt were also present in the old subseafloor basalt. The main differences observed were the higher frequency of Gamma-proteobacteria in subseafloor basalt, the restricted presence of Alpha-proteobacteria in seafloor basalt, and Beta-proteobacteria in subseafloor basalt. Fewer and more unequal distributions of the phylotypes in subseafloor samples suggest a decrease in biodiversity and biomass with burial and aging of the crust. The results from this study furthermore indicate that microbial communities inhabiting nonhydrothermal subseafloor basalts participate in the cycling of iron and in the production of methane from H2 and CO2. A major part of the microbial populations showed no close relation to previously characterized microorganisms. The correspondence between the microbial populations in nonhydrothermal seafloor (Lysnes et al., 2004a) and subseafloor basalts (Lysnes et al., 2004b) may reflect the low-temperature and oxidised conditions, as indicated by the secondary alteration phases and their chemical composition, in both environments. The genetic and physiological diversity of microorganisms in the nonhydrothermal oceanic basalt from the ADD area differ from those reported from hydrothermal fluids from hydrothermal active regions of the ocean crust.

Recently, evidence of the presence of also eukaryotic, fungal life in oceanic basalt was reported (Schumann et al., 2004). The filamentous structures, suggested to represent fossil fungi, were observed within carbonate-filled vesicles in 46 Ma subseafloor basalt recovered during the ODP Leg 200 in the Pacific ocean. These findings imply that fungal life may possibly still exist in the subseafloor basaltic environment.

Hydrothermal fluids
Several studies of the microbial diversity in hydrothermal fluids (see Hydrothermal Environments, Marine) associated with eruptions in the central volcanic active zone of spreading ridges have been carried out during the last years (Summit and Baross, 1998, 2001; Huber et al., 2002, 2003). These studies have shown that the vent fluids inhabit microorganisms that are different from indigenous seawater microorganisms, suggesting that the vent fluids carry endolithic microbial cells from the basaltic subsurface environment. In the studies by Huber et al. (2002, 2003) dominant subseafloor phylotypes affiliated with the Alpha-, Gamma-, Delta- and Epsilon-proteobacteria, Firmicutes, novel candidate divisions, Group I Crenarchaeota, and Group II Euryarchaeota were detected. Most of these phylotypes were reported to be unique, differing from those of surrounding seawater, and affiliated with both low- and high-temperature groups. Considerable overlap with seawater was only found within Alpha- and Gamma-proteobacteria. To explain the retrieved diversity, Huber et al. (2003) proposed a schematic model of the basaltic subseafloor at the sampling site, based on hydrothermal fluid and seawater mixing that included three different compartments: a deep, anaerobic zone dominated by the hot hydrothermal fluid that contains mostly thermophilic microorganisms; an intermediate zone with both seawater and hydrothermal fluid and the highest variability in microbial community composition, probably including anaerobes, aerobes, and microaerophilic organisms; and an uppermost low-temperature, aerobic zone that is seawater-dominated, possibly containing seawater organisms. Unique subseafloor microorganisms flushed out from this uppermost zone by the vent water could thus be similar to microorganisms detected in nonhydrothermal basalts. Some of the Gamma- and Epsilon-proteobacteria sequences retrieved from both nonhydrothermal seafloor and subseafloor samples (Lysnes et al., 2004a, b) were phylogenetic affiliated.
to sequences retrieved from the <50°C diffuse flow vent fluids associated with an eruptive event on the Juan de Fuca Ridge (Huber et al., 2003).

Investigation of 65°C subsurface fluids from aging (3.5 Ma) ocean crust by Cowen et al. (2003) also demonstrated the presence of a diverse bacterial and archaeal community in hydrothermal active areas of the mid-ocean ridge flanks. In this reduced, hydrothermal environment DNA sequences related to the ammonia-producing *Ammonifex degensii* and sulfate reducers of the *Delta-proteobacteria*, the low G + C Gram-positive genus *Desulfotomaculum*, and *Archaea* (*Archaeoglobus*) were dominant. A later study of 20°C subseaﬂow fluids from the same area demonstrated that the microbial community in the fluids composed of species indigenous to the subsurface, as well as species from seawater and sediments (Huber et al., 2006). No sequences retrieved from nonhydrothermal seaﬂow and subseaﬂow basalts resembled those obtained from these 65°C subseaﬂow fluids.

Using available published 16S rDNA sequences from basalt, hydrothermal fluids, basalt enrichments, and data from other relevant environments, Mason et al. (2007) examined the phylogenetic diversity of microbial communities associated with marine basalts. Based on these data, it was suggested that the microorganisms have a cosmopolitan distribution. Most groups were found in both basalts and sediments, but are distinct from microbes in other marine habitats. One group of Marine Group I *Crenarchaeota* seems furthermore to be specifically adapted to basalt.

**Biosignatures in ophiolites and greenstone belts**

During the last years, terrestrial fragments of ancient ocean crust (ophiolites) and greenstone belts have been investigated for similar biosignatures as those observed within in situ oceanic basalt. For example, the results from the well-preserved ~90 Ma Troodos ophiolite in Cyprus revealed comparable traces of microbial life in the glassy lava margins as reported from studies of in situ oceanic crust, except for DNA that could not be detected by specific staining (Furnes et al., 2001c). This, together with geochemical and isotopic data indicative of seawater alteration under oxidizing condition, imply that the main fraction of the alteration occurred prior to obduction during an early stage of ocean-floor alteration. Comparable textural, geochemical, and isotopic data obtained from lava margins from the ~3.5 billion-year-old Barberton greenstone Belt in South Africa further suggest subaqueous microbial activity during the early history of the Earth (Furnes et al., 2004) (see Early Earth). Thus, these biosignatures could be of great relevance in the search for life elsewhere in the universe (see Astrobiology).

**Conclusions**

Endolithic microbial communities have been documented in glassy margins of seafloor and subseafloor basaltic lavas from various locations of the ocean crust. In seafloor basalt the microbial cells are easily recognized by electron microscopy, and are shown to be closely associated with the alteration of the glass. In subseafloor basalt the microbial activity has mainly been recognized by the use of biosignatures such as characteristic cell-like alteration textures in the glass, elevated concentrations of carbon and nitrogen in these textures, and stable carbon isotope ratios of disseminated carbonate consistent with biological processes. DNA-specific staining indicates the presence of DNA in the cell-like alteration textures. Culturing and biomolecular studies have revealed the existence of diverse, endolithic microbial communities in the glassy margins of oceanic lavas. The results suggest that iron and manganese cycling are important microbial processes in cold, nonhydrothermal regions, while sulfur cycling plays a major role at elevated temperatures in hydrothermal active areas. The endolithic microbial life in oceanic basalt may have a significant impact on the alteration of the basalt and the chemical exchange between the ocean crust and seawater.

**Bibliography**


Beggiatoa, Figure 1 *Beggiatoa* filaments of 6 µm diameter seen under the light microscope with dark field illumination. The sulfur globules reflect the light and give the filaments their shining white appearance.

Beggiatoa, Figure 1

Beggiatoa filaments typically occur as mats on top of the sediment or within the upper few centimeters. They actively glide to the overlapping zone of oxygen, supplied from the open water, and sulfide evolving in the sediment. In addition to oxygen, the larger species can also use internally stored nitrate for the oxidation of sulfide and thereby increase their rates of sulfide oxidation or survive periods of anoxia. Sulfide is first oxidized to sulfur, which forms intracellular sulfur globules, and than further on to sulfate. If sulfide concentrations are high, the cells are filled with many sulfur globules, which are disappearing in the absence of sulfide. Apart from sulfur and nitrate, *Beggiatoa* filaments were also observed to store polyphosphate and poly-beta-hydroxybutyrate (PHB).
BIODETERIORATION (OF STONE)

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Definition
Biodeterioration: Undesirable alteration of the properties of materials caused by vital activities of organisms.

Introduction
Materials are exposed to weathering processes caused by physical, chemical, and biological factors, leading to an irreversible loss of our cultural heritage. The reduction of decay by conservation strategies is of particular importance to protect historical monuments or significant cultural artifacts.

Generally, the velocity of decay depends on the chemical nature and physical structure of materials as well as external factors associated to the locations where the objects are exposed. In many studies it was demonstrated that the process of weathering consists of various mechanisms acting together in coassociation with more or less intensity, making it impossible to separate individual aspects such as physical, chemical, or biological factors from each other (Koestler et al., 1994; Ehrlich, 2002). In many cases microbial activities can accelerate or trigger physicochemical processes. But the composition of microbial consortia, rather the microbial activity, is also affected by the surrounding chemical and physical factors.

Solar irradiation, wind, and variations in temperature and humidity predominate the physical effects. Wind and humidity, of course, lead to the erosion of particles from stone surfaces. Variations in temperature, due to diurnal and seasonal changes, cause tensile stresses that can break up the structure of the material, resulting in a loss of persistence. Humidity, in combination with frost and salt, can intensify this effect. The crystallization of water or saline solutions is connected with an increase in volume that leads to “frost shattering” or “saline shattering” (Steiger and Dannecker, 1995).

Chemical weathering may be caused by rain water which dissolves carbon dioxide originating from the ambient air or from respiration of present microorganisms. Generated carbon acid reacts with calcium carbonate, thereby producing calcium hydrogen carbonate with a higher solubility than calcium carbonate (Bell, 1993).

According to Krumbein and Schönborn (1987), biological damages of building material can be divided into different categories: (1) Anthropogenically induced damages due to atmospheric pollution as a result of the industrialization. These processes lead to an increasing level of inorganic (SO₂, NO₂) and organic (aliphatic and aromatic hydrocarbons) compounds in the air. This implicates acid rain that has a corrosive effect on stone buildings or other exposed materials (Pitzurra et al., 2003; Winkler, 1987). (2) Damages caused by higher plants and animals. The growth of higher plants, for example, leads to the physical destruction of materials. Moreover, excrement of birds contain ammoniac and nitrite, which can be utilized by chemolithotrophic bacteria. (3) Microorganisms such as bacteria, algae, fungi, and lichens can cause a degradation of materials. These damaging effects will be specified below.

Furthermore, biological colonization and the intensity of associated biodeterioration processes are strongly influenced by water availability. This is determined by specific material parameters such as porosity and permeability, as well as by environmental conditions.

Microbial degradation processes
How do microbial associations interact within these complex weathering processes? A considerable amount of studies have been done to figure out the role of microbiological agents in weathering processes (e.g., Urzi and Krumbein, 1994; Griffin et al., 1991; Warscheid and Braams, 2000). Generally, microorganisms play an important role within the regulation of the global cycles of materials, contributing to the function of the ecosystems. However, microorganisms also play a crucial role concerning the stability of materials. The interactions between microbial associations and materials can be divided into three different categories (Weber, 1993, cf. Figure 1): (1) The material can be decomposed enzymatically and used as a nutrient source by microbial associations. (2) Microorganisms can excrete metabolic compounds such as inorganic and organic acids that interact with mineral compounds of the material, thus causing dissolution and discoloration. (3) The material can be damaged by physical (mechanical) forces due to the growth of microorganisms.

For the most part, microorganisms conglomerate to biofilms of different natures such as crusts, fluffs, or slime, giving them the ability to colonize nearly all biotic and abiotic surfaces (Costeron et al., 1987). Biofilms can exhibit a highly spatial structured composition (Stoodley et al., 2002), thereby consisting of different prokaryotic and/or eukaryotic populations, extracellular polymeric substances (EPS), accumulated polyvalent cations, as well as solved and dissolved organic and inorganic particles. The formation of biofilms provides considerable advantages including mechanical stability, the facility of symbiotic and syntrophic interactions, the building of a diffusion barrier and an increased resistance against toxic environmental conditions, biocides, acidity, or pH variabilities (Wolfardt et al., 1999). However, the presence of biofilms can lead to biophysical determined damages of...
Surface structures of stone (Gorbushina and Krumbein, 2000; Gorbushina, 2007). Variations in temperature and humidity induce shrinking and swelling cycles of the film, leading to an alteration of hydrophobic–hydrophilic properties of rock surfaces. As a consequence, hydrophobic properties of stone surfaces can turn into hydrophilic.

Microorganisms involved in biodeterioration processes

The ecology of microbial associations colonizing rocks is highly complex and involves different groups of microorganisms with different physiological properties. These ecosystems can develop in various ways, depending on environmental conditions and physicochemical properties of the material. Therefore, only a small range of examples can be discussed in this framework, representing the complexity of the interactions between microorganisms and their substrate.

Phototrophic microorganisms

Phototrophic microorganisms such as algae, cyanobacteria and lichens, but also mosses and even higher plants cover stone surfaces, thus changing the face of new buildings or cultural heritage. In many cases they form biofilms or crusts to protect themselves from unfavorable environmental factors. These crusts or films can appear in a deep or light green color under humid conditions or in black tint under dry environmental conditions (Urzi and Krumbein, 1994; Krumbein and Warscheid, 1992). Under particular climatic conditions (e.g., tropical environments) these biofilms provide a protective effect, isolating the stone surface from aggressive environments and regulating humidity and temperature (Krumbein and Warscheid, 1992). But the formed biopatina can also cause damages by physical and chemical processes (Krumbein and Warscheid, 1992; De los Ríos et al., 2009). Associated biodeterioration processes can be induced by the excretion of corrosive acids (Arino et al., 1997), the uptake of substrate compounds such as sulfur and calcium and accumulation into their cells (Orgenta-Calvo et al., 1994), and by the alteration of stone forming minerals (Ortega-Morales et al., 2000; Crispim and Gaylarde, 2004). Finally, the penetration of hyphae and roots causes enlargement of pores and loosening of stone particles from the material (Ascaso et al., 1998). The formation of biofilms intensifies this loss of structural consistency (Orgenta-Calvo et al., 1991).

Due to the capability of fixing atmospheric nitrogen, phototrophic organisms are often the primary settlers of habitats especially in combination with fungi as lichens. In rural or strongly industrialized areas where the air is enriched with nitrogen from fertilizers or industrialization, their growth can be enhanced (Cadot-Leroux, 1996; Cicik et al., 2008). Some phototrophic microorganisms (epilithicphototrophs) may penetrate several millimeters into the stone pore system (Golubic et al., 1981). Additionally, some of these microorganisms can colonize extreme locations, whereby a special pigmentation protects them against strong light irradiation (Krumbein and Jens, 1981).
Chemolithotrophic and chemoorganotrophic microorganisms

Even the natural biomass accumulated by photosynthetic microorganisms – especially in combination with anthropogenic pollution – can serve as nutrient source for the establishment of heterotrophic microorganisms (Warscheid et al., 1991; Zanardini et al., 2000).

Besides, even without a primary settlement of phototrophic microorganisms an establishment of heterotrophic organisms is possible (Krasilnikov, 1949). A sufficient amount of inorganic compounds (e.g., sulfur, ammonium, sulfide) enables the appearance of chemolithoautotrophic bacteria (Kaufmann, 1960; Pochon and Jaton, 1967). The formation of nitrous acid (HNO₂ by *Nitrosomas*) or nitric acid (HNO₃ by *Nitrobacter*) and sulfuric acid (*Thiobacillus*) results in the solution of mineral rock compounds. Acidic metabolic compounds resulting from the oxidation of sulfur, for example, interact with calcium carbonate of the stone structure. This reaction leads to the formation of calcium sulfate, which is more soluble than calcium carbonate.

The most numerous microorganisms in natural building stone are chemoorganotrophic bacteria. Results of Warscheid et al. (1993) indicate that they colonize natural stone within weeks. They can excrete acid compounds but, generally, their destructive activity has been evaluated as low (Lewis et al., 1988). Nevertheless, they are extensively involved in the formation of biofilms, thus causing physicochemical properties of the mineral structure described above. Materials can contain or be associated with organic compounds such as dust, natural resin, wax, or atmospheric aerosols that can act as nutrient source for chemoorganotrophic microorganisms (Warscheid, 1990). As an effect of organic pollution, chemoorganotrophic microorganisms can be the first settlers on buildings in highly industrialized locations (Krumbein, 1966). Many chemoorganotrophic bacteria are able to use low degradable compounds, offering nutrients for other microorganisms of the microbial consortia (Warscheid et al., 1991; Zanardini et al., 2000).

Fungi

The colonization of stone surfaces or other materials by fungi causes damages, especially by physical (mechanical) forces (cf. Figure 2). The formation of penetration hyphae enables some fungi to enter actively into materials, despreading within the material and affecting the so-called biopitting structures on rock surfaces (Sterflinger and Krumbein, 1997). Furthermore, the production of aggressive acidic metabolites leads to biocorrosion (Kurakov et al., 1999). In comparison to bacteria, fungi can establish rapidly, especially in environments with low humidity due to their widespread system of hyphae (Weber, 1993). Their damaging effects also include the discoloration of surfaces due to the building and excretion of melanins (Gorbishina et al., 1993). The synthesis of special pigments such as melanin, carotenoids, and mycosporines provides protection against UV radiation and enables some species (e.g., black fungi) to colonize even at locations with extreme UV radiation (Chertov et al., 2004).

Measures and preventive treatments

In general, a microbial colonization is primarily dependent on the availability of water and nutrients and specific material parameters such as porosity, permeability, architectural conditions (considering buildings), and environmental factors. Natural selective processes can lead to the formation of populations that reveal a perfect adaption between organisms, substrate, and environment. A previous individual and comprehensive analysis of all factors and relationships is essential before preventive treatments or cleaning of the material is applied. A mechanical cleaning of historical buildings from dust or biological films should integrate a chemical analysis to characterize the surface dirt. Otherwise, the cleaning procedure can fail to have a long-lasting effect and even accelerate the degradation processes or alteration within the composition of microbial associations affecting changes of microbial activity (Dornieden et al., 2000).
Water cleaning can help remove soluble salts and biological infections, but in the long term it can lead to a wider dispersion of the microbial infection because of increased humidity. Furthermore, consolidation treatments can be applied, but their effectiveness is strongly connected to the physical structure of the stones. The application on stone surfaces with insufficient consistence and absorbent capacity can intensify physical damages (Sattler, 1992; Wendler, 1992; Zoghlami and Gómez-Gras, 2004). Other preventive agents impregnate surface structures and are often based on high polymer polysaccharide, silane, or siloxane. Various studies have shown that the application of consolidants or water repellents induce an increasing activity or colonization of microorganisms using these restoration agents as nutrient source (Krumbein et al., 1993; Petersen et al., 1991, cf. Figure 3). Studies of Urzi and De Leo (2007) and De Leo and Urzi (2003) have shown that water repellents alone do not stop microbial colonization, but in combination with biocides, microbial growth can be prevented.

Finally, it is important to mention that the treatment with biocides should only be applied in instances where biodeterioration processes cannot be controlled and chemical applications are unavoidable. An inappropriate treatment with biocides or other preventive arrangements can lead to an increasing selective microbial colonization that may accelerate the deterioration.

**Summary**

The process of weathering consists of physical, chemical and biological factors acting together with more or less intensity, leading to an irreversible loss of our cultural heritage. Microbial activities can accelerate or trigger physicochemical processes. But the composition of microbial community or rather the microbial activity is also affected by the surrounding chemical and physical factors. Interactions between microbial associations and materials contain the enzymatic decomposition of materials, excretion of metabolic compounds such as inorganic or organic acids, and damages by physical (mechanical) forces due to the growth of microorganisms.

**Bibliography**


**Biodeterioration (of Stone), Figure 3** Sandstone treated with (a) consolidation agents and (b) water repellents. A high microbial colonizations was observed 3 month after treatment and outdoor exposure, scale bar 10 μm (photograph by A. A. Gorbushina and C. Beimforde, Beimforde, 2006).
Bioerosion

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Definition

Bioerosion is defined as erosion, i.e., removal and transport of materials by the action of organisms. Bioabrasion refers to removal by mechanical means: scraping, rasping, drilling. Biocorrosion refers to removal by chemical means: etching, dissolving, boring. Bioerosion is often carried out by a combination of bioerosional tools. Bioerosion by microorganisms is referred to as microbioerosion as opposed to macrobioerosion performed by animals and plants. External bioerosion is carried out by organisms that live outside the substrate but remove it in the process of seeking food (grazing). Internal bioerosion is carried out by organisms that excavate the substrate for shelter and live inside as endoliths. They penetrate the substrate actively as euendoliths, or occupy existing cavities as cryptoendoliths or fissures in the rocks as chasmoendoliths.

Concept of bioerosion

The concept of erosion is usually discussed in geology, particularly in geomorphology, sedimentology, and soil sciences, in reference to degradation and transport of rocks and sediments by environmental forces such as wind, water, or ice. The participation of organisms in these processes and their long-term geological impact are often ignored. The role of organisms in sediment stabilization, organization, and buildup is becoming increasingly recognized as biosedimentation, and so is bioerosion, i.e., the participation of organisms in destruction of rocks and sediments.

Similar to weathering and erosion by environmental forces, the destruction of hard substrates by organisms, i.e., biological erosion or bioerosion (Neumann, 1966) includes two mechanisms: (a) biological corrosion, which refers to destruction of substrates by chemical means introduced by organisms, thus promoting dissolution of solid substrates, and (b) biological abrasion, which refers to mechanical destruction and removal of substrate by organisms (Golubic and Schneider, 1979). Sediment particles are frequently produced during biological abrasion and are redeposited (Neumann, 1966; Schneider and Torunski, 1983).

Bioerosion affects various substrates, most commonly the relatively soluble ones such as carbonates, phosphates, and sulfates. Bioerosion of siliceous rocks occurs at much lower rates and is rarely conspicuous. The objects of bioerosion include solid rocks, sediment particles, and mineralized skeletons of various live and dead organisms. Bioerosion takes place in a wide variety of aquatic and terrestrial environments. Its effects are especially noticeable in coastal carbonate rocks, coral reef structures, and molluscan calcareous skeletons, but are less apparent in skeletons of live corals (Le Campion-Alsumard et al., 1995a), encrusting coralline algae (Pantazidou et al., 2000; Tribollet and Payri, 2001; Ghirardelli, 2002), foraminiferas (Golubic et al., 1984), calcareous ooid sand particles (Al-Thukair and Golubic, 1991), and fish teeth (Underwood et al., 1999).

Many researchers describe penetration into hard substrate as boring activity, in contrast to burrowing, which describes digging into loose substrate such as sand and mud; however, some use the term burrowing for penetrating any substrate. Others suggested that activities of bioerosing organisms be distinguished on the basis of their objectives (e.g., food vs. shelter). Many bioerosing organisms are fully or partially embedded in the rocky substrate, occupying an endolithic habitat, while others erode the substrate from outside. Accordingly, they have been distinguished as internal versus external bioeroders. Many investigators view bioerosion as behavior (Boucot, 1990) and relate bioerosing organisms to the traces they leave in modern and ancient hard substrates. Researchers...
Agents of bioerosion

Agents of bioerosion cover a wide range of sizes. Organisms that actively penetrate hard substrates are for practical reasons treated separately as microborers (size <100 µm) and macroborers (size >100 µm). In addition to macro- and microboring organisms, which predominantly act as biocorrosive agents (internal bioeroders), bioerosion is strongly affected by bioabrasion by various grazing invertebrate and vertebrate animals (external bioeroders). Predators able to penetrate shells and attack the animal inside constitute a special group of bioeroders that includes predatory snails and cephalopods (called “predators” in this chapter). Bioeroding organisms are metabolically diverse; they employ different feeding strategies and thus affect substrates differently. Some organisms penetrate or perforate hard substrates in search of food, such as fungi and predatory snails. Others penetrate the substrate and use it as a habitat while depending on external energy sources. This is the case with boring phototrophs, such as cyanobacteria, algae, and lichens as well as with filter-feeding invertebrates.

Organisms that occupy the interior of rocky substrates are called endoliths, as opposed to epiliths that adhere to external rock surfaces. At the microscopic scale, hard substrates provide different habitats, which are occupied by different endolithic microorganisms (Figure 1). Cracks and fissures in rocks are occupied by chasmoendoliths, the existing pore spaces between grains in sandstones or structural pores in skeletons are occupied by cryptoendoliths; only euendoliths or microborers actively dissolve substrates and occupy spaces of their own making (Golubic et al., 1981).

The euendoliths are equipped with special chemical or mechanical means of bioerosion, although other inhabitants of the rock interiors may also affect the substrate by modifying the chemistry of the surrounding interstitial waters. Euendoliths, both micro- and macroborers, produce boreholes and tunnels in the substrate that comply closely to the outlines of their bodies (Pohowsky, 1978; Radtke and Golubic, 2005). At a finer scale, they leave etch marks that comply with and reflect crystalline composition of the rock (Golubic et al., 1975). Both, micro- and macroborings preserve well in the fossil record and provide valuable information about past environments (Vogel et al., 1987; Glaub et al., 2007; Bromley, 2004).

Microborers comprise phototrophs such as cyanobacteria and algae (mostly chlorophytes and rhodophytes) and organotrophs (heterotrophs) including fungi, foraminifera, and other, mostly unidentified prokaryotic and eukaryotic light-independent organisms. Euendolithic cyanobacteria (Figure 2) include members of all orders, Chroococcales (coccoid forms) (Figure 2a–c), Oscillatoriales (non-heterocystous filamentous forms), Nostocales, and Stigonematales (unbranched and branched heterocystous filamentous forms) (Figure 2d). Euendolithic algae include septate and siphonal chlorophytes (Figure 3) and endolithic “Conchocelis” stages in the life cycle of some rhodophytes (Bangiales). Euendolithic fungi include lower (Chytrids) and higher fungi (Ascomycetes, Basidiomycetes), but many are known only by the borings they employ a variety of experimental strategies and preparation techniques, including x-ray visualization (Bromley, 1970; Sammarco and Risk, 1990), replication (casting) of borings in polymerizing resins (Golubic et al., 1970), and digital imaging (Tapanila, 2008).

The research of geological history of bioerosion relies on traces left by bioeroding organisms in ancient sediments, so that the study of trace fossils constitutes a separate discipline with its own methods and rules of nomenclature. According to the rules of the International Code of Zoological Nomenclature (ICZN), which governs trace fossil investigations, the traces have to be named in special journals (e.g., Ichnos, Facies), and as proceedings of symposia and compendia (Carriker et al., 1969; Crimes and Harper, 1970; Frey, 1975; Bromley, 1990; Miller, 2007). The geological roles of bioeroding organisms are periodically reviewed regarding: (a) their diversity and evolution, (b) behavior, (c) recognition and classification of their traces and imprints in the substrate, (d) their impact on sedimentary systems, and (e) other environmental consequences of their activities (Hutchings, 1986; Radtke et al., 1997; Perry and Hepburn, 2008; Tribollet, 2008b). Bibliographies on past and present bioerosion are also displayed on line (see Cross-references below).
produce (Figure 4). Euendolithic and partially endolithic mode of life is known for benthic foraminifera, including fossil forms, and their boring traces are common. However, little is known about the biology or taxonomic identity of euendolithic foraminifera (Bromley et al., 2007).

The mechanisms of carbonate dissolution in the process of penetration into rocky substrate by microbial euendoliths are poorly understood. The observed variations in boring behavior indicate the existence of different mechanisms, which may include calcium pumps (Garcia-Pichel, 2006), respiratory carbonic acid (Tribollet, 2008b), or enzymes such as carbonic anhydrase. Microborers are mainly agents of biocorrosion.

Macroborers include specialized sponges (Figure 5), such as clionids (Rützler, 2002), various groups of worms including polychaetes (Wielgus et al., 2006), phoronids (Plewes, 1994) and sipunculids (Gherardi and Bosence, 2001), and other invertebrates including ctenostome bryozoans (Pohowsky, 1978; Todd, 2000), acrothoracic cirripedia (barnacles) (Kolbasov, 2000), and bivalves, such as lithophagid mussels (Figure 6) (Scott and Risk, 1988; Kleemann, 1996).

Boring sponges employ the enzyme carbonic anhydrase for biocorrosion combined with mechanical cleavage of cell-size particles, which are expelled into the environment (Pomponi, 1980). The research on boring sponges was recently reviewed by Schönberg (2008).

Polychaetes and pholadid mussels use a combination of chemical and mechanical means of penetration into rocks and shells. For example, polychaetes involve setae for mechanical removal of chemically loosened substrate, whereas the pholadid mussels use rotation movement of their shells (Hutchings, 1986).

Grazers are the principal agents of biological abrasion (external bioerosion), and comprise gastropods (Schneider and Torunski, 1983; Radtke et al., 1996), polyplacophores (Rasmussen and Frankenberk, 1990), echinoderms (Carreiro-Silva and McClanahan, 2001), and fish (Bruggemann et al., 1994), especially parrotfish on coral reefs (Hoey and Bellwood, 2008). Grazers are...
not endolithic and they do not perforate shells. Instead, they remove layers of the mineral substrate with their specialized hard mouth parts (e.g., “Aristotle’s lantern” of sea urchins, magnetite-enforced radulas of snails and beak-like jaws of parrotfish) while feeding on epilithic algal turf and also removing external and internal microbial biofilms (Golubic and Schneider, 2003).

Predators that successfully attack invertebrates protected by shells include snails of the families Naticidae, Muricidae, and five other families (Walker, 2007), as well as foraminifera (Cedhagen, 1994). Predatory snails use a combination of mechanical and chemical means of making a perforation in the shell of their pray and killing the pray (Carriker, 1969). Euendolithic fungi, which grow inside coral skeletons, from where they regularly attack coral polyps, can also be considered predatory. Fungi may compromise the coral’s ability to overcome environmental stress conditions or to resist diseases inflicted by other causative agents (Le Campion-Alsumard et al., 1995b; Bentis et al., 2000).

Diversity of bioeroding organisms

The ability to exploit resources by eroding rocks and sediment particles evolved in various prokaryotic and eukaryotic groups of organisms, often enhanced by their mutual interactions. Microbial euendoliths contribute significantly to the overall benthic primary production and support a variety of grazing invertebrates and vertebrates. Their continuing activity, however, depends on grazing, which maintains optimal light access to rocky surfaces.

Microbial euendoliths or microborers are ubiquitous. They are distributed globally: in marine (Tribollet, 2008b), freshwater (Schneider and Le Campion-Alsumard, 1999; Anagnostidis and Pantazidou, 1988), and terrestrial environments (lichens) (Golubic and Schneider, 2003). However, they are most diversified in marine environments, where they occupy a variety of ecological niches that range in distribution from the supratidal coastal spray zone to the abyssal depths. Macroborers in carbonate substrates are predominantly marine. There are a number of predatory snails in terrestrial and freshwater habitat, but shell perforation is known only for marine species.

Bioeroding organisms are also highly diversified and specialized, so that different environmental settings, different latitudes and regions are settled by different species and genera. Their regional distribution based on taxonomic distinctions and evolutionary history is beyond the scope of the present article. Instead, we will concentrate on environmental conditions and constraints that determine distribution of particular assemblages of euendoliths and their traces, so that they can be used as environmental and, by extension, as paleoenvironmental
and paleobathymetric indicators (Golubic et al., 1975; Vogel et al., 1987; Glaub et al., 2001, 2007). Further we will present case histories that illustrate synergistic interactions among bioeroding organisms that resulted from their coevolution.

**Depth distribution of microbial euendoliths**

Light is a critical factor for depth distribution of phototrophic euendolithic organisms, whereas the organotrophs are light-independent and depend on organic food supply. Light is absorbed selectively by the water column as well by the organisms and their pigment systems. The combination of both defines the euphotic and disphotic environments, and triggers adaptations to different light intensities and spectral qualities, as well as responses to quantitative and qualitative shading, resulting in microbial arrangement in strata. Other factors, such as water supply and chemistry, including redox potential and nutrient availability pose further limitations, challenges, and opportunities to which the organisms respond.

The intertidal ranges of carbonate coasts are densely populated by assemblages of endolithic cyanobacteria (Le Campion-Alsumard, 1979). More than half a million euendoliths commonly occupy a square centimeter of limestone rock surface (Figure 7) (Schneider and Le Campion-Alsumard, 1999). Intertidal barnacles and muscles are also subject to cyanobacterial borings whenever the protective periostracum layer of their shells is damaged. Extensive boring by euendolithic cyanobacteria has been shown to inflict lethal damage on muscles by destroying their shells (Kaehler and McQuaid, 1999).

An assemblage of intertidal cyanobacteria appears to have cosmopolitan distribution with characteristic morphotypes of *Hyella balani* (Figure 2b), *H. caespitosa*, *Plectonema terebrans*, *Mastigocoleus testarum* (Figure 2d), and *Kyrtuthrix dalmatica* (Figure 8), which are common on Mediterranean coasts (Le Campion-Alsumard, 1979; Le Campion-Alsumard and Golubic, 1985) and also on the coast of South Africa (Kaehler and McQuaid, 1999). Similar assemblages of euendolithic organisms and their traces have been found at different latitudes including northern seas (Wisshak et al., 2005), but their highest diversity and the highest rates of bioerosion have been recorded in lower latitudes (Wisshak, 2006; Tribollet and Golubic, 2005).

Special assemblages of euendolithic cyanobacteria occupy subtidal carbonates. In shoaling sands (oolids), mollusk shells, and shell fragments in subtropical regions of the Bahamas, and the Arabian Gulf, assemblages are dominated by a number of different cyanobacteria of the genus *Hyella* (Al-Thukair and Golubic, 1991; Al-Thukair et al., 1994) that produce boring traces of the ichnogenus *Fascichnus* (Figure 9) (Radtke and Golubic, 2005). Relatively stable carbonate substrates such as bivalve shells, coral skeletons, and carbonate hard grounds in illuminated marine waters are dominated by septeate euendolithic chlorophytes, *Phaeophila* and *Eugomontia*, which produce traces of the ichnogenus *Rhopalia* (Radtke, 1991; Golubic and Radtke, 2008) and by the siphalon chlorophyte, *Ostreobium quekettii*, which produces trace *Ichnoreticulina* (Figure 3) (Radtke and Golubic, 2005). Similar assemblages of euendoliths and their traces have been studied in profiles across coral reefs in Mexico (Günther, 1990) and Jamaica (Perry, 1998).

*Ostreobium* reaches considerable depths in clear oligotrophic tropical seas. This endolith dominates carbonate substrates between 10 and 150 m and is still present in the rocks at 300 m (Vogel et al., 2000). The maximum
depth for phototrophic euendoliths is reached by the cyanobacterium *Plectonema (Leptolyngbya) terebrans*, which was identified alive at 370 m depth (Lukas, 1978). Conchocelis stages of bangialean rhodophytes penetrate carbonate substrates to a depth of 78 m in the North Sea (Clokie et al., 1981). The actual depth of occurrence of all phototrophic euendolithic organisms depends on light penetration in the waters which can be affected by environmental factors such as terrigenous inputs of nutrients and sediments, and eutrophication in general. Depth zones have, therefore been defined by Glaub et al. (2001) in relative terms as: “shallow euphotic zone,” divided in three stages to cover the supratidal, intertidal, and shallow subtidal ranges, “deep euphotic zone,” “disphotic zone” (defined by light penetration between 1% and 0.001% of the incoming illumination at the surface), and “aphotic zone” beneath that.

Euendolithic fungi penetrate carbonate substrates that contain organic matter. They permeate carbonate substrates with their thin, branched hyphae (Figure 4) (Mao-Che et al., 1996). Fungi also parasitize on euendolithic algae (Bentis et al., 2000; Priess et al., 2000; Golubic et al., 2005) and form lichens with cyanobacteria (Le Campion-Alsumard and Golubic, 1985) and some chlorophytes (Golubic and Schneider, 2003). Euendolithic organotrophs such as bacteria and fungi may occur at any depth, including abyssal ranges (Golubic et al., 1984; Golubic et al., 2005). Their traces have been described in deep-water coral reefs (Wisshak, 2006).

**Phototrophic euendoliths in corals**

Distribution of phototrophic euendoliths deep into the disphotic zone of the oceans is paralleled by the depth of their penetration into carbonate substrates. *Plectonema* *terebrans* and *Ostreobium quekettii* penetrate over 1 mm deep into live and dead coral skeletons (Tribollet, 2008a). These euendolithic phototrophs can survive and grow at light intensities as low as 0.04% of the surface illumination and are, therefore, considered oligophotic (Fork and Larkum, 1989; Shashar and Stambler, 1992; Schlichter et al., 1997). The ability to harvest energy by extending the window of photosynthetically useful radiation is facilitated in cyanobacteria by the auxiliary phycobilin pigments, including optional chromatic adaptation in some taxa. Different strategies are employed by *Ostreobium*. This green alga can increase the proportion of Chlorophyll-b (Schlichter et al., 1997), or use light from the long-wave portion of the solar spectrum (Koehne et al., 1999; Wilhelm and Jakob, 2006).

The ecological niche that euendolithic algae occupy in the carbonate skeletons of live calcifying organisms such as growing corals requires a positive phototropic growth orientation and a rate of growth and carbonate penetration that keeps up with the host’s accretion rates. This condition is fulfilled by *Plectonema terebrans* and *Ostreobium quekettii* (Figure 10), and less frequently by Conchocelis stages in the life cycle of bangialean rhodophytes. *Ostreobium quekettii* is omnipresent in live corals (Lukas, 1973, 1974) as euendolith and as cryptoendolith (Schroeder, 1972) (Figure 11).

The distribution and abundance of *Ostreobium* in live corals and its possible interactions with the coral host has been the subject of several studies, leading to the...
suggestion of a symbiotic relationship. This proposal was supported by documenting an exchange of nutrients and metabolic products between the two partners living in close proximity (Shashar and Stambler, 1992; Schlichter et al., 1997). Chemistry of pore waters in corals was reviewed briefly by Risk and Muller (1983). Such an ectosymbiotic relationship between euendoliths and live coral polyps may help the survival and recovery of the latter following the stress of coral bleaching events (Fine et al., 2006).

**Ostreobium** responds to coral bleaching first by photoinhibition, followed by rapid accommodation to higher light intensities. This response leads to an increase in growth, identified as a blooms of **Ostreobium** (Fine et al., 2006). Uneven density of **Ostreobium** filaments and their pigmentation in coral skeletons is expressed as green bands (Lukas, 1973), which were interpreted as periodic blooms of the alga (Highsmith, 1981). Le Campion-Alsumard et al. (1995a) showed that euendolithic algae in coral skeletons tend to increase in density beneath the polyp layer whenever coral growth slows down (Figure 12). It is, therefore, conceivable that the green bands studied by Lukas and illustrated by Le Campion-Alsumard et al. (1995a, Fig. 7) represent a record of past coral bleaching events.

**Organotrophic euendoliths in corals**

Regular inhabitants of coral skeletons other than phototrophs are euendolithic fungi. Their penetration depends on the distribution of organic matter in the bored substrate, including that which was contributed by euendolithic phototrophs. It has also been documented that fungi attack endolithic algae in coral skeleton as well as the live coral polyps (Priess et al., 2000; Bentis et al., 2000). To protect itself, the coral responds by initiating deposition of a dense carbonate layer over the fungal borehole. As the fungus continues to penetrate, such deposits tend to accumulate, building conical structures reminiscent of deposition of pearls around foreign bodies by oysters (Le Campion-Alsumard et al., 1995b). The reported interrelations between fungi and corals (Priess et al., 2000; Bentis et al., 2000) have been recently complemented experimentally by demonstrating stimulation of cultured coral skeletogenic cells by the basidiomycete fungus *Cryptococcus*, independently isolated from a coral skeleton (Domart-Coulon et al., 2004). Several strains of ascomycete fungi were isolated earlier from corals in conjunction with the study of their parasitic role (Kendrick et al., 1982).

**Boring foraminifera**

Carbonate penetration by benthic foraminifera is a widespread but little known activity. Some euendolithic foraminifera construct their tests inside their boreholes (Cherchi and Schroeder, 1991), but more often, only boring traces of soft-bodied parts remain inside carbonate substrate, leaving only spine-like traces of their pseudopodia. Foraminifera include taxa that are strictly organotrophic as well as those that contain phototrophic endosymbionts (*Symbiodinium*, zooxantellae). The latter group is expected to be limited in distribution to illuminated parts of the ocean, but their relation to microboring activity is not known. Boring traces of foraminifera overlap in size the ranges usually assigned to micro- or macroendoliths. Most of them are small, possibly temporary anchoring devices. Boring foraminifera include also large forms (Rosalinidae) that parasitize on mollusks by perforating their shells (Smyth, 1988; Cedhagen, 1994). The mollusk responds to the attack in a way similar to coral’s defense from predatory fungi by producing layers.
of nacre over the area of penetration (Figure 13) (reviewed by Beuck et al., 2008). The naming of boring foraminifera became a subject of controversy when the organism described as Globodendrina, on the basis of the interpretation of its fossil trace (Plewes et al., 1993), was subsequently formally described as the trace Semidendrina (Bromley et al., 2007).

Distribution of macroborers

Marine macroborers are mostly filter-feeders, and thus need to maintain contact with the substrate surface in order to access food from the surrounding water column. Accordingly, these internal bioeroders tend to thrive in eutrophic coastal waters (Risk et al., 1995), and are less abundant in the offshore oligotrophic settings (Tribollet and Golubic, 2005), with particularly strong offshore decline recorded for the abundance of boring bivalves (Sammarco and Risk, 1990).

Depth distribution of macroboring organisms has not been assessed as vigorously as for microboring organisms. The boring sponges seem to show an increase in abundance with depth within the range between 10 and 30 m (Zundelevich et al., 2007). Most work on macroboring organisms concentrates on shallow marine habitats and reefs (Mariani et al., 2000). Macroboring traces, however, have been also observed in deep-sea corals and shells (Freiwald et al., 1997; Wisshak, 2006).

Depth of penetration of macroboring into substrates varies, depending on the organism and its size. Small polychaetes, larvae, and juveniles do not penetrate deep inside substrates, and the depth of penetration and volume of the substrate excavated increase with age of the macroborer (Figure 6). Some macroborers are nearly microscopic in size. The zooetia of boring bryozoa, for example, are connected by stolones that bore tunnels close in dimensions to those of endolithic algae and to borings of microbial heterotrophs described as Orthogonum (Radike, 1991). Larger euendoliths include cirriped crustaceans and various boring worms, such as polychaetes, phoronids, and sipunculids. These borers remove relatively small amounts of substrate due to their small size when their population density is low. In higher concentrations, they are known to effectively damage mollusk shells (Bergman et al., 1982).

Interaction between macroendoliths and their coral hosts has produced distinctive patterns and taphonomic alterations (Scoffin and Bradshaw, 2000), analogous to distinction between live and dead corals shown for microbial euendoliths. The exceptionally pervasive macroborers are clionids (Mariani et al., 2000), which produce extensive branched and interconnected systems of galleries within carbonate substrates (Figure 5) while communicating with the surface by numerous small openings; other boring sponges are partially endolithic with bodies that overgrow the substrate as well as penetrate inside (Rützler, 2002). Clionid sponges are known to attack the substrate at the cellular level by carving out silt-to sand-sized chips that are then expelled into the environment contributing to formation of fine-grained sediments (Pomponi, 1980). However, the chemical dissolution that accompanies this process removes more than three times the amount of carbonate than is removed by the mechanical chip removal (Zundelevich et al., 2007). Traces of sponge borings are known by the name of Entobia (Bromley and D’Alessandro, 1989), which are characterized by species-specific boring patterns. Like microborders and sponges, other macroborers, such as bryozoans, cirripeds, and bivalves excavate spaces that comply closely with the outlines of their bodies (e.g., Hutchings, 1986; Scott and Risk, 1988). All these macroboring organisms preserve well in the fossil record and provide useful information for paleoecological research.

Bioerosion over geological time

Commonly asked questions about the euendolithic mode of life are: Why do they bore? When and how did this habit evolve, and which selective pressures supported that habit. An early hypothesis that microbial euendoliths evolved seeking shelter to escape the grazing metazoans when those evolved around 540 million years ago, was rejected since Proterozoic euendoliths were discovered in much older strata (Campbell, 1982). Entire assemblages of fossil euendolithic cyanobacteria have subsequently been discovered in Neoproterozoic silicified ooids (Knoll et al., 1986). The oldest microbial euendoliths were found to bore ancient stromatolites in over 1,500 Myrs. old rocks (Zhang and Golubic, 1987).

The observation that many euendoliths enter the substrate via a small opening, from which they expand laterally, i.e., parallel to the surface, suggests that they
successfully avoid competition with epiliths for settlement ground. The phototrophic euendoliths evolved efficient light-harvesting systems to operate at low light levels exploiting different spectral ranges. This adaptation is important because the light available to euendoliths changes qualitatively and quantitatively as it is filtered by the water column, mineral substrate, coral endosymbionts, and/or epilithic turf and crusts (Fork and Larkum, 1989; Schlüchter et al., 1997; Koehne et al., 1999).

The evolution of invertebrates with mineralized skeletons in the course of the early Phanerozoic provided microbial euendoliths with new substrates. Euendoliths have occupied these substrates successfully, leaving a wealth of microboring traces throughout the Phanerozoic (Glaub and Vogel, 2004; Glaub et al., 2007). Organically preserved euendolithic rhodophytes were found occupying crinoid ossicles in rocks of Upper Silurian age (Kazmierczak and Golubic, 1976). They were identified and described as *Palaeoconchocelis starmachii* (Campbell, 1980). Many microborings remained preserved as natural casts due to differential solubility of borehole fill versus bored matrix (e.g., Harris et al., 1979); however, surprisingly high number of boreholes remained empty, so that they have been successfully cast by polymerizing resins and observed by scanning electron microscopy (Golubic et al., 1970). This finding illustrates the role of microborers in contributing to the microporosity of rocks, which serve as petroleum reservoirs (Cantrell and Hagerty, 1999).

Early diversification of microboring organisms, which was initiated during Proterozoic, continued throughout the Phanerozoic. In Middle Cambrian, both body and trace fossils of microbial endoliths were present (Stockfors and Peel, 2005), and entire microboring assemblages appear to be well-established by the Ordovician times (Figure 14), with about 35% of fossil taxa that survived to the present; this record also includes the first occurrence of borings attributable to euendolithic green alga *Ostreobium* and chytrid fungi (Glaub and Vogel, 2004). In general, microboring traces constitute attractive markers for identification of paleoenvironments (bio- and litho-facies), but are less reliable as stratigraphic markers. Although microborers in their protected niches persisted through periods of major extinction, they seem to show bursts of diversification at the base of the Mesozoic and Cenozoic eras (Glaub and Vogel, 2004).

Evidence of predatory macroboring has been found in late Proterozoic strata associated with the evolution of the earliest exoskeletal organisms (Bengtson and Zhao, 1992). The diversification of macroboring organisms proceeded during the early Paleozoic when representatives of most animal phyla appeared in the fossil record (Palmer and Plewes, 1993; Wilson, 2007). The sheltered habitat that macroendoliths occupy does offer an advantage and protection from predators and may have been a strategy favored by the early selective pressure. An overview of the evolutionary history of macroborers is provided by Taylor and Wilson (2003), and the use of macroborers in stratigraphy is discussed by Bromley (2004). Boring traces of sponges and worms (e.g., *Tripanites*) were observed from the Cambrian on. Macroborers diversified thereafter and became well-established by early Ordovician times (Ekdale and Bromley, 2001) when ctenostome boring bryozoans also occurred for the first time in addition to boring sponges and worms (Mayoral, 1991). Small stellate *Dendrina*-type borings diversified in Devonian (Vogel et al., 1987) and later in the Cretaceous (Hofmann, 1996). These are now attributed to boring foraminifera (Bromley et al., 2007).

**Bioerosion, Figure 14** Resin replicas of borings in late Ordovician brachiopod *Raphinesquiana alternata* from the shallow marine facies of Tanner Formation, Richmond Group, southeastern Indiana, USA. Two out of four boring morphotypes produced by phototrophic euendoliths include: coccoid cyanobacteria (*left*) and filamentous algae with changing diameter, a possible chlorophytes. Scale bar is 100 μm long. (From Golubic, S., Knoll, A. H., and Rann, W., 1980. Morphometry of late Ordovician microbial borings, American Association of Petroleum Geologists, Bulletin, 64, 713.)
The first borings attributed to endolithic mussels appeared in the Carboniferous, whereas the first boring cirripedia were documented in Permian strata (Simonsen and Cuffey, 1980). Modern type macroboring communities became established during the Jurassic times (Fürsich et al., 1994).

Synergistic relations among agents of bioerosion

Phototrophic microbial euendoliths are distributed over large areas of coastal limestone, coral rubble, and other carbonate substrates in illuminated marine benthic habitats. Their growth and substrate penetration stabilize at the light level of compensation (where the rates of photosynthetic activity equal respiration). If left alone, the bioerosion by phototrophic euendoliths would remain limited to a few millimeters at the substrate surface. However, as primary producers, euendolithic cyanobacteria and algae attract and support a variety of grazing animals, which periodically remove layers of carbonate substrate together with the resident epiliths and some endoliths. This synergism between euendoliths and their grazers converts an intrinsically self-limited process into a progressive one with significant bioerosive impact (Schneider and Torunski, 1983). For example, the removal of a thin surface layer of the rock by hard, magnetite reinforced radula of a grazing gastropod (Figure 15) causes a displacement of the compensation depth for phototrophs deeper into the rock, leading to a resumption of boring. With continuing grazing, the horizon with euendoliths moves like a front through the rock. The abraded carbonate particles break along cyanobacterial perforations, as they pass through the digestive system and can be recognized in the sediment (Figure 16). The interaction between microboreurs and their grazers degrade coastal rocks and contribute to fine-grain sediment accumulation in the environment. A different mechanism with similar results was described for macroboring clionid sponges (Schneider and Torunski, 1983).

Grazing is a normal condition on coral reefs, so that the composition, diversity, and integrity of primary producers, which coevolved with the grazers, also depend on them. The rates of grazing and microbioerosion by euendoliths are positively correlated (Tribollet and Golubic, 2005). Moderate rates of grazing appear to have a regulatory effect by promoting high productivity-to-biomass ratio among the epilithic and endolithic cyanobacteria and algae (Vooren, 1981). In addition, moderate grazing provides new settling grounds for endoliths and coral larvae (Sammarco, 1980). The zone of active microbioerosion at any time contains only the “residual standing crop” of microboreurs, which does not include the microbial contribution already removed by grazers. These dynamic relations need to be taken into account when rates of bioerosion are calculated and attributed to different components of the system (Chazottes et al., 1995; Tribollet and Golubic, 2005).

Without grazing, the reefs would be overgrown by soft-bodied algae, as it is achieved locally by aggressive territorial damselfish that deter sea urchins and other grazing fish. Damselfishes are known to maintain and weed algal
farms, thus influencing macrobioeroding communities in the process (Sammarco et al., 1987). The results of grazer-exclusion experiments (Wanders, 1977), which showed a successional shift in species composition in favor of macroalgae and in a loss of some normally abundant and structurally important species of coralline algae, confirmed independently the experimentation performed by damselfish.

In contrast, excessive grazing is expected to be self-limiting and thus cyclical. When grazing pressure is too intense, as in the case of populations of grazing urchins studied on la Reunion Island, Indian Ocean (Chazottes et al., 2002), or in pollution impacted Faa reef at the airport of Papeete (French Polynesia; Pari et al., 1998), grazing and microbioerosion become negatively correlated (Figure 17). When grazing is too intense, euendoliths may be completely removed, thus reducing grazing while new euendoliths recolonize the barren grounds.

The optimal conditions for the interaction between euendoliths and their grazers are exemplified by the formation of intertidal bioerosional notch (Neumann, 1966) (Figure 18). Tidal ranges on rocky coasts represent an ecozone environment, a transitional zone between ecosystems that are adapted either to marine or to terrestrial environments. This transitional zone may be perceived as extreme for both. It is characterized by a gradient of variable water supply, solar irradiation, and salinity. Organisms along that gradient are arranged in distinct zones (Figure 19). Both, microbial euendoliths and their grazers optimize their activity in dependence on local water supply. Their activity, in turn, improves local water retention. The upper tidal ranges are harsh environments dominated by euendolithic cyanobacteria, often by a single species. Accordingly, the rates of bioerosion are modest. In contrast, the lower ranges with regular water supply by tides are more diversified, inviting competing microboring and grazing taxa. The coastal profile reflects the resulting bioerosion like a diagram, with the location of the notch corresponding to maximum bioerosion. The pattern of bioerosional notch formation is repeated around the margins of flat-bottom intertidal and supratidal rockpools although the species composition of the euendolith and grazer populations of the pools may be distinct from those of the large-scale coastal notches (Radtke et al., 1996).

In the supratidal ranges, the distribution of wave spray, water retention, and solar irradiation selects and controls the combined boring and grazing activity of darkly pigmented euendolithic cyanobacteria and small grazing gastropods. Consequently, the coastal limestone rocks are biodegraded selectively, a process that enhances the relief of the coastal rock surface, leading to formation of elaborate sharp-edged rock forms called biokarst (Figure 20). The term emphasizes the biological modification of the limestone dissolution forms that are characteristic of world’s karstic regions (Schneider and Torunski, 1983).

Macroborers are orders of magnitude larger than microborers (compare Figures 6 and 7) and can excavate significantly larger volumes of carbonate then microbial euendoliths but, unlike the microborers, the macroborers are not subjected to grazing pressure. Macroboring self-stabilize once they reach their adult size and thus are not part of the advancing bioerosion. Macroboring activity may lighten the reef structure but does not necessarily imperil the structural integrity of the reef’s framework (Scott and Risk, 1988); although, excessive damage to corals by macroborers makes them vulnerable to physical forces, usually associated with the passage of tropical storms (Clark and Morton, 1999). Macroboring increases porosity of rocks and promotes formation of new settling grounds and additional ecological niches, an activity that can be viewed as a positive contribution to the reef ecosystem.

Bioerosion, Figure 17 Heavy grazing by Echinometra mathaei has bypassed the experimental exposure blocks and undermined their basis. Coral reef under the influence of pollution at Faa, near Papeete, French Polynesia. The blocks are about 10 cm wide. (Courtesy of M. Peyrot-Clausade.)

Bioerosion, Figure 18 Bioerosional notch on limestone coast in protected shallow lagoonal environment at Lee-Stocking Island, Bahamas.
Production and cementation of sediment is another process that reinforces reef framework, and is in part facilitated by endolithic organisms. There is good evidence that some of the carbonate dissolved by microbial euendoliths is re-precipitated, thus contributing to formation of micritic encrustations in marine (Kobluk and Risk, 1977; MacIntyre et al., 2000) and freshwater environments (Schneider et al., 1983). Ostreobium quekettii dissolves carbonate, which then precipitates around its filaments when the alga exits the substrate and inhabits the coral pore spaces as cryptoendolith (Figure 21). Precipitation of carbonate on filaments of Ostreobium, was observed first in corals (Schroeder, 1972) and later in stromatolites and live corals (Feldmann and McKenzie, 1998; Nothdurft et al., 2007).

Rates of bioerosion
Quantification of bioerosion rates includes direct observations of behavioral patterns of particular organisms such as grazers (Hoey and Bellwood, 2008) and their selective impact (Rojtan and Lewis, 2005). A different approach involves measuring grazers’ carbonate consumption rates (Mokady et al., 1996). Other approaches involve assessment of long-term substrate removal from experimentally exposed carbonate blocks without (Kiene and Hutchings, 1992), or with identification of participating bioeroders (Chazottes et al., 1995, 2002; Tribollet and Golubic, 2005; Tribollet, 2008a). The latter approaches assess the total bioerosion rates (macrobioerosion + microbioerosion + grazing) by measuring changes in substrate size and density over time (Chazottes et al., 1995; Tribollet and Golubic, 2005). The techniques involve measurements of eroded areas in surface projection and in vertical sections, using light and scanning electron microscopy with
The euendolithic filaments of this chlorophyte produced the perforation in the skeleton (arrow), whereas the cryptoendolithic ones are covered by precipitate. Scale bar is 10 μm long.

Bioerosion, Figure 21 View of the fractured skeleton of the coral *Montastrea*, invaded by *Ostreobium quekettii*.


to grazer settlement and excluding grazers by a 250 μm size nylon mash, was estimated to be 350 g m⁻² y⁻¹ (Tudhope and Risk, 1985). These rates should vary with depth (Vogel et al., 2000), type of substrate (Perry, 1998; Zubia and Peyrot-Clausade, 2001), grazing pressure (Reaka-Kudla et al., 1996; Chazottes et al., 1995, 2002), sedimentation (Tribollet and Golubic, 2005), and eutrophication (Carreiro-Silva et al., 2005).

Tribollet and Golubic (2005) showed that carbonate dissolution by euendoliths in synergy with grazers along an inshore–offshore profile across the Great Barrier Reef was the dominant bioerosion process over time. They also quantified the combined yearly rates of grazing per 1 m² of exposed surface area to be between 0.04 and 1.8 kg of CaCO₃. Macroborers as filter-feeders were important only on inshore reefs where waters were rich in particulate matter (Risk et al., 1995). Macroborers eroded between 0.03 and 0.38 kg m⁻² y⁻¹. Combined interactive activity of grazing and microbioerosion contributes more to the total bioerosion of the reefs than macrobioerosion (Tribollet and Golubic, 2005).

Grazing rates vary depending on the organism. Rates by sea urchins are often very high. Grazing rates measured on Galapagos Islands reach up to 22 kg CaCO₃ m⁻² y⁻¹ (Reaka-Kudla et al., 1996). Mokady et al. (1996) measured consumption of grazing echinoids in the Red Sea. They observed that a meal of a sea urchin contained on the average three times more carbonate per weight than organic food. According to their calculations, removal of carbonate by a single sea urchin could reach 1 kg CaCO₃ m⁻² y⁻¹, depending on the size of the animal. The actual removal per area then depends on grazer population densities, which are commonly reciprocal to the average size of the animal. Grazing by extremely dense populations of small gastropods has been studied in the intertidal ranges (Schneider and Torunski, 1983), but has largely been ignored in the coastal subtidal ranges and on the reefs where there are also large numbers of small gastropods.

Summary: bioerosion and the carbonate budget of coral reefs

A carbonate ecosystem with a positive carbonate budget occurs when carbonate production, due to growth of calcifying organisms and precipitation of CaCO₃, is higher than carbonate dissolution and/or exportation of carbonate sediments to the open ocean (e.g., Le Campion-Alsumard et al., 1993). An unbalanced carbonate budget in favor of destructive forces can be observed in coral reef ecosystems, which are mostly made of carbonates (Peyrot-Clausade et al., 1995; Reaka-Kudla et al., 1996; Pari et al., 1998). Such a disequilibrium between reef constructive forces (production of carbonates) and reef destructive forces (mainly bioerosion) can lead to physical losses of reef framework and consequently to a loss of reef diversity (Hallock, 2005; Wilkinson, 2004).

The balance between constructive and destructive forces is critical for coral reefs, particularly in view of global warming and the expected rise in sea level. It is predicted that the rising atmospheric pCO₂ due to human activities and the consequent ocean acidification (Tribollet et al., 2006), in conjunction with the rising seawater surface temperature, overfishing, and eutrophication (Szmant, 2002), will increase the rate of coral morbidity and mortality (Mumby et al., 2001; Hallock, 2005). This may lead to a shift from coral-dominated to algal-dominated reefs, with a loss of corals as one of the main frame-builders in the reefs (Szmant, 2002; Hallock, 2005). Although the effects of the above combined factors on bioerosion processes are poorly known, the integrity of coral reefs is increasingly in jeopardy (Hoegh-Guldberg, 1999; Wilkinson, 2004; Kleypas et al., 2006).
Coral reefs protect tropical coastlines and oceanic islands (Spencer and Viles, 2002). In the past they were able to grow and keep up with changes in seawater level. In order to better monitor and to preserve coral reefs it is essential to quantify carbonate budgets accurately. Different techniques have been used to achieve this goal, including alkalinity method of seawater passing over the reefs, which measure both constructive and destructive effects simultaneously. The alkalinity method provides an instantaneous measurement of reef net calcification rate (kg CaCO3 m⁻² of planar reef area d⁻¹). This provides a fast estimate of whether the reef is accreting or dissolving (Gattuso et al., 1999; Langdon et al., 2000). However, this method does not show which organisms are responsible for carbonate production, and which for bioerosion, nor does it predict the reef’s carbonate budget over time. Additional techniques were introduced, which include monitoring of chemical and biological variables such as coral cover, coral recruitment and settlement, coral growth and calcification rates, and rates of macrobioerosion (Edinger et al., 2000). These studies have shown that inshore reefs, disturbed by sedimentation and eutrophication, had a negative carbonate budget, even without considering microbioerosion and grazing rates. However, budget calculations of “pristine coral reefs” according to the same study (Edinger et al., 2000) have to be considered with caution, because grazing and microbioerosion were not quantified, and these processes are often greater in “pristine” oligotrophic reefs than at the “disturbed” inshore reefs (Tribollet and Golubic, 2005). The studies of carbonate budget in reefs stress the importance of simultaneous analyses of constructive and destructive forces over time and under changing environmental conditions if the state of health of coral reefs, and more generally of carbonate coastal ecosystems, has to be maintained and improved.

**Bibliography**


**Cross-references**

*Algae (Eukaryotic)*

*Animal Skeletons, Advent*

*Basalt (Glass, Endoliths)*

*Biodeterioration (of Stone)*

*Biogeochemical Cycles*

*Biomining (Mineral Bioleaching, Mineral Biooxidation)*

*Biosignatures in Rocks*

*Calkified Cyanobacteria*

*Calcite Precipitation, Microbially Induced*

*Carbon Cycle*

*Carbonate Environments*

*Carbonates*

*Cyanobacteria*

*Endoliths*

*Extracellular Polymeric Substances (EPS)*

*Extreme Environments*

*Foraminifera*

*Fungi and Lichens*

*Glass*

*Geomycology*

*Ichnology*

*Karst Ecosystems*

*Microbial Biominalization*


**Biofilms, Figure 1** Cyanobacterial biofilm forming a modern aragonitic stromatolite (Walker Lake, Nevada). Biofilm is dominated by filamentous cyanobacteria – Calothrix (C). Few coccolid cyanobacteria are also present (CC). Within the youngest lithified part, remains of cyanobacterial sheets are visible (LS).

many common processes. Geoscientists have increasingly investigated the rock- and mineral-forming potentials of biofilms in the 90s of the last century (e.g., Ferris, 1991; Pedley, 1992; Reitner, 1993). Biofilms were also found in deep subsurface environments and, significantly for the Deep Biosphere, up to the upper known temperature limit of life (110–120°C) (Pedersen, 1993; Fyfe, 1996; Gold, 1999; Stetter, 1994, 1996).

Organo-films are special organic coatings which are formed by various macromolecules and do not contain living cells. Organo-films are taphonomic remains of biofilms or newly formed from biogenic degradation products (geopolymers sensu Killops and Killops, 1997; Gold, 1999). Adsorption on mineral surfaces and self-organizing catalytic processes play a central role during formation (Ferris et al., 1996; Reitner, 1993).

Stromatolites, thrombolites, and generally microbialites, are lithified products of bio- and organofilms, as well as thick microbial mats (Figure 1).

**Bibliography**


**BIOFILMS AND FOSSILIZATION**

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**Synonyms**  
Carbonaceous bacterial biofilms; Hautschatten

**Definition**  
Microbial biofilms cover normally in aquatic environments dead organisms and control partly the process of decay. The soft tissues of the body outline are preserved as a layered structure which comprises a carbonaceous microbial biofilm, in the literature often called “Hautschatten” (Figure 1). Fossils with soft part preservation are extremely rare, but under certain circumstances, like suboxic or anaerobic conditions, remains of their soft tissues with detailed histological fabrics are found. The evidence of non-biomineralized soft tissue normally degrading completely during decay is linked with special microbial processes. This exceptional preservation requires soft tissues to be replicated by authigenic minerals or pseudomorphed by bacterial biofilms (e.g., Wuttke, 1983; Toporski et al., 2002; Briggs, 2003). The different organs in many cases often exhibit different microbially controlled lithified biofilms with an unusual preservation of involved microbes.

**Geobiological implications and examples**  
Understanding the relationship between decay and mineralization is of fundamental importance to palaeontologists interested in the record of soft-bodied organisms. Soft-bodied fossils also provide insights into early diagenetic processes in sediments and give basic information on related organic geochemistry. The lack of biomineralized hard parts in many organisms is the normal condition in the fossil record. However, the mode of soft-tissue decay is far from uniform and the sum of various processes results in a preservation in so-called Konservat-Lagerstätten (Seilacher, 1970; Seilacher et al., 1985).

Derek Briggs and his group have made fundamental experiments on how the preservation of soft-tissues works (e.g., Briggs, 1999, 2003; Briggs and Kear, 1993; Briggs and Wilby, 1996; Grimes et al., 2001; Gupta et al., 2006). For example, they could demonstrate that initial pyritization of a plant tissue can be an extremely rapid process (within 80 days) and is driven by anaerobic bacterially mediated decay. They have made comparable experiments mainly with arthropods. Toporski’s and coworkers’ (Toporski et al., 2002) investigation on the Oligocene Enspel Maar black shale is a milestone in the research on soft-tissue preservation via microbial biofilms. The multitask biogeochemical analyses give the deepest insight into the taphonomy of soft tissue and microbial-related decay processes. The most famous locality with preserved soft tissues is the Eocene Messel locality. From this locality, soft tissues covered with lithified biofilms were first described by Wuttke (1983). Thick microbial biofilms are generally much darker than the surrounding black-shale and form the so-called “Hautschatten” and were for a long time believed to represent fossilized soft tissues. The biofilms trace back the decaying soft tissue in an anaerobic environment and “casting” of the former soft tissues. Often the digestive process...

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**Biofilms and Fossilization, Figure 1**  
A very well-preserved mouse (*Apodemus atavus*) from the late Pliocene fossil lagerstätte Willershausen (Lower Saxony, Germany) with perfectly preserved biofilm-related “Hautschatten” with visible remains of the former fur (Geoscience Museum University of Göttingen).
system of large fossils is excellently preserved (Franzen, 1994). Fundamental studies on biofilm soft-tissue relationship were carried out by Richter (1994) and Liebig (1997). Another intriguing example is the “soft-tissue” preservation in dinosaur bones (Kaye et al., 2008). Kaye and coworkers have found microbiobially altered soft tissue in a dinosaur bone and they give a critical explanation of this fact. Within their paper they discuss the processes of tissue preservation and also demonstrate the importance of recent microbial biofilm invasion and late diagenetic alteration of primarily formed pyrite frambooids. The clou of this investigation is also that there is no real soft-tissue preservation—however, preserved biofilms cast the former tissue and give an idea about the original structures.

All “Hautschatten” soft-tissue preservation are probably preserved lithified biofilms and not real soft tissues.

Conclusion

Biofilms play a significant role in suboxic and anaerobic milieu preserving soft tissue remains. The body outline of dead organisms is exactly traced by the margins of the mineralised biofilms and give a perfect image of the entire fossil. These structures are often called after the German word “Hautschatten”-perservation and is commonly found under black shale condition.

Bibliography


Cross-references

Bacteria

Beggiatoa

Biofilms

Biomarkers (Molecular Fossils)

Black Shales

Detachment

Ediacaran Biota

Mat-Related Sedimentary Structures

Microbial Biomineralization

Microbial Communities, Structure, and Function

Microbial Degradation

Organomineralization

Thirotrophic Bacteria

Whale and Wood Falls

BIогЕохЕмICAL CYCLES

A biogeochemical cycle defines the pathways by which chemical elements occurring in organisms are cycled between different living and nonliving compartments on the Earth (e.g., biosphere, atmosphere, hydrosphere, geosphere, etc.). Typical driving forces of a biogeochemical cycle are the metabolisms of living organisms, geological processes, or chemical reactions. For specific reading on major biogeochemical cycles, please refer to “Carbon Cycle,” “Nitrogen,” “Sulfur Cycle,” “Phosphorus, Phosphorites.”

BIОLOGICAL CONTROL ON DIAGENESIS: INFLUENCE OF BACTERIA AND RELEVANCE TO OCEAN ACIDIFICATION

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Introduction

Soon after carbonate mineral particles are deposited, a series of chemical, physical, and biological processes start that can significantly alter their mineralogy, isotopic composition and chemistry, and the fabric of the accumulating sediment. These processes include dissolution and precipitation reactions, abrasion, and transport,
ingestion and boring by organisms. The processes occurring after deposition fall into the category of diagenesis. In particular, carbonate sediments consisting of the minerals low (<4 mol% MgCO₃) and high (>4 mol% MgCO₃) biogenic and inorganic magnesian calcite and aragonite and organic matter deposited in shallow waters are repeatedly deposited, resuspended, and then deposited again during periods of elevated turbulence, which are common especially during storms. Consequently, carbonate minerals may be subjected to several depositional environments before being permanently buried in a sediment. The study of carbonate diagenesis is further complicated by the fact that significant amounts of shallow-water carbonates are commonly associated with reefs, for which traditional models of sediment diagenesis are not always appropriate.

Soon after deposition, chemical and physical changes occur in carbonate sediments as a result of a myriad of diagenetic processes. Among the most important are dissolution-precipitation reactions and major alterations of pore water chemistry by bacterially mediated oxidation of organic matter. These changes generally occur most rapidly in the upper few to tens of centimeters of sediment. It is this topic that is most fully addressed in this article. In addition, transport within the sediment of both solid and dissolved components is also of fundamental importance in diagenesis. It can be caused by physical stirring resulting from the movement of infaunal organisms (e.g., worms, bivalves, shrimp), and is called bioturbation. Closely associated with bioturbation is bioirrigation, which is a result of organisms pumping water through their burrows. Deeper within the sediment and thus later in its history, advection of pore water and molecular diffusion in pore water are generally the dominant transport processes.

The diagenetic equation

Since the early 1960s, considerable effort has gone into modeling the diagenesis of sediments. A central theme of most models is that diagenetic change results from the sum of transport and chemical and biological reactions of various types. Diagenetic models can be divided into those assuming constant conditions (steady-state models) and those taking into account factors that may vary with time (e.g., temperature, salinity, material input rates, water depth). The primary idea in the steady-state models is that a property in the sediment at a given depth does not change with time. Steady-state models are generally more amenable to a mathematical solution than non-steady-state models. However, diagenesis in many shallow-water carbonate sediments is significantly influenced or even dominated by non-steady-state processes.

Dissolution of carbonates

Continental shelves and slopes comprise about 20% of the Earth’s surface, and contain more than half of the sediments in the ocean. Recent estimates of marine carbonate burial rates indicate that between 35% and 70% of Holocene carbonate deposition has taken place on continental shelves and today’s production of shallow-water biogenic carbonate (“calcareous”) skeletons and tests represents about 28% of the total carbonate produced in the world’s oceans.
A substantial fraction (>50%) of shallow-water produced CaCO₃ is at present time permanently buried in the sediments. In contrast, most of the CaCO₃ produced in the open ocean mainly by planktonic organisms such as coccolithophorids, forams, and pteropods dissolves before it reaches the ocean floor. As much as 50–90% of the material may dissolve in the upper 1,000 m owing to microbial decomposition of organic matter creating microenvironments corrosive to CaCO₃ particles. Typical examples of such environments include inside the guts of zooplankton or within aggregates of CaCO₃ and organic material referred to as “marine snow.” Carbonate material making it past the depth of the saturation horizon, the thermodynamic equilibrium boundary between the forward and backward reactions of precipitation and dissolution, will consequently become subject to dissolution regardless of the activity of microbes and the formation of microenvironments.

Since most shallow-water environments (coastal zone, continental shelf, banks, reefs, etc.) at present time are immersed in seawater supersaturated with respect to calcite, aragonite, and most Mg-calcite phases, dissolution of sedimentary CaCO₃ minerals in these environments is completely driven by microbially mediated processes associated with oxidation of organic material in the pore water-sediment system or boring autotrophic or heterotrophic microorganisms referred to as endoliths. In the presence of oxygen, the extent of CaCO₃ dissolution is almost completely controlled by the amount and the extent of organic matter decomposed or respired, but under anoxic conditions, oxidation of organic matter accompanied by reduction of sulfate can produce radically different results. Most studies of the impact of chemical diagenesis on the carbonate chemistry of anoxic sediments have focused primarily on the fact that sulfate (SO₄²⁻) reduction results in the production of alkalinity (mostly dissolved bicarbonate [HCO₃⁻], carbonate [CO₃²⁻] and carbonic acid [H₂CO₃] and CO₂ chemical species), which can cause precipitation of carbonate minerals (e.g., Berner, 1971). The reduction of the sulfate is promoted by bacteria, particularly Desulfovibrio desulfuricans, splitting the sulfur–oxygen bond and leading to the accumulation of dissolved chemical species of reduced sulfur (mainly H₂S and HS⁻; these sulfur species may contribute to the total alkalinity of the pore waters) in the pore waters and in sediments containing iron as ferrous sulfide minerals (mainly pyrite, “fool’s gold,” FeS₂) and free solid sulfur. Although the reaction is complex, this process can be schematically represented, using the average composition of organic matter of phytoplankton as substrate material, as:

\[
(CH₂O)_{106}(NH₃)_{16}H₃PO₄ + 53SO₄²⁻ = 106HCO₃⁻ + 53HS⁻ + 16NH₃ + H₃PO₄ + 53H⁺,
\]

which results in the commonly observed decrease in sulfate ion, and pH (measure of hydrogen ion concentration, H⁺), and increase in total alkalinity, hydrogen sulfide (H₂S), phosphate (PO₄³⁻), ammonia (NH₃), and total dissolved inorganic carbon (DIC) in sediment pore waters with increasing depth below the sediment–water interface.

During the early stages of sulfate reduction (~2 to 35%) (Figure 2), this reaction may not cause precipitation,
but rather results in undersaturation conditions with respect to carbonate minerals because the impact of lowered pH (increasing H+) has a larger negative effect on the total alkalinity than the positive effect on this chemical property arising from reduction of sulfate. Carbonate ion activity decreases rapidly as it is “titrated” by CO₂ from organic matter decomposition abetted by bacteria leading to a decrease in pore water saturation state. This process is evident in observational data for Fe-poor shallow water carbonate sediments from the Bahamas and has been confirmed in recent studies of sediments from Florida Bay, in the Checker Reef of Kaneohe Bay, Oahu, Hawaii, and in organic-rich sediments of Mangrove Lake, Bermuda (Morse and Mackenzie, 1990) and elsewhere. In Mangrove Lake, it was shown based on model calculations that the degree of undersaturation of anoxic pore waters with respect to carbonate mineral compositions can depend significantly on the carbon to nitrogen ratio (C/N) of the labile organic matter undergoing bacterial oxidation in the sediment pore water (Figure 3).

This sulfate reduction reaction in anoxic carbonate sediments has potential importance for carbonate dissolution in shallow-water, marine environments, but its global significance remains a question. An observation of interest is that even complete sulfate reduction can return the saturation state of the pore water only to about half of its original value. Thus, the sulfate reduction reaction by itself may not promote carbonate precipitation as total alkalinity increases and partial sulfate reduction may result in carbonate dissolution. The combination of the sulfate reduction reaction and reactions primarily with iron and manganese oxide minerals in the sediments can lead to significant calcium carbonate precipitation. This type of net process can be represented schematically as (where CH₂O is a simplified formula for organic matter):

\[
\text{9CH}_2\text{O} + 4\text{SO}_4^{2-} + 4\text{FeOOH} = 4\text{FeS(solid)} + 9\text{HCO}_3^- + \text{H}^+ + 6\text{H}_2\text{O}. \tag{3}
\]

Reaction 3 results in the pH of the waters, in the absence of carbonate precipitation, being buffered at higher pH values. It is, therefore, reasonable to expect that the effectiveness of sulfate reduction in producing carbonate dissolution or precipitation may depend in part on the availability of reactive iron. This conclusion has been demonstrated in a study of the pore water geochemistry of aluminosilicate and carbonate-rich sediments from Kaneohe Bay, Oahu, Hawaii. The aluminosilicate sediments contain abundant pyrite whereas the pyrite content of the carbonate sediments in the bay is low. Pore waters collected from the aluminosilicate sediments have higher pH values than those collected from the carbonate-rich sediments. This observation is a result of the pH values in the pore waters of the aluminosilicate sediments being buffered at higher values than those for carbonate sediments because of Reaction 3 (Mackenzie et al., 1981).

There are several processes that have been identified that can cause undersaturation of pore waters with respect to carbonate minerals, in addition to the previously discussed undersaturation conditions that may result during the early stages of sulfate reduction. These are early post-death microenvironments within organisms, oxidation of organic matter by processes preceding sulfate reduction, and oxidation of sulfides. In addition, both autotrophic and heterotrophic endoliths actively penetrate carbonate substrates by creating corrosive conditions with respect to these mineral phases. These processes commonly will be most important near the sediment–water interface. The most important process occurring prior to the onset of bacterial sulfate reduction is extensive organic matter degradation due to bacterially mediated oxygen reduction, a process important in both shallow-water and deep-sea carbonate sediments. The influence of benthic bacterial activity under aerobic conditions on carbonate mineral dissolution was nicely demonstrated by Moulin et al. (1985) for pore waters from sediments of the Gulf of Calvi, Corsica. Under aerobic conditions, the oxidation of organic matter may be written:

\[
\text{CH}_2\text{O} + \text{O}_2 = \text{CO}_2 + \text{H}_2\text{O} = \text{H}_2\text{CO}_3, \tag{4}
\]

and if carbonate minerals were to dissolve because of the CO₂ produced by organic matter decomposition, then the reaction:

\[
\text{CaCO}_3 + \text{H}_2\text{CO}_3 = \text{Ca}^{2+} + 2\text{HCO}_3^- \tag{5}
\]

The overall net Reaction of 4 + 5 is:

\[
\text{CaCO}_3 + \text{CH}_2\text{O} + \text{O}_2 = \text{Ca}^{2+} + 2\text{HCO}_3^- \tag{6}
\]
Reaction 6 is possible only if the pore waters are undersaturated with a carbonate phase, and there are no other competing reactions, like significant ammonia release from decaying organic matter, to increase pore water saturation state. Figure 4 shows the increase in total alkalinity versus increase in total CO₂ (DIC) as observed in shallowly buried pore waters in the Gulf of Calvi Lithothamnion sp. algal-rich sediments and in low and high magnesian calcite- and aragonite-rich sediments from Mangrove Bay, Bermuda. The slopes of the regression lines for the relationships are close to 1. The slope indicates that Reaction 6 most likely gives rise to the increased total alkalinity observed in these pore waters; that is, the H₂CO₃ produced by organic matter oxidation is stoichiometrically utilized in dissolution of a carbonate mineral phase in the sediment. Close inspection of the regression line for the Gulf of Calvi sediments in Figure 4 shows that it does not pass through the origin. This is because about 0.1 mmol kg⁻¹ of H₂CO₃ has been added to the pore waters from aerobic bacterial respiration prior to carbonate dissolution. This initial input of H₂CO₃ lowers the saturation state of the shallowly buried pore waters to that required for carbonate mineral dissolution. These pore water observations have been confirmed in experiments using a biological reactor, and from solid phase mineralogical studies. High magnesian calcites are dissolved preferentially in these sediments, although the sediment contains a mixture of nearly pure calcite, aragonite, and magnesian calcites with up to 20 mol% MgCO₃. In the Gulf sediments composed principally of the red alga Lithothamnium sp., this early diagenetic reaction has resulted in dissolution of 75% of the carbonate initially deposited. Furthermore, in the case of the study of Mangrove Bay, the pore waters were sampled every few hours during a complete, 24 h, diel cycle. The trend and relationship between changes in total alkalinity and total DIC are essentially the same as observed in the sediments of Gulf of Calvi. In addition, sea grasses, such as Thalassia, which covers vast areas of the bottom in Mangrove Bay probably enhances carbonate dissolution in these sediments by releasing oxygen through its roots and rhizomes into the sediments that consequently enhances decomposition of organic matter and production of H₂CO₃. Evidence from the Bahamas bank has clearly demonstrated enhanced calcium carbonate dissolution owing to this process (Burdige and Zimmerman, 2002).

Other reactions of less importance than those above leading to undersaturated conditions with respect to calcium carbonate near the sediment–water interface include nitrate reduction and fermentation (e.g., Aller, 1980). Such reactions may also be important near the sediment–water interface of continental shelf and slope sediments, where bioturbation and bioirrigation can result in enhanced transport of reactants. Generally, as the water depth increases over continental slope sediments, the depths increase within the sediment at which significant sulfate reduction and reactions other than sulfate reduction influence carbonate chemistry.

**Carbonate dissolution and ocean acidification**

Future increases in atmospheric CO₂ owing to human activities, such as burning of fossil fuels and land-use changes, result in increased uptake of part of this CO₂ by ocean surface seawater and formation of carbonic acid. As the term implies, dissociation of carbonic acid produces hydrogen ions, which results in increasing acidity and decreasing pH of seawater and therefore the name ocean acidification. Surface seawater pH has already decreased by approximately 0.1 pH units since the onset of the Industrial Revolution and is anticipated to decrease by another 0.2–0.3 pH units by the end of the twenty-first century (e.g., Caldeira and Wickett, 2003). Oceanic uptake of CO₂ also produces an increase in the seawater concentration of bicarbonate ions (HCO₃⁻) and a decrease in the carbonate ion concentration (CO₃²⁻). As a consequence, the saturation state with respect to calcium carbonate minerals (Ω), which is determined by dividing the in situ ion concentration product of calcium and carbonate ions by an experimentally determined solubility product (Ω = [Ca²⁺][CO₃²⁻]/Kₛ), will decrease. A lower carbonate saturation state of surface seawater implies that the initial composition of marine sediment pore waters will be closer to undersaturation with
respect to carbonate minerals, and thus, microbial decomposition of less organic material is required to produce undersaturated conditions and subsequent dissolution of carbonate mineral phases in the pore water–sediment system. Furthermore, while most shallow-water environments at present time are supersaturated with respect to calcite, aragonite, and most Mg-calcite phases (in general, Mg-calcite containing 8–12 mol% MgCO3 or more is more soluble than both calcite and aragonite), surface seawater in high and intermediate latitude environments could become undersaturated with respect to most Mg-calcite phases and even aragonite during the present century. Consequently, as a result of human-induced ocean acidification, dissolution of carbonate minerals and sediments in shallow-water ocean environments is most likely to increase.

Increased dissolution of calcium carbonate minerals and sediments owing to human-induced ocean acidification will most likely occur selectively according to mineral stability. This could lead to a change in the average mineral composition of contemporary carbonate sediments in favor of stable mineral phases such as calcite and low Mg-calcite phases over metastable phases such as aragonite and high Mg-calcite. Although such a transition is slow relative to the rate at which seawater chemistry is changing owing to human activities, evidence for increasing carbonate dissolution and changing sediment composition exists based on numerical model simulations, satiumetry, and dissolution experiments, and mesocosm experiments, as well as from observations from natural environments. One such environment is Devil’s Hole, located within Harrington Sound, Bermuda. During summertime, the water column of Devil’s Hole becomes significantly stratified owing to the exponential decrease of solar irradiance as a function of depth. Organic matter produced in the surface mixed layer sinks and is decomposed by microbes in the subthermocline layer. As a consequence, the CO2, pH and the seawater saturation state with respect to carbonate minerals in this layer reach values typically not observed for most shallow-water environments today, but similar to values anticipated during the next several decades of the twenty-first century as a result of ocean acidification. In spite of significant production of fine grained Mg-calcite minerals throughout Harrington Sound, for example, by coralline algae, forams, and echinoderms, as well as the abundant occurrence of these mineral particles in sediments located at depths above the thermocline, little evidence exists for their presence in the sediments of Devil’s Hole. These mineral phases are most likely selectively dissolved, a conclusion that is also supported by observed changes in seawater carbonate chemistry in the subthermocline layer in Devil’s Hole (Figure 5; Neumann, 1965; Andersson et al., 2007).

In addition to the chemical changes imposed on surface seawater owing to rising atmospheric CO2, future dissolution of shallow-water carbonate minerals could also increase owing to increased deposition and subsequent increased microbial decomposition of organic matter in marine coastal sediments. Human activities on land have significantly increased the amount of organic matter transported via rivers and runoff to the global coastal ocean relative to preindustrial conditions, and the total amount is expected to continue to increase with increased
deposition of this material to the sediments. Furthermore, input of nutrients to the global coastal ocean region have also significantly increased, which may stimulate increased production of organic matter and subsequent deposition of this material to the sediments within this region. As long as surface seawaters are supersaturated with respect to most carbonate phases, microbial decomposition of organic material is the main driver of carbonate dissolution. Consequently, even if the organic material deposited to the sediment–water remained constant, but the microbial activity increased, for example, owing to increasing temperature, carbonate dissolution would also increase. For the next several decades, human activities are likely to continue to lead to acidification of surface seawater and continuous increases in the amount of organic material being deposited to shallow-water sediments, and concurrently with increasing atmospheric concentrations of CO2, surface and sea surface temperatures are likely to increase with potential increases in microbial and endolithic activity. As a result, dissolution of calcium carbonate minerals, particularly in coastal ocean environments, probably will increase due to all of these forcings. It is possible that under a business as usual scenario of fossil fuel CO2 emissions, the production of carbonates in the global coastal ocean will be exceeded by their dissolution by year 2150 or earlier.

**Summary**

Diagenesis refers to those physical, chemical and biologic processes that alter the original physical and chemical characteristics and properties of minerals and sediments. Calcium carbonate minerals constitute a substantial fraction of shallow-water sediments and may be subject to significant diagenetic alterations after deposition including dissolution and precipitation reactions. These reactions are largely mediated by microbial processes including oxidation of organic matter under both oxic and anoxic conditions as well as by endoliths, which actively penetrate carbonate substrates. The extent of calcium carbonate dissolution is to a large degree controlled by the amount of organic matter decomposed. Carbonate dissolution is likely to increase in the future in response to human-induced ocean acidification arising from the burning of fossil fuels, but also due to increased riverine transport of organic matter and nutrients to the coastal ocean, enhanced production in situ of organic matter in coastal waters, and subsequent deposition and microbial decomposition of the organic matter in marine sediments.

**Acknowledgments**

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**Bibliography**


**Cross-references**

Alkalinity
Biogenic
Calcrete Precipitation, Microbially Induced
Carbon (Organic, Degradation)
Carbonate Environments
Carbonates
Endoliths
Iron Sulfide Formation
Microbial Biominalization
Pore Waters
Sediment Diagenesis – Biologically Controlled
Sulfate-Reducing Bacteria

**BIOLOGICAL VOLCANIC ROCK WEATHERING**

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**Definition**

The biologically induced breakdown of volcanic rocks, including crystalline rocks and glasses.

**Volcanic rock weathering and global processes**

The weathering of volcanic minerals is known to make a significant contribution to the global silicate weathering budget (Dessert et al., 2003), thus influencing carbon dioxide drawdown and climate control, since carbon
dioxide is consumed in mineral weathering reactions. For example, on land, the Deccan Traps in India, with an estimated area of $10^6$ km$^2$, is thought to account for about 5% of the global silicate weathering flux (Dessert et al., 2003). In total, basalt rocks may account for over 30% of the global carbon dioxide drawdown in silicate weathering (Gaillardet et al., 1999). The weathering of volcanic rocks also contributes a significant flux of nutrients into the biosphere, and through the weathering of rocks and the formation of clays and secondary minerals, microorganisms accelerate the rate of production of volcanic soils (Andisols) from their parent minerals. Thus, understanding how volcanic rocks weather and what role a biota might play in these processes has importance for understanding climate control, soil formation, and the mechanisms governing the release and distribution of nutrients in the biosphere.

The biological role
The role of plants in accelerating the weathering of volcanic rocks has been established, primarily from studies in Hawaii and Iceland. In Hawaii, plants have been estimated to increase weathering rates by over an order of magnitude compared to unvegetated areas (Cochran and Berner, 1996). In geological terms, the acceleration of weathering caused by plants following their early evolution has been proposed as one mechanism for carbon dioxide drawdown in the late Silurian, resulting in a general cooling trend (Berner, 1992). Plants increase weathering rates by, inter alia, physically disaggregating the rock with their roots, the production of rock weathering organic acids, the removal of ions from the subsurface (which changes chemical equilibrium in favor of rock dissolution), and by indirectly producing nutrients and favorable microenvironments for prokaryotes and fungi within the rhizosphere, which themselves weather rocks (see below).

A microbiological involvement in volcanic rock weathering has been established. The primary effect of microorganisms is to accelerate the breakdown of the rock both physically and chemically, often with the formation of secondary minerals such as poorly ordered aluminosilicates and clays (Figure 1). Early studies on the biological weathering of volcanic rocks focused on the role of lichens (Jackson and Keller, 1970; Adamo and Violante, 1991, 2000; Banfield et al., 1999). The earliest study of biological volcanic rock weathering was the seminal study by Fry (1927) who showed that lichens can cause etching, fragmentation, and weakening of obsidian, a silica-rich volcanic glass. Lichens accelerate the physical disaggregation of volcanic rocks as the lichen thallus penetrates the rock substrate. The production of organic acids, organic complexing agents, and the excretion of oxalic acids are just some of the mechanisms by which lichens contribute to the chemical dissolution of the rock substrate (Adamo and Violante, 2000). Oxalic acids are produced by the fungal component in the lichen symbiosis. Calcium oxalate at the lichen–rock interface, although not ubiquitous, is one widely reported compound formed on lichen-weathered rocks, which suggests the role of oxalic acids reacting with mineral-derived calcium, although other oxalates, such as copper- and manganese-rich compounds, have been reported.

Lichens have been shown to induce the formation of considerably thicker weathering rinds on crystalline basalts in Hawaii compared to control rocks, which in addition to directly contributing to weathering, may secondarily accelerate the rate of abiotic chemical weathering (Jackson and Keller, 1970). Lichens may also accelerate the dissolution of the silicate from the rock matrix, causing it to be more susceptible to being washed away, promoting the preferential formation of oxides and other secondary minerals from iron. Iron oxide formation is reported at the interface between lichens and basaltic rocks in Hawaii and near Vesuvius, Italy.

Early studies on lichen weathering of volcanic rocks were the first to elucidate two important factors in microbial rock weathering in general: (1) the role of changes in the microenvironment induced by microorganisms at the rock surface, which include changes in pH, water retention, and redox state that alter the equilibrium chemistry of rock dissolution or secondary mineral formation, accelerating rock weathering; and (2) the differential susceptibility of minerals to microbial rock weathering. Calcium-containing plagioclase tends to weather faster than minerals, such as K-feldspars, and these differential effects are determined by the susceptibility of minerals to weathering agents produced by microorganisms or the changes in chemical equilibria.

Since early studies on lichens, many other microorganisms have been examined and found to be capable of accelerating rock weathering. Mechanisms of microbial weathering include: the production of organic acids; the secretion of protons, which changes the local pH at the
Microorganisms do not merely accelerate the weathering of all elements from volcanic rocks. A number of studies have shown that biotic weathering can cause preferential leaching or enrichment of elements at the weathering front or within the sediments produced by volcanic rock weathering. Staudigel et al. (1998), in a study of basaltic glass weathering using a microbial enrichment culture from Loihi seamount, Hawaii, showed that the population of microorganisms, which included heterotrophic bacteria, cyanobacteria, and diatoms, induced an enrichment of calcium in the sediments produced by weathering, but a loss of magnesium, in contrast to the controls which showed the opposite trend. These results, and many others, show that microorganisms can cause a differential rate of leaching of different elements from rocks. Bacteria on their own have been shown to induce the release of iron and manganese. Investigations using a single bacterial species (Burkholderia fungorum) (Wu et al., 2007) showed that iron release is induced by changes in the pH at the rock surface. Phosphorus released from the apatite within the basalt rock apparently also helped sustain bacterial growth. All of these experiments show that microorganisms potentially have an important role in modulating the release of different elements from the crust. The oxidation of iron by bacteria in the deep-sea probably gives them an important role in iron cycling and thus weathering of the basalt crust (Edwards et al., 2003).

These data also highlight the general point that although the role of microorganisms in enhancing mineral dissolution has been a major focus of research, it is also important to understand that the weathering of elements from rocks might be influenced by the retardation of elemental release caused by microbial action. For example, the production of oxidized iron from reduced iron in basaltic minerals such as olivine has been suggested to retard leaching of elements from the rocks (Santelli et al., 2001). Thus, through changes in oxidation states of elements and the production of secondary minerals at rock surfaces, the microbial involvement in volcanic rock weathering may be much more complex than merely enhancing weathering rates, instead changing the leaching rates of specific elements in ways that are either retarded or accelerated compared to abiotic conditions.

The formation of clays and poorly ordered aluminosilicates, such as smectites and allophane-like materials, has been reported around bacterial aggregates in volcanic ash near Sakurajima volcano, Japan (Kawano and Tomita, 2001). The authors, by carrying out batch culture experiments in the laboratory using bacteria isolated from the ash, suggest that the natural populations are involved in biomineralization and therefore play an important part in bacterial alteration of the weathered volcanic ash. In this case mineral formation is likely to have been facilitated by the bacterial cell surface. Although this work did not show active weathering of the primary rock minerals, it illustrates that the formation of clays and other minerals from weathered volcanic ash by microorganisms can play an important role in defining the chemical characteristics of the weathering environment, which itself will alter the environment for other members of the microbial community which may be directly involved in weathering. The work underlines the important link between rock weathering (dissolution and alteration) and mineral formation (biomineralization) during the transition of primary volcanic minerals to soils such that both processes must be considered to truly understand biological volcanic rock weathering and soil neogenesis.

As a logical development of the work of Fry, Herrera et al. (2008) examined bacterial communities within obsidian in Iceland and showed a diverse population of bacteria, which by the use of hybridization probes were shown to be associated with weathering alteration fronts in the rock. Basaltic rocks in terrestrial environments, as with the oceanic environments described above, have been shown to harbor a high prokaryotic diversity (Gomez-Alvarez et al., 2007).

The mechanisms by which microorganisms weather rocks are still based primarily on chemical measurements such as organic acid production or changes in solution pH. New molecular methods are likely to elucidate pathways by which microorganisms can weather rocks and strengthen our understanding of the link between the organisms and the weathering process.

**Microbial borings in volcanic glass**

Other evidence for a direct role of microorganisms in volcanic rock weathering has been presented in the form of channels or pitted and corroded granular textures, in reactive volcanic glasses. The most commonly studied of these are endolithic borings into volcanic glasses, including...
deep ocean pillow lavas (Fisk et al., 1998; Torsvik et al., 1998; Thorseth et al., 2003; Storrie-Lombardi and Fisk, 2004). Furnes and Staudigel (1999) suggest a dominant role for bioalteration of volcanic glasses in the upper 300 m of oceanic crust. Glass recovered by drilling from more than 250 m below the sea floor shows channels into altered zones that are 1–10 µm wide and penetrate to a depth of 150 µm. In other settings, larger tubes up to 20 µm in diameter and with a depth of 0.5 mm have been observed. The provenance of some of these borings has been controversial since the organisms responsible for the borings have not been cultured. Abiotic ambient inclusion trails, generated by the pressure forcing of mineral grains, such as sulfides, through glass have been suggested as a mechanism for the production of channel features (Figure 2). To address this possibility a number of approaches have been used to show an involvement of biological activity. These include isotopic and elemental analysis. Binding of the nucleic acid stain DAPI (4',6-diamidino-2-phenylindole) to DNA and fluorescence signals obtained using fluorescent in situ hybridization (FISH) probes against both archaea and bacteria in the channels suggest that organisms were responsible for the weathering. Microprobe analysis shows the presence of carbon not associated with carbonates. Low carbon isotope values were also suggested as evidence supporting a biological role in glass alteration.

Early Earth studies and astrobiology

Volcanic terrains would have dominated the landscape of the Archean Earth when life emerged about 3.5 billion years ago. One reason for the keen interest in weathering textures in volcanic glass is the discovery of similar features in ancient Archean volcanic rocks (Furnes et al., 2004). Insofar as channels are morphologically more distinctive than, for example, irregular pitted features, then they might offer evidence for life in early Earth environments. The age dating of materials with biological isotopic signatures has been used to argue that fossils of putative ancient microbial volcanic glass borers are the same as the age of the rocks (Banerjee et al., 2006). The study of microbial volcanic rock weathering and the textures and minerals left by such interactions may also offer a means to assay ancient Martian terrains for evidence of life (McLoughlin et al., 2007), particularly since Mars, in the absence of plate tectonics, is a planet dominated by volcanic terrains. On a more general level, understanding the interaction of microorganisms with volcanic rocks may provide a means to understand whether the formation of clays and other secondary minerals from basalts on Mars is a purely abiotic process or whether it could ever have been biologically mediated.

Conclusion

Both field and laboratory observations show that plants and microorganisms play an important role in volcanic weathering. The mechanisms by which they achieve this are diverse, but reduce to some common principles which include physical disruption, production of compounds that attack minerals, alteration of chemical equilibria in favor of dissolution, and changes in redox states of metals which favors dissolution. Through their actions, the biota generally accelerates rock weathering, contributing to the release of rock nutrients into the environment and silicate weathering reactions which themselves contribute to long-term carbon dioxide drawdown.

Bibliography


Cross-references

Astrobiology
Basalt (Glass, Endoliths)
Biodeterioration (of Stone)
Bioerosion
Biosignatures in Rocks
Fluorescence In Situ Hybridisation (FISH)
Glass

BIOMARKERS (MOLECULAR FOSSILS)

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Synonyms

Biological marker molecules; Geochemical fossils; Molecular fossils

Definition

Biomarkers are the molecular fossils of lipids and other natural products. In sedimentary environments, lipids that escape the remineralization process are commonly chemically reduced to hydrocarbon skeletons. Encased in sedimentary rocks, these skeletons can remain intact over hundreds of millions of years. The structure of biomarkers is often directly related to their precursor lipids and may be diagnostic for a specific group or groups of organisms. Diagnostic biomarkers are used to obtain information about the composition of past (microbial) ecosystems or to determine the first occurrence of organisms in the geological record. As many organisms prefer specific habitats, and as lipid compositions of individual organisms are frequently adjusted to changing physical and chemical conditions, biomarkers may also serve as paleoenvironmental proxies, for example for salinity, temperature, and oxygen availability. In the petroleum industry, biomarkers are utilized to elucidate the extent of biodegradation of crude oil and to obtain information about the thermal history, lithology, depositional environment, and geological age of hydrocarbon source rocks.

BIOMARKERS (MOLECULAR FOSSILS)
Other meanings of “biomarker”

**Environmental studies:** intact and predominantly functionalized biomolecules in recent sediments, including ancient DNA. **Medicine/pharmacology:** a natural or introduced molecule or substance in an organism that indicates the presence or state of a specific disease. **Toxicology:** a molecule or substance that indicates exposure of an organism to a toxin. **Cell biology:** a molecule that facilitates the detection and isolation of specific cell types. **Genetics:** a DNA fragment that causes disease or contains information about susceptibility to a disease. **Paleobiology:** mineralogical, elemental, isotopic, and morphological indicators for the presence and activity of life in the geological record. **Astrobiology:** anything that could yield experimental evidence for the existence, past or present, of life in our solar system and beyond.

**History**

Today, crude oil is indisputably recognized as a concentrate of biomarkers and other molecular alteration products of biological organic matter. The hypothesis about a biological origin of petroleum was probably first formulated by the Russian scholar Mikhail Lomonosov in 1757. However, in the nineteenth century, partly based on observations that organic molecules can be generated in the laboratory from inorganic ingredients, most scientists supported an inorganic theory of petroleum formation. The prominent French chemist Marcellin Berthelot suggested in 1866 that petroleum is the product of the reaction of water with inorganic carbides (Berthelot, 1866 and references therein), and Dmitri Mendeleev (1878) insisted that petroleum is abiogenic and forms deep in the Earth’s crust. The first studies that yielded systematic evidence for an organic origin of crude oil were carried out by petroleum geologists in the late nineteenth to early twentieth century (see Durand, 2003). Geologists found correlations between the location of petroleum reservoirs and the thermal maturity of associated sedimentary organic matter, and chemists were able to generate petroleum-like oils by heating animal products and black shales under the exclusion of oxygen (pyrolysis). As a consequence of these studies, it was well accepted among North American geologists in the 1930s that petroleum is generated by thermal degradation of organic matter in sedimentary rocks. In Russia in the 1920s, the organic theory of petroleum formation was defended by Vladimir Vernadsky. Vernadsky, clearly ahead of his time and now regarded as one of the founders of organic geochemistry, had already integrated his concepts of petroleum formation into a general framework of biogeochemical cycles (e.g., Vernadsky, 1934). However, the first undeniable proof that bitumen in sedimentary rocks contains molecules of biological origin was presented by Alfred Treibs. In 1934, Treibs isolated a vanadyl-porphyrin complex (vanadyl-deoxyphylloerythroetioporphyrin I) from a bituminous black shale. He found that the nitrogen-carbon skeleton of the red-colored sedimentary porphyrin was virtually identical to the green plant pigment chlorophyll $a$ II. He showed that the fossil pigment had been generated from a biogenic precursor through replacement of the central magnesium ion with vanadyl oxide and loss of several functional groups. Alfred Treibs (1936) subsequently solved the degradation pathway of chlorophyll $a$ in sedimentary environments, and is now celebrated as the father of biomarker geochemistry.

Crude oil is a complex mixture of thousands of different hydrocarbons and heteroatomic components containing C, S, N, and O. In the 1960s and 1970s, the analysis of such complex systems was facilitated by the rapid development of chemical separation and detection techniques such as gas chromatography (GC), gas chromatography-mass spectroscopy (GC-MS), and elemental analysis. The earliest biomarker studies were strongly inspired by the search for clues to the evolution of early life on the Earth (Barghoorn, 1957; Eglinton et al., 1964; Meinschein et al., 1964; Barghoorn et al., 1965; Burlingame et al.,
However, the main driving force of biomarker research later in the twentieth century was the petroleum industry. Organic geochemists employed biomarkers to understand the transformation of organic matter into petroleum, to estimate the thermal maturity and geological age of reservoir oil, and to correlate crude oil with potential source rocks (e.g., Philippi, 1965; Albrecht and Ourisson, 1969; Hunt, 1979; Tissot and Welte, 1984; Peters and Moldowan, 1993). Flowing from applications in the petroleum industry, and in the spirit of Vernadsky, biomarkers also became of increasing interest in the understanding of remineralization and burial of organic matter as crucial aspects of the global carbon cycle (e.g., Hedges et al., 1997; Eglinton and Repeta, 2004), the study of organisms and metabolisms that otherwise rarely leave a fossil record (e.g., Moldowan and Talyzina, 1998; Hinrichs et al., 1999; Summons et al., 1999; Kuyper et al., 2001), and as paleoenvironmental proxies (e.g., Brassell et al., 1986; Summons and Powell, 1986; Schouten et al., 2002).

Declining oil prices in 1998 led to the closure of most biomarker laboratories in the petrochemical industry, and biomarker research strongly shifted to universities with a focus on paleoenvironmental and geomicrobiological applications. In these applications, an integration with modern microbiological techniques and genomics will be a crucial aspect of biomarker geochemistry in the future (e.g., Pearson et al., 2003; Sinninghe Damsté et al., 2004b; Rashby et al., 2007).

Formation and alteration of sedimentary organic matter (diagenesis)

In most planktonic environments, organic carbon from primary producers is nearly quantitatively remineralized back to carbon dioxide and water. On an average less than 0.1% permanently accumulate in bottom sediments (Holser et al., 1988). Most biogenic compounds such as carbohydrates, proteins, nucleic acids, and small metabolites are prone to biological degradation under most conditions and are recycled rapidly back to carbon dioxide and water. Molecules that escape the mineralization process are commonly altered by diagenetic processes in the water column and subsequently, in the sediment. Diagenesis includes a multitude of chemical and biological reactions that lead to the formation of macromolecular aggregates from smaller molecules through condensation and cross-linking reactions, but also to fragmentation, oxidation, reduction, isomerization, and epimerization products (Tissot and Welte, 1984). The relative concentration of different alteration products depends on prevailing physical and chemical conditions in the sediment during and after burial. Analysis of the diagenetic products may, thus, yield information for example, about redox conditions and the presence of catalytic substances such as active mineral surfaces. Under prevailing reducing conditions and over geological periods of time, biolipids eventually lose their functional groups and are transformed to hydrocarbon skeletons (e.g., hopanepolyols III to hopanes IV, or okenone V to okenane VI). The hydrocarbon products may remain stable over hundreds of millions of years encased in sedimentary rock.

The exact chemical nature of organic matter in sediments primarily depends on the composition of the source organisms and the mechanisms of preservation. In the so-called selective preservation pathway, molecules that are highly resistant to chemical and biological degradation, such as aliphatic algal biopolymers or cuticular waxes of plant leaves, have a high potential to escape remineralization and may form a major fraction of sedimentary organic matter (Tegelaar et al., 1989; De Leeuw et al., 2006). Smaller molecular building blocks, including monomers generated by hydrolysis of biopolymers, may undergo random condensation to a highly cross-linked macromolecular product that is resistant to further degradation (degradation/recondensation pathway) (e.g., Larter and Douglas, 1980; Tissot and Welte, 1984). Under transitional oxidizing conditions, fatty acids and other lipids may also become cross-linked via ether and carbon–carbon bonds to form resistant aliphatic geomacromolecules (“oxidative polymerization pathway”) (e.g., Kuyper et al., 2002; Versteegh et al., 2004). One of the most
significant pathways for the preservation of biomarkers in sediments is natural sulfurization (or natural vulcanization) (Sinninghe Damsté and De Leeuw, 1990). In anoxic environments, bacterial sulfate reduction may lead to the accumulation of hydrogen sulfide and polysulfides. These reduced sulfur species can react with functional groups of organic molecules during early stages of diagenesis, either already in an anoxic water column or in the sediment. Abiotic addition of hydrogen sulfide to double bonds followed by reductive desulfurization is a crucial mechanism in the transformation of labile, oxygen-sensitive lipids into their more stable reduced counterparts (Hebting et al., 2006). Intramolecular incorporation of inorganic sulfur may also lead to the generation of small sulfur-containing molecules that are more resistant to biodegradation, and intermolecular vulcanization may protect smaller molecules by incorporation into a macromolecular network via sulfide and polysulfide bridges (e.g., Adam et al., 1993; Kohnen et al., 1993; Schaeffer et al., 1995; Wakeham et al., 1995). Finally, lithology also has an important influence on the presence and preservation of biomarkers. High concentrations of well-preserved organic matter are most commonly found in fine grained sedimentary rocks such as shales and marls. This has several reasons. Firstly, very fine suspended mineral particles and particulate organic matter have similar hydrodynamic properties and are likely to be deposited at the same location. Secondly, organic matter has a high affinity to clay minerals, and adsorption to mineral surfaces protects organic matter against attack by heterotrophic microorganisms and digestive enzymes, a phenomenon described as the “sorptive protection pathway.” Thirdly, the fineness of clay particles prevents the circulation of oxygenated water in the sediment, promoting development of anoxic and sulfidic conditions that exclude aerobic heterotrophic organisms and support natural sulfurization.

Thermal alteration of biomarkers and sedimentary organic matter (catagenesis)

The aggregates that form through degradation/recondensation, oxidative polymerization, and natural sulfurization, coalesce with degradation-resistant biopolymers to form kerogen. Kerogen is a highly complex, amorphous macromolecular network of organic matter in sedimentary rocks. Viewed in thin section, it commonly has a yellow, brown, or black color, depending on thermal maturity (De Leeuw and Largeau, 1993). With accumulation of sediment over the top and increasing depth of burial, geothermal heat will induce catagenesis, the thermal alteration and degradation of sedimentary organic matter. The cracking of chemical bonds in kerogen releases smaller molecular fragments that may form a liquid bitumen phase. Formally, bitumen is defined as the fraction of organic matter in sediments and sedimentary rocks that can be extracted using organic solvents. Bitumen released by the thermal decomposition of kerogen typically consists of saturated and aromatic hydrocarbons, including biomarkers, and lower concentrations of polar (NSO) compounds. Carbon—sulfur and sulfur—sulfur bonds in kerogen are cleaved at relatively low temperatures (~60°C), while biomarkers covalently attached to kerogen via carbon-oxygen and carbon-carbon bonds are released at progressively higher temperatures (e.g., Koopmans et al., 1997). Increasing pressure, partially caused by the release of carbon dioxide and methane from kerogen, may eventually lead to the expulsion of liquid hydrocarbons from the source rock and their possible release at hydrocarbon seeps or accumulation in oil reservoirs.

At catagenic temperatures, hydrocarbon biomarkers also undergo thermal isomerization, epimerization, and cracking reactions, and the relative concentration of the thermal alteration products can be used to elucidate the thermal history of bitumen and crude oil. Thus, maturity parameters based on biomarkers and other compounds are used in the petroleum industry to estimate the oil- and gas-generation potential of sedimentary basins. A large variety of different maturity parameters for different temperature stages is available and is summarized in Peters et al. (2004). For instance, the ratio of 22S/(22S + 22R) isomers of 17α(H)-homohopanes measures the gradual transformation of the biological 22R configuration to the more stable 22S epimer (see carbon atom marked ** in IV). The 22S/(22S + 22R) ratio reaches a thermodynamic equilibrium at a value of ~0.6, indicating a thermal maturity roughly corresponding to the early phase of oil generation (e.g., Zumbeerge, 1987). However, it is not possible to translate biomarker maturity parameters into absolute temperatures, as the rates of molecular isomerization and degradation also strongly depend on the availability of catalytically active mineral surfaces and reactive species such as free radicals. Therefore, biomarker maturity parameters may be widely different in rocks of different lithology and need to be recalibrated for different basins and formations.

 Destruction of biomarkers at high temperatures (metagenesis)

One of the most intriguing topics in biogeology is the examination of early microbial ecosystems using molecular fossils extracted from Precambrian rocks (>542 Ma [million years]). The oldest clearly indigenous bitumens come from the ~2.5 Ga (billion years) old Mt McRae Shale of the Hamersley Group in northwestern Australia (Brocks et al., 2003a). However, these bitumens were severely altered by pyrolytic temperatures and predominantly contain polycyclic aromatic hydrocarbons. The oldest molecules of proven provenance that contain biological information come from the 1.64 Ga old Barney Creek formation of the McArthur Basin in northern Australia (Summermns et al., 1988). The distribution of biomarkers in the Barney Creek formation is distinct from younger bitumens and strongly dominated by bacterial lipids and pigments (Brocks et al., 2005). While the biomarkers of the Barney Creek formation are clearly
indigenous (Brocks et al., 2008), reports of biomarkers from older successions have to be regarded with caution as the concentration of extractable hydrocarbons are commonly minute and molecular maturity parameters are inconsistent with the thermal history of the rock. Moreover, the extracted biomarkers are often found concentrated on rock surfaces (Brocks et al., 2008) and their composition is always conspicuously similar to Phanerozoic petroleum products (e.g., Brocks et al., 2003b).

All known sedimentary successions older than \( \sim 1.8 \text{ Ga} \) have suffered burial metamorphism to zeolite facies or higher, probably at minimum temperatures of 175–280°C. At these temperatures, metagenetic processes transform kerogen to a black, hydrogen-poor, and highly aromatic carbon phase, and residual bitumen is cracked to gas and expelled from the rock. However, the existence of residual biomarkers in rocks heated to about 220°C is theoretically not impossible. Modern deep-subsurface petroleum reservoirs produce commercial quantities of oil at present-day temperatures of up to 200°C, and these oils apparently still contain intact biomarkers. Biomarkers were also observed in bitumens extracted from sedimentary rocks that currently exist at temperatures of 200–223°C (for a review see Brocks and Summons, 2004). The persistence of aliphatic hydrocarbons over geological periods of time at up to 250°C is also supported by kinetic models of petroleum degradation (Pepper and Dodd, 1995; Domé et al., 2002). Therefore, if pockets of organic-rich sedimentary rocks of Paleoproterozoic to Archean age have escaped temperatures above \( \sim 220°C \), it should still be possible to discover intact molecular fossils to reconstruct the Earth’s oldest microbial ecosystems.

**Biological interpretation of biomarkers**

Most fossil hydrocarbons have multiple biological precursors that are distinguished by different functional groups and stereochemistries. Moreover, most of these biolipids may occur in a wide range of organisms that are not necessarily closely related. Therefore, for a meaningful interpretation of molecular fossils, it is crucial to identify all extant biological sources.

Some biomarkers contain little biological information because they are abundant across all domains of life. For example, the fossil hydrocarbon \( \beta \)-carotane VII contains virtually no taxonomic information. There are several hundred different biological carotenoids with a \( \beta \)-carotane skeleton that are distinguished by different combinations of functional groups such as double and triple bonds, oxo-, hydroxy-, epoxy-, and carboxy-groups and functional ornamentations such as carbohydrate side chains (e.g., Liaaen-Jensen, 1979). These carotenoids occur across a vast array of lineages in all three domains of life, and most are likely to yield \( \beta \)-carotane VII during diagenesis. Therefore, without additional information, \( \beta \)-carotane can not be assigned to any particular biological source.

In contrast to the ubiquitous occurrence of carotenoids with the \( \beta \)-carotane skeleton, the only known potential precursor of okenane VI is the purple-red colored carotenoid okenone V. As okenone is exclusively known from planktonic species of Chromatiaceae, a family of purple sulfur bacteria, it is interpreted as a diagnostic marker for this group of organisms (Brocks and Schaeffer, 2008). Isorenieratene VIII, an aromatic carotenoid like okenane VI, is an intermediate case. The known biological precursor isorenieratene IX is the major accessory pigment in brown strains of green sulfur bacteria (Chlorobiaceae). Chlorobiaceae are anoxygenic phototrophs and strictly require anaerobic and sulfidic conditions in the presence of light. In planktonic environments, Chlorobiaceae inhabit a zone in the chemocline of stratified waters wherever sulfidic conditions arise into the light penetrated zone (photic zone euxinia). Therefore, isorenieratene is commonly interpreted as a biomarker for Chlorobiaceae and photic zone euxinia (see below). However, a second potential microbial source of aromatic carotenoids with the isorenieratene skeleton are genera of the actinobacterial order Actinomycetales, such as *Mycobacterium*, *Streptomyces*, and *Brevibacterium*. For instance, the orange-colored growths on the surface of cheese is often due to isorenieratene, 3-hydroxyisorenieratene, and 3,3'-dihydroxyisorenieratene from *Brevibacterium linens*, an actinomycete that belongs to the cheese ripening flora (Guyomarc’h et al., 2000). Generally, Actinomycetales are regarded as a microbial group that is restricted to terrestrial environments, particularly soils. However, the group is also known to be ubiquitous, and occasionally abundant, in marine environments (Ward and Bora, 2006). Actinomycetales were detected in the marine water column, in marine snow, in sea sediments, including deep sea sediments, and as symbionts in vertebrates and invertebrates, particularly sponges. It is not yet known just how widespread aromatic carotenoids in marine actinomycetes are. However, at least *Streptomyces griseus*, an actinomycete known from terrestrial as well as marine environments, has the capacity to biosynthesize
isorenieratene (Krügel et al., 1999). Therefore, the fraction of isorenieratene in marine sediments that is derived from terrestrial and marine actinomycetes may be significant, and an actinomycetal origin of isorenieratene in sedimentary rocks from marine depositional environments cannot be ruled out.

The occurrence of isorenieratene in distantly related groups of bacteria, Actinomycetales and Chlorobiaceae, highlights our lack of understanding of the distribution of genes involved in carotenoid biosynthesis in extant organisms. For a full interpretation of isorenieratene, we have to learn whether isorenieratene biosynthesis was present in the common ancestor of Chlorobiaceae and Actinomycetales, whether the biosynthetic capacity was transferred horizontally between the two groups, or whether the pathways developed independently. While these questions may best be answered in the future using genomic information, isorenieratene derived from Chlorobiaceae can principally be distinguished from other sources by a diagnostic isotopic enrichment in $^{13}$C (Summons and Powell, 1987; Grice et al., 1996a) (also see Biomarkers (Organic, Compound-Specific Isotopes)).

In summary, strictly, biomarkers have to be interpreted as indicators for biosynthetic pathways, not as markers for taxonomic groups. In the above example, isorenieratene is a biomarker for $\beta$-carotene desaturase, the enzyme responsible for the convergence of $\beta$-carotene to isorenieratene, and for $crtU$, the gene that codes for this enzyme. In some cases, it is possible to discriminate between different biological sources of a biomarker using carbon isotopic compositions (see also Biomarkers (Organic, Compound-Specific Isotopes)).

Biomarkers for the domains bacteria, archaea, and eukarya

A selection of biologically informative molecular fossils, their potential biological origins and environmental interpretations are summarized in Table 1 (more comprehensive accumulations can be found in Peters and Moldowan [1993], Brocks and Summons [2004], and Peters et al. [2004]). Table 1 also includes biomarkers that are not hydrocarbons but are stable over geological periods of time such as maleimides X and archaeal glycerol ethers (XI and XII). Molecules with limited or no biological information were not included.

Biomarkers of eukaryotes

Next to bacterial hopanoids, eukaryotic steroids are probably the most common polycyclic biomarkers in the geological record. Sterols, such as cholesterol, ergosterol, and sitosterol, are essential membrane components of all eukaryotic organisms. Eukaryotes produce a large variety of different sterols that are distinguished by the number and position of double bonds, hydroxy-, oxo- and alkyl-groups and other, often complex, substituents. Functionalized sterols detected in recent sediments can be diagnostic for a wide range of taxonomic groups, particularly algae (e.g., Volkman, 2003). However, the number of different diagenetic hydrocarbon products is comparatively low (Table 1). The most abundant steranes in bitumens and oils from the late Neoproterozoic to the Cenozoic are cholesterol XIIIa (C$_{27}$), ergostane XIIIb (24-methylcholestane, C$_{28}$) and stigmastane XIIIc (24-ethylcholestane, C$_{29}$). Biological precursors of cholesterol are abundant in animals and red algae (Rhodophyceae), and precursors of ergostane occur in fungi and several groups of algae, including coccolithophorids and diatoms, while sterols with the stigmastane skeleton are very widespread in higher plants, eustigmatophytes, chrysophytes, and green algae (Chlorophyceae) (Volkman, 1986; Volkman, 2003). However, C$_{27}$ to C$_{29}$ sterols are not restricted to these organisms and occur across all major eukaryotic clades. Even individual organisms may contain sterols with all three carbon skeletons. Thus, cholestanes, ergostenes, and stigmastanes in sedimentary rocks are not specific for any particular group of eukarya. However, the relative abundance of these three steroids shows systematic variations through the Phanerozoic and can be used to obtain an estimate of the age of crude oil (Grantham and Wakefield, 1988).

The biosynthetic capacity to produce steroids also occurs in at least three independent groups of bacteria, the Myxococcales, Methylococcales, and Planctomycetales.
<table>
<thead>
<tr>
<th>Group of organisms</th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Eukarya</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eukaryotes in general</td>
<td>Ergostane XIIIb, stigmastane XIIIc and their aromatic analogs</td>
<td>Most environments, Neoproterozoic to present</td>
<td>Possible minor contribution from myxococcales (myxobacteria)</td>
<td>(Volkman et al., 1998; Volkman, 2003; Summons et al., 2006) (Kohl et al., 1983; Bode et al., 2003)</td>
</tr>
<tr>
<td>Pelagophyte algae (“brown tides” and Sarcinochytries)</td>
<td>24-n-Propylcholestane XIIIId</td>
<td>Commonly found only in marine environments</td>
<td></td>
<td>(Moldowan et al., 1990)</td>
</tr>
<tr>
<td>Dinoflagellates</td>
<td>4-Methylergostane, 4-methylstigmastane</td>
<td>Mostly Mesozoic and Cenozoic (minor concentrations in Paleozoic, possibly of “protodinoflagellate” origin)</td>
<td>Minor component in other eukaryotes</td>
<td>(Robinson et al., 1984; Volkman et al., 1993; Moldowan and Talyzina, 1998)</td>
</tr>
<tr>
<td>Dinosterane XIV</td>
<td>C25 HBI alkanes XV</td>
<td>Marine environments</td>
<td></td>
<td>(Nichols et al., 1988; Volkman et al., 1994; Sinninghe Damsté et al., 2004b)</td>
</tr>
<tr>
<td>Diatoms</td>
<td>24-Norcholestane (C26)</td>
<td>Cretaceous to Cenozoic</td>
<td>C25 HBI in pennate diatoms</td>
<td>(Holba et al., 1998a, b)</td>
</tr>
<tr>
<td>Centric diatoms (genus Rhizosolenia)</td>
<td>C25 XV and C30 highly branched isoprenoid (HBI) alkanes</td>
<td>Marine environments, Upper Turonian to present</td>
<td></td>
<td>(Nichols et al., 1988; Volkman et al., 1994; Sinninghe Damsté et al., 2004b)</td>
</tr>
<tr>
<td>Pennate diatoms (phylogenetic cluster including Haslea, Pleurosigma, Navicula)</td>
<td>C25 HBI alkanes XV</td>
<td>Marine environments</td>
<td>Centric diatoms (genus Rhizosolenia)</td>
<td>(Nichols et al., 1988; Sinninghe Damsté et al., 2004b)</td>
</tr>
<tr>
<td>Prymnesiophyte algae</td>
<td>C37-C39 di- to tetr-unsaturated alkenones</td>
<td>The degree of unsaturation changes with water temperature, and this is used in the photic zone temperature proxy Uk37</td>
<td></td>
<td>(Brassell et al., 1986)</td>
</tr>
<tr>
<td>Botryococcus braunii (Chlorophyte alga)</td>
<td>Botryococccanes XVI, cyclobotryococccanes, and polymethylsqualanes</td>
<td>Fresh to brackish water, Tertiary</td>
<td>Gammacerane precursors were also observed in a fungus, a fern, and the ubiquitous α-proteobacterium Rhodospseudomonas</td>
<td>(Metzger et al., 1985; Huang et al., 1988; Metzger and Largeau, 1999; Summons et al., 2002)</td>
</tr>
<tr>
<td>Glococapsomorphopsis prisca (uncertain affinity, possibly an alga)</td>
<td>Outstanding concentrations of n-C15, n-C17, and n-C19</td>
<td>Cambrian to Devonian</td>
<td></td>
<td>(Fowler, 1992; Blokker et al., 2001)</td>
</tr>
<tr>
<td>Ciliates</td>
<td>Gammacerane XVII</td>
<td>The main source is possibly bacterivorous ciliates inhabiting the chemocline of stratified waters</td>
<td></td>
<td>(Ten Haven et al., 1989; Sinninghe Damsté et al., 1993)</td>
</tr>
<tr>
<td>Terrestrial plants in general</td>
<td>n-Alkanes &gt; C24 with odd-over even carbon number preference, derived from higher plant waxes</td>
<td>Proximity to terrestrial organic matter sources; post-Silurian age</td>
<td>Non-marine algae</td>
<td>(Hedberg, 1968; Tissot and Welte, 1984)</td>
</tr>
</tbody>
</table>
## Biomarkers (Molecular Fossils), Table 1 (Continued)

<table>
<thead>
<tr>
<th>Group of organisms</th>
<th>Biomarker or biomarker pattern</th>
<th>Environmental interpretation and age constraints</th>
<th>Other known potential sources</th>
<th>Further informationa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lupane, various plant sources</td>
<td>Common in coal and lignites, Cretaceous to Cenozoic</td>
<td>Possible occurrence in algae and microorganisms</td>
<td>(Wang and Simoneit, 1990)</td>
<td></td>
</tr>
<tr>
<td>Isopimarane, retene, simonellite, eudesmane, and fichtelite</td>
<td>Predominantly Upper Cretaceous to Cenozoic</td>
<td>Precursors also occur in lichens and ferns in minor concentrations</td>
<td>(Moldovan et al., 1994)</td>
<td></td>
</tr>
<tr>
<td>Angiosperms</td>
<td>Oleane X VIII, precursors are betulins, taraxerenes, and others triterpenoids</td>
<td>Carboniferous to Cenozoic</td>
<td>(Cox et al., 1986; Van Aarssen et al., 1992)</td>
<td></td>
</tr>
<tr>
<td>Angiosperms (e.g., dipterocarpaceae)</td>
<td>Cadinanes, bicadinanes XIX (from degradation of polycadinene resins)</td>
<td>Devonian to Cenozoic, often abundant in coal</td>
<td>Lower concentrations in other land plants and possibly algae</td>
<td>(Noble et al., 1985)</td>
</tr>
<tr>
<td>Conifers</td>
<td>Phyllocladanes, beyeran, kaurane, and atisane</td>
<td>Neoproterozoic to present, with higher relative abundance in the late Neoproterozoic to early Cambrian</td>
<td>(McCaffrey et al., 1994; Love et al., 2009)</td>
<td></td>
</tr>
<tr>
<td>Sponges (demospongiae)</td>
<td>24-Isopropylecholestan ste XIIe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Archaea</td>
<td>C31-C40 head-to-head linked acyclic isoprenoids (e.g., XXI), archaeol XI, and other glycerol ethers Abundant squalane XX, abundant regular acyclic isoprenoids</td>
<td>Only reported from a Mid-Cretaceous oceanic anoxic event First appearance in the Cretaceous</td>
<td>Bacteria and eukaryotes, but commonly in lower concentrations</td>
<td>(Vink et al., 1998; Kuypers et al., 2001)</td>
</tr>
<tr>
<td>Marine pelagic Crenarchaeota</td>
<td>2,6,15,19-Tetramethylicosane (TMI) Crenarchaeol XII</td>
<td>Evaporitic environments, salt lakes</td>
<td>Other archaena</td>
<td>(Grice et al., 1998b)</td>
</tr>
<tr>
<td>Haloarchaea</td>
<td>Regular acyclic isoprenoids with 21-30 carbon atoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaerobic methane oxidizers (ANME)</td>
<td>Crocetane XXII</td>
<td>Typical habitats are subsea gas sources, gas hydrates, and mud volcanoes</td>
<td>Degradation product of diaromatic carotenoids</td>
<td>(Thiel et al., 1999; Bian et al., 2001)</td>
</tr>
<tr>
<td>Methanogenic and methanotrophic archaea</td>
<td>2,6,10,15,19-Pentamethylicosane (PMI)</td>
<td></td>
<td></td>
<td>(Schouten et al., 1997; Elvert et al., 1999; Thiel et al., 1999)</td>
</tr>
<tr>
<td>Bacteria</td>
<td>C30-hopanes</td>
<td>Some cryptogams, ferns, mosses, lichens, filamentous fungi, and protists</td>
<td></td>
<td>(Rohmer et al., 1984)</td>
</tr>
<tr>
<td>Bacteria in general</td>
<td>Hopanes with extended side chain (homohopanes (C31) – pentakishomohopane IVa (C35))</td>
<td></td>
<td></td>
<td>(Rohmer et al., 1984; Ourisson and Albrecht, 1992)</td>
</tr>
<tr>
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<tr>
<td>Cyanobacteria</td>
<td>Abundant monomethylalkanes and dimethylalkanes</td>
<td>Other bacteria, sponges</td>
<td>(Shiea et al., 1990; Kenig et al., 1995; Köster et al., 1999; Dembitsky et al., 2001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-Methylhopanes IVc</td>
<td>Pseudomonadaceae, rhizobiales</td>
<td>(Summons et al., 1999; Rashby et al., 2007)</td>
<td></td>
</tr>
<tr>
<td>Type-I methanotrophic bacteria (methylcocaceae)</td>
<td>3β-Methylhopanes IVb</td>
<td>Methylo trophic, acetic acid bacteria</td>
<td>(Zundel and Rohmer, 1985a, b, c; Summons and Jahnke, 1992; Farrimond et al., 2000)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4-Methycholestanes, 4,4- dimethylcholestanes</td>
<td>Dinoflagellates, minor component in all eukaryotes</td>
<td>(Bird et al., 1971; Brocks et al., 2005)</td>
<td></td>
</tr>
<tr>
<td>Green sulfur bacteria (Chlorobiaceae)</td>
<td>Chlorobactane XXIII (green pigmented species)</td>
<td>Anoxic and sulfidic conditions in the absence of light in microbial mats or planktonic environments (photic zone euxinia)</td>
<td>(Schaeffer et al., 1997; Grice et al., 1998a, 2005; Brocks et al., 2005)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isorenieratane VIII (brown pigmented species)</td>
<td>Photic zone euxinia</td>
<td>Some sponges, Actinomycetales</td>
<td>(Hartgers et al., 1993; Grice et al., 1996a; Koopmans et al., 1996b; Bosch et al., 1998; Pancost et al., 1998; Putschew et al., 1999; Simons and Kenig, 2001; Sinninghe Damsté et al., 2001)</td>
</tr>
<tr>
<td></td>
<td>3-Isobutyl-4-methylmaleimide Xc</td>
<td>Photic zone euxinia</td>
<td>Chloroflexaceae</td>
<td>(Grice et al., 1998a; Brocks and Schaeffer, 2008)</td>
</tr>
<tr>
<td>Purple sulfur bacteria (Chromatiaceae)</td>
<td>Okenane VI</td>
<td>Photic zone euxinia, planktonic conditions</td>
<td>Cyanobacterial synechocyanin?</td>
<td>(Brocks et al., 2005; Brocks and Schaeffer, 2008)</td>
</tr>
<tr>
<td></td>
<td>2,3,4-Trimethyl aryl isoprenoids</td>
<td>Photic zone euxinia</td>
<td>Sponges or sponge symbionts</td>
<td>(Brocks et al., 2005; Graham and Bryant, 2008)</td>
</tr>
<tr>
<td></td>
<td>Renieratane</td>
<td>Photic zone euxinia</td>
<td>Sponges or sponge symbionts, cyanobacterial synechocyanin?</td>
<td>(Graham and Bryant, 2008; Brocks and Schaeffer, 2008)</td>
</tr>
<tr>
<td></td>
<td>Renierapurpurane</td>
<td>Photic zone euxinia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gemmata (Planctomycetales)</td>
<td>Lanostane</td>
<td>Minor in all eukaryotes and steroid producing bacteria</td>
<td>(Pearson et al., 2003)</td>
<td></td>
</tr>
<tr>
<td>Phototrophs in general</td>
<td>Pristane, phytane</td>
<td>From chlorophylls of cyanobacteria and phototrophic eukaryotes, BChl a and b of phototrophic bacteria</td>
<td>Tocopherols and other acyclic isoprenoids, archael membrane lipids</td>
<td>(Peters et al., 2004)</td>
</tr>
</tbody>
</table>
Phylogenetic trees of oxidosqualene cyclase (OSC), the first enzyme in the sterol biosynthetic pathway, show that sterol biosynthesis was laterally transferred between eukaryotes and the three bacterial groups (Pearson et al., 2003). However, bacteria do not possess the enzymes required to alkylate the steroid side chain, and fossils steroids alkylated at C-23 or C-24 (e.g., ergostane \( \text{XIIIB} \), stigmastane \( \text{XIIIC} \) and dinosterane \( \text{XIV} \)) must still be regarded as diagnostic biomarkers for eukaryotes.

**Pelagophytes and dinoflagellates.** In contrast to C\(_{27}\) to C\(_{29}\) steranes, steroids with 30 carbon atoms can be highly specific. For example, 24-n-propylcholestane \( \text{XIIId} \) is a diagnostic marker for algae of the order Sarcinochrysidales and the so-called brown tide algae (Volkman, 2003). These organisms are strictly marine, and 24-n-propylcholestane is, thus, regarded as a reliable indicator for marine depositional conditions.

Another steroid with a unique carbon skeleton is dinosterane \( \text{XIV} \) (4,23,24-trimethylcholestane). The biological precursors of dinosterane \( \text{XIV} \), dinosterol, and related compounds (Robinson et al., 1984), are the dominant steroids in many dinoflagellate species (see references in Volkman, 2003). Only one other organism, a diatom species, is known to contain sterols with the same carbon skeleton. Therefore, dinosterane is regarded as a relatively reliable biomarker for dinoflagellates (Summons et al., 1987). In the microfossil record, the first modern dinoflagellates appeared in the Mesozoic, and this is also reflected by a significant increase in dinosterane concentrations in contemporaneous rocks (Moldowan et al., 1996; Moldowan and Talyzina, 1998; Summons et al., 1987, 1992). Traces of dinosteranes in Paleozoic sedimentary rocks were identified as the likely molecular remains of ancestral dinoflagellates (Moldowan et al., 1996; Talyzina et al., 2000). Dinoflagellates are probably also the dominant source of other steranes and aromatic steroid that bear a methyl group at C-4 (4-methylcholestane, 4-methylergostane, and 4-methylstigmastane; compare structure \( \text{XIII} \)). However, these biomarkers are less specific, and multiple other biological sources are known (Volkman, 2003).

**Diatoms.** An unusual sterane common in post-Jurassic marine sediments is 24-norcholestane. Although the biological precursor lipids remain unknown, indirect evidence points to diatoms as the major source (Holba et al., 1998a). Consistent with this assignment, the relative concentration of 24-norcholestanes in crude oils rises in concert with the radiation of diatoms from the Jurassic to the Tertiary (Holba et al., 1998b). Other specific biomarkers for diatoms are “highly branched isoprenoid” (HBI) alkanes \( \text{XV} \) with 25 and 30 carbon atoms

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**Biomarkers (Molecular Fossils), Table 1 (Continued)**

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<tbody>
<tr>
<td><strong>Anthropogenic contaminants</strong></td>
<td></td>
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</tr>
<tr>
<td>Polyethylene plastic bags</td>
<td>5,5-Diethylalkanes with odd-over-even carbon number preference, and other branched alkanes with quaternary carbon (BAQCs)</td>
<td></td>
<td>(Grosjean and Logan, 2007; Brocks et al., 2008)</td>
<td></td>
</tr>
<tr>
<td>Detergents</td>
<td>Phenylalkanes</td>
<td></td>
<td>(Harvey et al., 1985)</td>
<td></td>
</tr>
<tr>
<td>Plasticizers</td>
<td>Phthalates</td>
<td></td>
<td>(Mayer et al., 1972)</td>
<td></td>
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</tbody>
</table>

*additional information taken from Peters et al. (2004) and Brocks and Summons (2004)*
(e.g., Volkman et al., 1994). The unsaturated precursors of HBI alkanes are found in two discrete phylogenetic clusters of diatoms. C_{25} and C_{30} HBIs occur in centric diatoms of the genus Rhizosolenia, and a second cluster, comprising three genera of pennate diatoms, produces C_{25} HBI alkenes (Sinninghe Damsté et al., 2004b). Remarkably, the C_5 building blocks of HBI alkenes in the centric diatoms are biosynthesized using the mevalonate (MVA) pathway, while the isoprenoid building blocks of HBI alkenes in pennate diatoms are constructed following the methylenelythritol (MEP) route (Massé et al., 2004). The distribution of C_{25} HBIs XV in marine sediments and petroleum constrains the radiation of rhizosolenid diatoms quite accurately to the Upper Turonian, 91.5 ± 1.5 Ma ago (Sinninghe Damsté et al., 2004b). The biological origin of HBIs in marine sediments with 20, 21, 22, and 26 carbon atoms is still unknown, but diatoms are also a likely source (Sinninghe Damsté et al., 2005). Although C_{20} HBI has been identified in field samples of the green macro algae Chara and Enteromorpha (Rowland et al., 1985; Jaffé et al., 2001), it is likely derived from epiphytic micro algae colonizing the macrophytes (Volkman et al., 1998; Atahan et al., 2007) (see also Biomarkers (Organic, Compound-Specific Isotopes)).

Green algae. The green unicellular microalga Botryococcus braunii is found in freshwater, brackish lakes, and reservoirs at varying latitudes (Tyson, 1995 and references therein). Three distinct races (A, B, and L) of this alga are recognized and are classified according to their hydrocarbon composition. The A race biosynthesizes n-alkadienes and trienes with an odd carbon number between C_{25} and C_{31} (Metzger et al., 1986, 1991). The B race produces C_{30} to C_{37} branched isoprenoidal hydrocarbons called botryococcenes giving rise to botryococcanes (e.g., XVI) (Maxwell et al., 1968; Metzger and Largeau, 1999) and a wide variety of cyclobotryococccenes (Metzger et al., 1985; David et al., 1988), and polymethylsqualenes (Summons et al., 2002). The L race makes a C_{40} isoprenoid hydrocarbon, lycopa-14(E),18(E)-diene (Metzger and Casadevall, 1987; Metzger et al., 1991). Macromolecular alkane biomarkers with 15-34 carbon atoms are derived from Botryococcus cell wall material (Audino et al., 2002). Although botryococccanes are quite rare in petroleum, they are among the most specific biomarkers and indicate a lacustrine origin and Tertiary age.

Straight chain alkanes (n-alkanes) are the most abundant class of saturated hydrocarbons in all bitumens and oils that have not suffered biodegradation. n-Alkanes have a vast variety of biological precursors such as saturated and unsaturated fatty acids and their esters, alcohols, alkenes, plant waxes, and polymethylenic biopolymers. As biochemicals with n-alkyl skeletons occur in all branches across the three domains of life, the structure of single n-alkanes has no taxonomic value (although their carbon and hydrogen isotopic composition may yield valuable environmental and biological clues - see Biomarkers (Organic, Compound-Specific Isotopes)). However, some n-alkane profiles, i.e., the relative concentration of n-alkanes with different carbon numbers, can be taxonomically informative. For instance, bitumens and oils from the early Paleozoic often show elevated abundances of n-alkanes with odd carbon numbers in the range n-C_{15} and n-C_{19}. This age diagnostic n-alkane profile is attributed to Gloeocapsomorpha prisca, an extinct marine alga (or other organism) of unknown affinity (Hoffmann et al., 1987; Fowler, 1992).

The cell wall of many marine and lacustrine chlorophytes and marine eustigmatophytes is composed of algaenan, an insoluble, polymethylene macromolecule (Derenne et al., 1992; e.g., Gelin et al., 1997; Blokker et al., 2000). Cracking of algaenan during burial and catagenesis releases large volumes of n-alkanes that contribute significantly to the formation of liquid petroleum (Tegelaar et al., 1989). There is evidence that a major radiation of chlorophytes in the late Neoproterozoic also contributed to the formation of the oldest commercial reservoirs of petroleum (Grantham et al., 1988). These oils are characterized by high concentrations of long-chain n-alkanes derived from the degradation of algaenan or related macromolecules (Klomp, 1986; Fowler and Douglas, 1987; Höld et al., 1999). The same oils contain unprecedented high relative concentrations of stigmastanes XIIIc, the major steroid derived from green algae (Volkman, 2003).

Ciliates. Some bacterivorous ciliates produce the pentacyclic triterpenoid tetrahymanol (Harvey and McManus, 1991). The fossil product of tetrahymanol, gammacerane XVII, is often abundant in sedimentary rocks that were deposited under stratified and anoxic waters (Sinninghe Damsté et al., 1995). In these environments, predatory ciliates may inhibit the chemocline feeding on microaerophilic and anaerobic microorganisms. Gammacerane can reflect the often unusual carbon isotopic composition of the microbial food source (see Biomarkers (Organic, Compound-Specific Isotopes)) (Sinninghe Damsté et al., 1995; Grice et al., 1998a).

Terrestrial plants. In the geological record, plant-derived biomarkers start to appear in bitumens and oils from the Devonian, and can be exceedingly abundant in sediments from the late Mesozoic to Cenozoic. The most common plant signature is a suite of n-alkanes derived from plant waxes with more than 27 carbon atoms and a
predominance of odd-over-even carbon numbers (i.e., C_{27}, C_{29}, C_{31} etc.) (Hedberg, 1968). In coals and other sediments with significant input of terrestrial organic matter, high-molecular-weight alkanes with more than 40 and up to 110 carbon atoms are the degradation products of cuticular waxes and plant-derived biopolymers such as cutan and suberan (e.g., Nip et al., 1986; Tegelaar et al., 1995; Del Rio and Philip, 1999). One of the most common polycyclic terpenoids derived from plants is oleanane \text{XVIII}. Oleanane is a biomarker for flowering plants (angiosperms), and the relative concentration of oleanane in bitumens from the early Cretaceous to Tertiary parallels the radiation of angiosperm families, as documented in the pollen record (Moldowan et al., 1994). The thermal degradation of polycadinene resins of tropical angiosperms generates terpenoids of the cadinane family. Cadinane, bicadinanes \text{XIX}, and tricadinanes are found in sedimentary rocks from the Carboniferous to Cenozoic and are potentially diagnostic for Dipterocarpaceae, a group that today comprises mainly tropical lowland rainforest trees (Cox et al., 1986; Van Aarssen et al., 1990). The degradation of conifer resins releases a large variety of bicyclic and tricyclic terpenoids such as phyllocladane, beyeran, kaurane, atisane, retene, and simonellite that are abundant in coal (Table 1). However, many of these biomarkers are also found in lower concentrations in marine sedimentary rocks or rock of pre-Devonian age, and it appears likely that they also have algal or prokaryotic sources. For instance, the triaromatic hydrocarbon retene can be abundant in Tertiary shales and coals, and used to be interpreted as the degradation product of conifer resins (Villar et al., 1988). However, retene was also detected in marine rocks of late Neoproterozoic to early Paleozoic age (Jiang et al., 1995), and in pyrolysis products of algal and cyanobacterial tissue (Wen et al., 2000).

\textit{Sponges}. Surprisingly, 24-isopropylcholestane \text{XIIIe}, indicative of the class Demospongiae, is the only known hydrocarbon biomarker that appears to be specific for any group of animals. In the geological record, the concentration of 24-isopropylcholestane \text{XIIIe} (relative to algal 24-n-propylcholestane \text{XIIIId}) is particularly high from the latest Neoproterozoic to early Phanerozoic age, a distribution that may reflect the radiation and abundance of early sponges during this time (McCaffrey et al., 1994; Love et al., 2009).

\textbf{Biomarkers of archaea}

Archaea play a key role in the geochemical cycles of hydrogen, nitrogen, methane, and sulfur in a wide variety of marine, lacustrine and terrestrial environments. Although many archaea inhabit "extreme" environments such as hot springs and crystallizer ponds, they are also cosmopolitan inhabitants of the open ocean (e.g., Delong, 1992; Karner et al., 2001). Therefore, it is not unexpected that archaean biomarkers can be found in a large number of oils and bitumens of all ages. Membrane lipids of archaea are very distinct from eukaryotes and bacteria. They are predominantly based on regular or irregular, acyclic or cyclic isoprenoids commonly bound to a glycerol backbone via ether links. The most common hydrocarbons derived from archaea in mature bitumens and oils are C_{21+} regular acyclic isoprenoids, and squalane \text{XX}, an irregular isoprenoid that comprises two tail-tail linked C_{15} isoprenoid units. Although precursors of these isoprenoids also occur in other organisms, archaea are presumed to be the major source. The abundance of archaean biomarkers in bitumens is usually low in comparison to eukaryotic and bacterial lipids. However, under specific environmental conditions, archaean organic matter may constitute a significant fraction of total sedimentary organic carbon, including marine (Kuyper et al., 2001) and hypersaline (Grice et al., 1998b) depositional environments.

\textit{Marine Crenarchaeota}. According to cell counts based on whole-cell rRNA-targeted fluorescent in situ hybridization, pelagic crenarchaeota constitute 20% of picoplankton cells in the world ocean, and may even outnumber the bacteria in the mesopelagic and bathypelagic zones (Karner et al., 2001). Cell numbers computed from the concentration of archaean membrane lipids at different water depth in the Arabian Sea confirm this estimate (Sinninghe Damsté et al., 2002). The membrane lipids of crenarchaeota are typically glycerol dialkyl glycerol tetraethers (GDGTs) (De Rosa and Gambacorta, 1988). GDGTs consist of two glycerol units linked by two tetramer (C_{40}) chains that may be acyclic (biphytane \text{XXI}) or contain one to three cyclopentane moieties.
The biomarker ratio TEX86 is a linear function of annual the number of pentacyclic rings in GDGTs (expressed as GDGTs are considered to be a mechanism for temperature surface temperatures across the PETM based on TEX86 including crenarchaeol (see also Biomarkers (Organic, Compound-Specific Isotopes)).

The rings incorporated into the biphytanyl chains of GDGTs are considered to be a mechanism for temperature adaptation in archaea. Schouten et al. (2002) found that the number of pentacyclic rings in GDGTs (expressed as the biomarker ratio TEX86) is a linear function of annual mean sea surface temperatures. The correlation $T = 10.8 + 56.2 \times \text{TEX86}$ is calibrated for temperatures from 5 to $35^\circ C$ (Kim et al., 2008) and possibly $40^\circ C$ (Schouten et al., 2007). As an example, TEX86 measurements revealed that average sea surface temperatures in the late Cretaceous were very high, $\sim 15^\circ C$ in the Arctic ocean (Jenkyns et al., 2004), and $>32$–$36^\circ C$ at low latitudes in the North Atlantic (Schouten et al., 2003). Temperature measurements on GDGTs also contributed significantly to the understanding of the Paleocene/Eocene thermal maximum (PETM) 55 Ma ago. The PETM was one of the most rapid global heating events in Earth’s history. It was accompanied by environmental changes and a sharp global negative carbon isotopic excursion, although the precise order of events remained uncertain for a long time. Sluijs et al. (2007) obtained a high-resolution record of sea surface temperatures across the PETM based on TEX86 from sediments in New Jersey. The record indicated that sea surface temperatures and ecological changes preceded the carbon isotopic excursion by $\sim 3,000$ years. This discovery is consistent with the hypothesis that increasing temperatures of marine bottom waters caused dissociation of submarine gas hydrates and injection of isotopically light methane carbon into the atmosphere/ocean system. TEX86 measurements also indicated that sea surface temperatures near the North Pole increased during the PETM from 18 to 23°C, temperatures that are more than $10^\circ C$ higher than predicted by previous models (Sluijs et al., 2006).

**Halophilic archaea.** Archaea of the order Halobacteriales are frequently the dominant organisms in hypersaline environments such as salt lakes and pools of evaporating sea water (Oren, 2002). The membrane lipids of extremely halophilic archaea, which may thrive at halite concentrations as high as saturation levels, contain glycerol ethers with $C_{20}$ and $C_{25}$ regularly branched acyclic isoprenoid side chains (e.g., XI). The degradation products of these glycerol ethers are often preserved in sedimentary rocks associated with halite deposits. For example, the dominant hydrocarbons in Miocene/Pliocene sediments from the Dead Sea Basin, Israel, are regular isoprenoids with 19–25 carbon atoms (Grice et al., 1998b). Halophilic archaea also biosynthesize carotenoids with highly characteristic skeletons with 50 carbon atoms such as bacterioruberin. However, the fossil equivalents of these carotenoids have not yet been detected in the geological record.

**Methanogenic and methanotrophic archaea.** Methane metabolizing archaea biosynthesize generic glycerol diethers and tetraethers, and diagnostic hydroxyarchaeols, macrocyclic archaeols, and irregularly branched acyclic isoprenoid hydrocarbons such as 2,6,10,15,19-pentamethyllicosane (PMI). Methanotrophic archaea oxidize methane under anaerobic conditions in consortium with sulfate reducing bacteria. The sulfate reducers make the process thermodynamically viable by removing the hydrogen that is generated as a by-product of the methanotrophic process. Crocetane XXII, an irregular tail-to-tail linked $C_{20}$ isoprenoid, is commonly found at methane seeps, and is diagnostic for methanotrophic archaea if it has a light carbon isotopic composition (Greenwood and Summons, 2003). For more details about biomarkers at methane venting sites and of methane metabolizing Archaea, see Biomarkers (Organic, Compound-Specific Isotopes).

**Biomarkers of bacteria**

By mass, bacterial hopanoids stored in sedimentary organic matter and oil deposits were described as the most abundant natural products on the Earth (Ourisson and Albrecht, 1992). Fossil hopanes IV and aromatic hopanoids can be found in virtually all thermally well-preserved bitumens and oils from the Paleoproterozoic to the present. The biological precursors in bacteria are bacteriohopanepolyols III with a hydrophilic side chain that is biosynthetically derived from a pentose sugar. The polar side chain commonly carries three to five hydroxy groups and may additionally be ornamented with a terminal amino group, peptidic derivatives, carboxydrates, glucosamines, nitrogenous bases, and many more (Ourisson et al., 1987). Bacteriohopanepolyols are thought to play a role as membrane modifiers analogous to sterols in eukaryotes, but the exact function of the large structural variety of hopanoids remains a major area for future research. Although terpenoids with a $C_{10}$ hopane skeleton have been observed in some cryptogams, ferns, mosses, lichens, filamentous fungi, and protist, $C_{35}$ bacteriohopanoids III with a pentose-derived side chain appear to be diagnostic for bacteria. Although most hopanoid producers are aerobic, there is now a growing number of reports of anaerobic bacteria that contain...
homanols as well (Sinninghe Damsté et al., 2004a; Fischer et al., 2005). Generally, bacterial lineages that produce hopanoids are widely distributed across phylogenetic trees without apparent systematic patterns, and less than 10% of all species contain the genes required for hopanoid biosynthesis (Pearson et al., 2007). Thus, hopanes in sediments cannot be attributed to any specific bacterial source without additional information (Rohmer et al., 1984; Farrimond et al., 1998). However, hopanoids with alkyl substituents at ring A appear to be limited to a restricted number of groups. For instance, 3-methylhopanoids appear to be exclusive to microaerophilic proteobacteria (acetic acid bacteria, methanotrophs, methylotrophs) (Summons and Jahnke, 1992), and 2-methylhopanoids are abundant in, although not exclusive to, cyanobacteria (Summons et al., 1999).

**Cyanobacteria.** Many cyanobacteria produce relatively high concentrations of hopanepolyols that are methylated at C-2. The hydrocarbon derivatives of these lipids, 2α-methylhopananes IVe, are found in most oils and bitumens of all ages. To gauge the contribution of cyanobacterial debris to the sedimentary carbon pool, Summons et al. (1999) introduced the 2α-methylhopane index (2-MHI) that expresses the concentration of C31 2α-methylhopane relative to general bacterial C30 hopane. On an average, the 2-MHI is higher in the Precambrian (Summons et al., 1999). However, not all cyanobacteria produce hopanoids (Rohmer et al., 1984), and hopanoid biosynthesis in marine cyanobacteria appears to be rare (Pearson et al., 2007). Thus, absence or low concentrations of 2-methylhopanes should not be used as evidence for low activity of these organisms. Moreover, 2-methylbacteriohopanepolyols also occur in methylo trophic and nitrogen fixing proteobacteria. 2-Methylhopanoid biosynthesis in the γ-proteobacterium *Rhodopseudomonas palustris* occurs under anaerobic conditions, and this further complicates the interpretation of 2-MHI as an indicator for oxygenic photosynthesis (Rashby et al., 2007).

Low-molecular-weight (C₁₄−C₁₉) monomethyl alkanes and other acyclic alkanes with one or more alkyl groups occur in all oils and bitumens, but are particularly abundant in the Precambrian and in sediments with remnant microbial mat assemblages (Summons and Walter, 1990). Although methylalkyl compounds occur in many microorganisms, the predominant sources are probably the mono- to tri-methyl branched alkanes that are abundant in extant cyanobacteria (Köster et al., 1999).

**Methanotrophs,** methylotrophs, and acetic acid bacteria. Several groups of microaerophilic proteobacteria - type-I methanotrophs, methylotrophs, and acetic acid bacteria - biosynthesize hopanoids with an additional methyl group in 3β-position (Zundel and Rohmer, 1985a; Summons and Jahnke, 1992). Although 3β-methylhopanes IVb in sedimentary rocks and oils may be derived from any of the above groups, hopanoids of methanotrophs are probably the major source and can be distinguished by a pronounced depletion in 13C compared to co-occurring compounds (see *Biomarkers (Organic, Compound-Specific Isotopes)*) (Collister et al., 1992; Jahnke et al., 1999). A second unusual group of lipids produced by type-I methanotrophs are sterols with a cholestane XIIIa skeleton (C₃₇) that carry one or two additional methyl groups at C-4 (Bird et al., 1971; Summons and Capon, 1988). 4-methyl and 4,4-dimethyl cholesterol esters are also widespread in eukaryotes because they are the early intermediates in the biosynthesis of higher sterols. However, as intermediates, concentrations in eukaryotes are commonly very low, and very high relative concentrations of 4-methyl- and 4,4-dimethylcholestanes and their aromatic analogs in bitumens and oils likely indicate activity of methanotrophic bacteria. The oldest biomarkers of type-I methanotrophic bacteria come from the 1.6 Ga Barney Creek Formation in the McArthur Basin, northern Australia (Brocks et al., 2005). The well-preserved, organic-rich dolostones of the Barney Creek Formation were deposited in a deep rift basin that was, at least intermittently, stratified and sulfidic. In these ancient bitumens, 4-methylated triaromatic cholesteroids constituted >90% of the entire steroid population and, together with high relative concentrations of 3β-methylhopanes, suggest that aerobic methanotrophic bacteria were abundant members of the microbial community. As aerobic methanotrophs are usually only abundant in sulfate-starved environments (<0.5 mM) (Hoehler et al., 1998), the biomarkers suggest that sulfate concentrations in this Paleoproterozoic basin were well below the present marine level of about 28 mM.

**Green sulfur bacteria (Chlorobiaceae).** Green sulfur bacteria (Chlorobiaceae) are strictly anaerobic, obligately phototrophic organisms that exclusively utilize photosystem I (PS I). They require, with few exceptions, reduced sulfur species such as sulfide and sulfur as an electron donor. Thus, biomarkers of Chlorobiaceae, including specific aromatic carotenoids and bacteriochlorophyll breakdown products, are proxies for anoxic and sulfidic conditions in the presence of light, either in microbial mats or marine and lacustrine stratified waters (photic zone euxinia). Green pigmented Chlorobiaceae contain the carotenoids chlorobactene and hydroxychlorobactene, and the diagenetic product of these pigments, chlorobactane XXIII, is regarded as a highly diagnostic biomarker. The major carotenoids of brown pigmented strains of Chlorobiaceae are isorenieratene IX and β-isorenieratene that form the geologically stable fossil analogs isorenieratane VIII and β-isorenieratene. Isorenieratene IX also occurs in Actinomycetes, bacteria
that predominantly inhabit soil but have also been observed in marine sediments. However, Chlorobiaceae and Actinomycetes follow different carbon fixation pathways, and the carbon isotopic compositions of isorenieratane VIII can be used to determine the exact biological source (see Biomarkers (Organic, Compound-Specific Isotopes)). An unusual isomer of isorenieratane VIII, palaerenieratane XXIV with a 3,4,5-trimethyl aryl substitution pattern, is exclusively known from sedimentary rocks of Paleozoic age. A biological precursor of palaerenieratane XXIV has not yet been discovered in extant organisms, but a strong carbon isotopic enrichment indicative of carbon assimilation via the reverse tricarboxylic acid (TCA) suggests that it is derived from unknown Chlorobiaceae (Hartgers et al., 1993).

Other biomarkers that are diagnostic for Chlorobiaceae are degradation products of specific bacteriochlorophylls (BChl). BChls c and d exclusively occur in green filamentous bacteria (Chloroflexaceae) and Chlorobiaceae. BChl e (as a major pigment) is restricted to brown strains of Chlorobiaceae. During diagenesis, oxidative degradation of the tetrapyrrole skeleton of chlorophylls can lead to the formation of maleimides (1H-pyrrole-2,5-diones) that are highly stable under geological conditions (Grice et al., 1996b). While Chl a found in oxygenic phototrophs yields 3,4-dimethyl Xa and 3-ethyl-4-methylmaleimides Xb upon oxidation, BChls c, d and e yield the diagnostic biomarker 3-isobutyl-4-methylmaleimide Xc. Whether Xc in a particular sample is derived from Chlorobiaceae or Chloroflexaceae can be distinguished by compound-specific carbon isotopic measurements (Grice et al., 1996b) (see also Biomarkers (Organic, Compound-Specific Isotopes)).

Purple sulfur bacteria (Chromatiaceae). Purple sulfur bacteria (families Chromatiaceae and Ectothiorhodospiraceae) are anoxygenic phototrophs that form a well-separated group in the γ-subgroup of Proteobacteria. Although purple sulfur bacteria are generally metabolically more versatile than green sulfur bacteria, they also predominantly utilize sulfide and sulfur as their electron source. In microbial mats and stratified water bodies, they commonly inhabit a layer above green sulfur bacteria within the light penetrated zone directly beneath the oxic–anoxic interface. Twelve known genera of Chromatiaceae contain the purple coloured carotenoid okenone V that possesses a unique carbon skeleton with a 2,3,4-trimethyl aryl substitution pattern (Brocks and Schaeffer, 2008). The molecular fossil, okenone VI, is believed to be a diagnostic biomarker for Chromatiaceae (Brocks et al., 2005). Okenone has not been detected in microbial mat environments, and it may function as a specific accessory pigment for planktonic Chromatiaceae, adjusting the light harvesting systems to lighting conditions at relatively shallow water depth of less than ~25 m. Thus, okenone can be regarded as a biomarker for euxinic water systems with particularly shallow stratification.

The oldest biomarkers of green and purple sulfur bacteria were discovered in the Paleoproterozoic Barney Creek Formation in the McArthur Basin in northern Australia (Brocks et al., 2005). The 1.64 Ga old dolostones were deposited in a deep intracratonic rift basin that probably only had restricted connection to the ocean. Extracts of sedimentary rocks yielded high relative concentrations of okenane VI and chlorobactane XXIII, but only minor amounts of isorenieratane VIII. In comparison with modern ecosystems with similar pigment distributions, the biomarkers probably indicate a basin with a very shallow thermocline (~12 m). Chromatiaceae probably formed major blooms in a layer directly beneath the anoxic–oxic boundary, underlain by a layer of green pigmented Chromatiaceae and, yet deeper, minor communities of brown pigmented strains. In modern ecosystems, a strong activity of Chromatiaceae and green pigmented Chlorobiaceae is often associated with a low abundance of oxygenic phototrophs in the overlying water column. In the Barney Creek Formation, this is confirmed by a paucity of biomarkers diagnostic for eukaryotic algae (Brocks and Schaeffer, 2008).

Conclusions
The development of new metabolic pathways that permitted microorganisms to utilize previously untapped energy sources, such as iron reduction, oxygenic photosynthesis, and methanotrophy probably had profound effects on the chemical evolution of Earth’s atmosphere, crust, and oceans. It is a major aim of geobiology to correlate these chemical and isotopic changes in the geological record with the appearance of new microbial physiologies. One of the most informative methodologies to recognize the radiation of a new microbial group in the geological record is molecular fossils. To assign molecular fossils to specific organisms, or groups of organisms, we rely on information about the occurrence of precursor lipids in extant organisms, and almost all this information is derived from organisms that were grown in culture. However, according to some estimates, more than 99% of microorganisms currently defy isolation, and their physiologies, ecological roles, and lipid biosynthetic capacities remain largely unknown. This gap in knowledge is the main limitation for the application and interpretation of biomarkers. Promising ways to fill this gap are offered by cultivation-independent genomic and microbiological techniques such as 16S rRNA clone libraries, group-specific fluorescence in situ hybridization (FISH), and simultaneous community genomic (Tyson et al., 2004) and proteomic (Ram et al., 2005) studies. The technologies will help to describe which organisms are present in natural environments,
which groups are quantitatively important, and what are their major lipid products under specific conditions (Brocks and Banfield, 2009). Ideally, it should become possible to collate quantitative data about the rate of horizontal gene transfer and convergent evolution in lipid biosynthetic pathways, and this should make it possible to estimate the probability with which a biomarker from an ancient rock can be assigned to a specific biological source.

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**Cross-references**

*Algae (Eukaryotic)*

*Anaerobic Oxidation of Methane with Sulfate*

*Archaea*  

*Astrobiology*  

*Bacteria*  

*Biomarkers (Organic, Compound-Specific Isotopes)*  

*Cold Seeps*  

*Cyanobacteria*  

*Diatoms*  

*Isotopes and Geobiology*  

*Methane, Origin*  

*Nitrogen*  

*Origins of the Metazoa*  

*Sulfate-Reducing Bacteria*  

*Sulfur Cycle*

**BIOMARKERS (ORGANIC, COMPOUND-SPECIFIC ISOTOPES)**

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**Synonyms**

Stable isotopes of individual biomarkers

**Definition**

Stable isotopes as opposed to radiogenic (unstable) isotopes do not decay; thus, their natural abundances stay relatively constant, even over geological time. However, variations in stable isotopic composition, or isotopic fractionation, occur in nature as a consequence of chemical and physical processes, due to different isotopes of an element having different physical and chemical properties. Equilibrium processes and kinetic processes can lead to isotopic fractionation. Equilibrium isotope effects occur as a result of temperature-dependent equilibrium isotope-exchange reactions resulting in a change of the isotope distribution between different chemical materials, between varying phases, or between individual molecules (Hoefs, 1987). For example, the equilibrium reaction:

\[ A_1 + B_2 \rightleftharpoons A_2 + B_1 \]

Subscripts indicate that A and B contain either the light (1) or heavy (2) isotope.

In these cases, there is no net variation in the chemical process, i.e., the products are chemically similar to the reactants, with the exception that the stable isotopes are distributed differently between them. An example is the evaporation/condensation cycle of water as shown in the following equation (D stands for “deuterium,” the heavy hydrogen isotope, \( ^2H \)
The stable isotopic composition of organic matter is determined by its genetic origin. The elements hydrogen and carbon are the main constituents of organic matter and play key roles in many biochemical, ecological, environmental, hydrologic, and atmospheric processes. Therefore, stable isotopic compositions preserved in organic matter can provide powerful insights into these processes. Early isotopic investigations were chiefly based on bulk carbon isotope analysis. Continuous flow isotope ratio monitoring gas-chromatography mass spectrometry (irn-GCMS) also commonly referred to as compound-specific isotope analysis (CSIA) is important for determining the stable isotopic compositions of individual organic components in complex mixtures (e.g., petroleum, natural gases, sediments, soils, groundwater, potable waters, and extracts from plants and other media). As with gas-chromatography-mass spectrometry (GC-MS), compounds are separated according to mass differences. In an isotope ratio mass spectrometer, different isotopes of the same compound are separated based on relative mass differences. A gas analyte stream is prepared and it is then ionized by an ion source. The electron multiplier detects the ions of different isotope masses over a range of mass/charge ratios. This technique has been applied for several decades to the analysis of biomarkers of lipids (also see Chapter Biomarkers (Molecular Fossils)) and other natural products such as chlorophylls/bacteriochlorophylls found in intricate mixtures of petroleum, and in bitumens and kerogens extracted from sediments and sedimentary rocks. John Hayes pioneered the development of CSIA and its applications in the field of organic geochemistry based on his practice and principles of isotopic measurement and calibration, inter- and intramolecular isotopic distributions and
a fundamental understanding of carbon and hydrogen isotopic fractionation within biosynthetic pathways (Matthews and Hayes, 1978; Hayes, 1983, 1993, 2001; Summons et al., 2008). The first carbon isotopic measurements of individual fossil hydrocarbons was performed by Summons and Powell in 1986, and within the fields of organic geochemistry and biogeochemistry such measurements have become important for palaeoclimate reconstruction and are also used to elucidate aspects of biogeochemical cycling within a broad range of microbially mediated processes (Freeman et al., 1990; Summons et al., 1994; Summons et al., 1996).

**Instrumentation**

The irm-GCMS, whereby the gas chromatograph (GC) is linked to an isotope ratio mass spectrometer via a combustion interface for determining $^{13}\text{C}/^{12}\text{C}$ of individual organic components, in complex mixtures of petroleum and organic extracts from sedimentary material (Matthews and Hayes, 1978). The combustion interface either consists of a quartz tube containing CuO pellets (850°C) or a ceramic tube containing twisted CuO/Pt wires (850°C) (Matthews and Hayes, 1978; Hayes, 1983) yielding a gas analyte of CO$_2$ and by-product H$_2$O for each GC-separated component. H$_2$O is removed either with a Nafion trap or a liquid N$_2$ trap at -100°C. The isotope ratio mass spectrometer measures the abundances of the ions m/z 44 ($^{12}\text{CO}_2$), 45 ($^{13}\text{CO}_2$) and 46 ($^{12}\text{C}^{18}\text{O}_{16}\text{O}_2$). D/H CSIA requires that a GC is linked to an isotope ratio mass spectrometer via a pyrolysis furnace – either ceramic with a glassy carbon coating (1,400°C) or quartz packed with sieved chromium pellets (1,050°C) yielding a gas analyte of H$_2$ for each GC-separated component (e.g., Prosser and Scrimgeour, 1995; Burgoyne and Hayes, 1998). For this application the mass spectrometer measures the abundances of the ions m/z 3 ($^2\text{DH}$) and 2 ($^2\text{H}_2$). Contributions from H$_2^+$ produced in the ion source are corrected following m/z 3 analyses at two different pressures of the H$_2$ reference gas to determine the H$_2$ correction factor. Developments also include irm-GCMS instruments capable of measuring $^{15}\text{N}/^{14}\text{N}$ and $^{18}\text{O}/^{16}\text{O}$ (Merrit, 1993; Brand et al., 1994; Kashiwakuma et al., 2007).

In brief, biomarkers are the molecular fossils of biolipids and other natural products and are diagnostic for certain organisms such as algae, higher plants (e.g., Brassell et al., 1986), methanotrophic bacteria (Summons et al., 1994), and a wide range of other heterotrophic organisms (Grice et al., 1998a and references therein). Many biomarkers can be detected by GC-MS, and stable isotopic values are now routinely measured with the advent of CSIA. The identity, isometric arrangement and stable isotopic composition of biomarkers have been used in studies of petroleum and sedimentary organic matter to assess biological sources, and paleoenvironmental and depositional information.

In the petroleum industry, CSIA has been used for source rock–oil, oil–gas, and gas–gas correlation studies (e.g., Fusetti et al., 2010a, b).

**Separation techniques**

To obtain accurate and precise isotope ratios, components must be amenable to analysis using GC. However, biomarkers may be chemically bound to kerogen, or they may be highly functionalized, making them difficult to resolve by GC, thus presenting significant analytical challenges for CSIA. With the advent of hydropyrolysis (HyPy) (e.g., Love et al., 2005 and references therein) and micro-scale-sealed-vessel pyrolysis (MSSV) (Horsfield and Dueppenbecker, 1991; Beramendi-Orosco et al., 2006; Greenwood et al., 2006; Meredith et al., 2006) we can now release biomarkers from kerogen under mild conditions and convert highly functionalized lipids into hydrocarbons that are more amenable to GC-MS and CSIA.

Sample preferences for CSIA include: (1) low-molecular weight compounds, (2) at least 60 ng component concentration, and (3) complete chromatographic separation (i.e., to baseline) of component peaks of interest. CSIA has much more critical requirements for the GC resolution of products than standard molecular characterization by GC-MS. Compounds containing a higher propensity of $^{13}\text{C}$ atoms elute slightly earlier than their isotopically lighter counterparts. Thus, incomplete chromatographic resolution results in an overlap between $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ analyte signals from the co-eluting compounds, making it impossible to accurately determine their individual isotopic values. To avoid co-elution, separation techniques such as molecular sieving isolate fractions based on molecular size (e.g., 5A, ZSM-5, 13X, Armanios et al., 1993; Ellis et al., 1994; West et al., 1990; Grice et al., 2001; Audino et al., 2004; Tolosa and Ogrinc, 2007; Grice et al., 2008a). Other separation techniques include preparative thin layer chromatography (TLC), liquid chromatography (LC), gel permeation chromatography (Inaba and Suzuki, 2003), and High Performance LC-MS (HPLC-MS) (e.g., Smittenberg and Sachs, 2007). However, in petroleum, there are many minor components that are not routinely analyzed by CSIA due to lack of techniques to isolate suitable fractions. Even after fractionation of petroleum using state-of-the-art separation techniques, the resulting mixtures can still be extraordinarily complex and may not be amenable to CSIA. It is also important to establish that separation procedures do not cause isotopic fractionation.

For polar components, it is usually necessary to derivatize and improve chromatographic separation using derivatization agents such as BSTFA (N-O-bis(trimethylsilyl) trifluoroacetamide) (Jones et al., 1991) or MTBSTFA (N-(tert-butylimidemethylsilyl)-N-methytrifluoroacetamide) (e.g., Grice et al., 1996a). For CSIA, choice of the derivatization reagent is crucial to ensure accurate $\delta^{13}\text{C}$ analysis
Isotopic interpretation of biomarkers

The δ13C and δD of individual biomolecules in sediments and organisms depend on the complex isotopic fractionation associated with the biosynthetic network that is responsible for their formation (e.g., Hayes, 1993; Collister et al., 1994; Hayes, 2001; Monson and Hayes, 1982; Melzer and Schmidt, 1987; Schwender et al., 1996; Sessions et al., 1999; Chikaraishi et al., 2004a, b; Liu and Huang, 2005; Chikaraishi and Naraoka, 2006; Rommerskirchen et al., 2006). Lipids produced by different biosynthetic pathways can differ in δ13C by up to 20‰ within an individual organism (e.g., Summons et al., 1994; Schouten et al., 1998; and van der Meer et al., 1998). However, the exact mechanisms leading to these isotopic differences are not well-understood (Hayes, 2001). Other factors that impose carbon isotopic variation in living organisms include the partial pressure of CO2 (pCO2), cell size and geometry (Goericke et al., 1994; Popp et al., 1998), the growth rate of phytoplanktonic cells (Laws et al., 1995; Bidigare et al., 1997), and in land plants, water-use efficiency as, e.g., observed in C3, C4, and CAM plants (Ehleringer et al., 1993).

When reconstructing environments of deposition based on CSIA 13C/12C of biomarkers it is important to determine an isotopic reference point against which values for various components can be compared. C27 steranes and the C30 regular isoprenoid phytane are especially useful components since they are commonly reported in lacustrine and marine settings and mostly deriving from C27 sterols and chlorophyll a, respectively. Hence, δ13C of sedimentary steranes and phytane provide a reference point for lipid components predominantly biosynthesized by the algae in the upper water column. In sediment samples from the Permian Kupferschiefer, the δ13C of phytane was found to be similar to co-occurring algal-derived cholesterol, consistent with a common algal origin for both biomarkers. However, other sources of phytane cannot be excluded, such as chlorophyll a of cyanobacteria (Grice et al., 1996b), and C27 sterols are also a significant component in the cell membranes of most animals. The Permian Kupferschiefer also contains the biomarker methyl ethyl maleimide, an oxidative degradation product predominantly derived from the macrocycle of chlorophyll a (Grice et al., 1996b, 1997). The maleimide was 3.5–4.5‰ more enriched in 13C than phytane from the phytyl side chain, a magnitude of difference to be expected from a common chlorophyll origin (Grice et al., 1996b, 1997). When using CSIA 13C/12C of biomarkers in sediments organic matter to reconstruct paleoenvironments, it is understood that stable carbon isotopic fractionation effects associated with the biosynthesis of sterols and isoprenoids as well as cell sizes and geometry of the phytoplankton community, may be averaged out for sediment samples covering hundreds to millions of years. CSIA 13C/12C has also been applied to feeding studies. Zooplankton feeding on algae does not alter the stable carbon isotopic compositions of the algal lipids, which these organisms consume, retain, metabolize to cholesterol, and/or excrete (e.g., Grice et al., 1998a). Zooplanktons are selective feeders and the preferred algae may have distinct δ13C. δ13C signals of steroids (sterols and steranes) in sediments are probably representative of a phytoplankton mixture, although individual communities of the mixture have different δ13C compositions. This limitation should be borne in mind when using δ13C of sterols and steranes as isotopic reference points. Furthermore, a co-occurrence of algal lipids can complicate the interpretation of δ13C of phytoplankton biomarkers, since individual algal species are known to biosynthesize lipids with quite different carbon isotopic compositions (see Schouten et al., 1998). Thus, differences between phytane and cholesterol in organic matter may not be due to different source organisms, since there may be distinct effects associated with the biosynthesis of each by a single organism. Schouten et al. (1998), however, still suggest cholesterol is one of the most suitable isotopic reference points of the primary producers living in the euphotic zone of ancient water columns. Until recently, phytol and its diagenetic alteration products were thought to be useful indicators of phytoplanktonic isotopic reference points (e.g., Freeman et al., 1990; Hayes et al., 1990; Grice et al., 1998a). However, recent phytol isotopic data reported from a range of algae, land plants, and bacteria suggest that the disparities in carbon isotopic value of phytane and of a given degradation (e.g., the C19 isoprenoid pristane) may be due to isotopic heterogeneity within molecules (Schouten et al., 2008). However, the stable carbon isotopic composition will depend on whether pristane and phytane are exclusively derived from phytol or if there are additional sources (e.g., archaeal diether lipids, tocopherols, bacteriochlorophylls a/b) with potentially different stable carbon isotopic compositions (see below).

It is well-established that diagenetic and catagenetic processes over geological time (millions of years) promote significant hydrogen exchange between organic biomolecules in the surrounding environment with formation water (Rigby et al., 1981; Alexander et al., 1984; Schimmelmann et al., 1999; Leif and Simoneit, 2000; Schimmelmann et al., 2001; Sessions et al., 2004; Dawson et al., 2005). D/H studies of extant organisms have shown that biosynthetically produced
l lipids are depleted in D relative to the total biomass (e.g., Estep and Hoering, 1980). Furthermore, polyisoprenoid lipids are depleted in D relative to n-alkyl lipids (Estep and Hoering, 1980; Sessions et al., 1999). The magnitude of the fractionation between growth water and n-alkyl lipids is ca. \(-150\%\), while the fractionation between water and isoprenoid lipids is ca. \(-235\%\) (e.g., Smith and Epstein, 1970; e.g., Estep and Hoering, 1980; Sessions et al., 1999).

D/H of biomarkers can be strongly influenced by thermal maturation reflecting a strong relationship with the alteration of indigenous D/H signatures. Dawson et al. (2005) demonstrated the use of D/H ratios of sedimentary hydrocarbons (n-alkanes, pristane, and phytane) to evaluate the maturity of source-rocks and crude oils from the Perth Basin (WA). Pristane and phytane were significantly depleted in D compared to n-alkanes, although this difference reduces with increasing maturity due to thermally promoted isotopic exchange. The n-alkane–isoprenoid D/H signature of crude oils of different source facies was consistent with values associated with mature-rate mature sediments, i.e., representative of the peak oil-generative window. The average D/H ratios for Pr and Ph correlate well with \% equivalent vitrinite reflectance, which further supports a relationship between maturation and isotopic enrichment in isoprenoids. The work by Dawson et al. (2005, 2007) and Pedentchouk et al. (2006) suggests that the D/H measurement of sedimentary hydrocarbons may represent a useful maturity parameter which also accounts for different source-effects.

Green algae

Biomarkers derived from Botryococcus braunii (also see Chapter Biomarkers (Molecular Fossils)) have been shown to be significantly enriched in \(^{13}\)C compared with other phytoplanktonic biomarkers in crude oils (Dowling et al., 1995) and sediments (Boreham et al., 1994; Huang et al., 1995; Grice et al., 1998b; Huang et al., 1999; Audino et al., 2001a, 2002a, b; Grice et al., 2001) have been previously identified in torbanites and Indonesian crude oils containing remains of the freshwater alga. Based on \(\delta ^{13}\)C data it has been suggested that these compounds are derived from the algaean of Botryococcus braunii formed by an olefin metathesis reaction. The hydrogen isotopic ratios of lipids from Botryococcus braunii, including alkanediene, botryococcenes, heptadecenes, fatty acids, and phytadiene, closely follow the \(\delta D\) of the assimilated water (Zhang et al., 2007). A \(\delta D\) saw-toothed profile for odd and even n-alkanes in the torbanites is attributed to a dual-source system, a predominant B. braunii input with a second minor contribution from land plants (Dawson et al., 2004). \(\delta D\) of n-alkanes also reflected the depositional palaeolatitude/palaeoclimate of the sediments, attributed to the \(\delta D\) composition of meteoric waters in the environment where the sediments were deposited. For example, Torbanites deposited in a mid-latitude region under cool-temperate conditions contain n-alkanes with \(\delta D\) values falling in between those of n-alkanes from tropical (isotopically heavier) and glacial (isotopically lighter) environments (Dawson et al., 2004).

Photosynthetic sulfur bacteria

Green sulfur bacteria (Chlorobiaceae) fix CO\(_2\) via the reversed tricarboxylic acid cycle (Evans et al., 1966) leading to an enrichment of \(^{13}\)C in biomass (Quandt et al., 1977; Sirevag et al., 1977) by around 15\% relative to oxygenic phototrophic organisms utilizing the Calvin–Benson cycle for carbon fixation (e.g., Summons and Powell, 1986, 1987; Sinninghe Damsté et al., 1993; Hartgers et al., 1994a, b; Grice et al., 1996a, b). Chlorobiaceae also produce a range of relatively specific biomarkers such as the carotenoid derivatives isorenieratane and chlorobactane, certain aryl isoprenoids, methyl isobutyl maleimide, and farnesane (see Chapter Biomarkers (Molecular Fossils)). Detection of carbon isotopically strongly enriched lipids of this kind

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**Biomarkers (Organic, Compound-Specific Isotopes)**

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in the rock record is evidence for the activity of green sulfur bacteria. As Chlorobiaceae are strictly anaerobic phototrophs and utilize reduced sulfur species as electron donor, isotopically heavy isorenieratane and related aromatic compounds in geological samples have been widely used as molecular indicators for the presence of H2S in the zone of light penetration (“PZE” photic zone euxinia). In addition, more recently the biomarker crocetane in thermally mature samples has been attributed to carotenoids of Chlorobiaceae (Maslen et al., 2009).

The characteristic isotopic enrichment of Chlorobiaceae has also been observed in chlorobactane derived from the accessory pigment chlorobactene (Grice et al., 1998d) and in certain bacteriochlorophylls (Grice et al., 1996a, b). The structurally specific bacteriochlorophylls (BChl) of Chlorobiaceae (BChl c, d (e)) give rise to biomarkers such as 3-methyl-4-isobutylmaleimide and farnesane derived from the bacteriochlorophyll macrocycle and lipid farnesyl side chain, respectively. Farnesane has been reported to be depleted in 13C by about 3% compared to methyl isobutyl maleimide consistent with an origin of bacteriochlorophylls of Chlorobiaceae (Grice et al., 1996a, b). These data are supported by a recent study of the isotopic composition of lipids at different depths of saline meromictic Lake Kiiike, Japan (Ohkouchi et al., 2007). The carbon isotopic compositions of tetrapyrroles (chlorophyllides) were considerably enriched in 13C relative to the side chains (phytol or farnesol) of chlorophyll a, bacteriochlorophyll a, and bacteriochlorophylls e.

Along with S isotopic evidence from sedimentary pyrite minerals, Chlorobiaceae-derived biomarkers have provided evidence for the presence of PZE conditions during the largest extinction event of the Phanerozoic, at the end-Permian mass extinction (Grice et al., 2005a; Nabbefeld et al., 2010a, b). It has been proposed that the release of toxic H2S to the ocean surface and atmosphere played a critical role in the extinction and protracted recovery (Knoll et al., 1996). Similar conditions during the end-Permian have been reported in the contemporaneous seas off southern China, W. Canada, E. Greenland, and Kashmir-Tibet (Summons et al., 2006). It has been proposed by Kump et al. (2005) that a chemocline upward excursion probably provided a trigger for the extinction, releasing toxic H2S to the ocean surface and atmosphere.

Lipids derived from purple sulfur bacteria of the family Chromatiaceae, such as the carotenoid pigment okonene (Schaeffer et al., 1997; Brocks and Schaeffer, 2008), may be significantly depleted in 13C compared to phytoplankton markers. Carbon fixation in both Chromatiaceae and phytoplankton follows the Calvin–Benson Cycle and should, in principle, lead to similar carbon isotopic compositions of organic matter. Chromatiaceae inhabit deeper layers just beneath the thermocline of stratified euxinic waters where they may utilize CO2 derived from remineralized organic matter, and a CO2 source that is typically strongly depleted in 13C.

### Dinoflagellates

CSIA 13C/12C has been used to distinguish dinoflagellates and methanotrophic bacterial sources of sedimentary 4-methyl and 4, 4-dimethylcholesteroids (Bird et al., 1971). The δ13C values of the 4-methylsteroids in Chinese hypersaline lacustrine sediments (Grice et al., 1998d) are significantly enriched in 13C compared to co-occurring phytoplankton markers. These data are inconsistent with an origin from methanotrophic bacteria (see below).

In a study by Ramos et al. (2003) CSIA of individual fatty acids was performed to establish the transfer of primary photosynthetic carbon from the euphotic zone to benthic/hypertrophic environments in Conception Bay (Newfoundland, Canada). δ13C of fatty acids were highest at the height of the phytoplanktonic bloom, and became 13C-depleted at the end of the bloom cycle.

### Diatoms

Highly branched C25 and C30 isoprenoid (HBI)alkane biomarkers of sedimentary organic matter (Robson and Rowland, 1986; Sinninghe Damsté et al., 1989) are sourced from diatoms (Nichols et al., 1988; Volkman et al., 1992, 1994). The δ13C of these components are heavier than other phytoplankton biomarkers (e.g., Freeman et al., 1994; Summons et al., 1993), which have been attributed to a seasonal blooming of certain diatoms. Non-blooming diatoms are also reported to use a bicarbonate pumping mechanism leading to their biomass being 13C-rich (Summons et al., 1993). In samples of particulate organic matter from the water column and sediments from Ellis Fjord (eastern Antarctica) the C25:2 HBI alkene-assumed precursor of C25 HBI was found to have an enriched stable carbon isotopic composition consistent with a diatom source, probably belonging to the *Navicula* genus in the sea ice (Sinninghe Damsté et al., 2007).

### Epiphytic algae

C20 HBI and its monoene have been reported in both marine and freshwater sediments (Rowland et al., 1985). The structural similarity of the C20 HBI to C25 and C30 HBIs, led Sinninghe Damsté et al. (2005) to propose a common diatom source for each of these. The C20 HBI has not been identified in cultured diatoms (Volkman et al., 1998; Sinninghe Damsté et al., 2005), but has been identified in field samples of macrophytes *Chara* sp. and *Enteromorpha prolifera* (Rowland et al., 1985; Jaffe et al., 2001) pointing toward an epiphytic algal source (Volkman et al., 1998). Similar δ13C and δD of the C20 HBI to the C31 n-alkane attributed to land plants waxes in a series of Holocene sediments from China (Atahan et al., 2007; Grice et al., 2008b, 2009) implies a common source organism, or organisms utilizing similar carbon sources. Therefore, an epiphytic algal source is suggested for C20 HBI.
Haptophytes

Long-chain C\textsubscript{37} and C\textsubscript{38} alkenones (de Leeuw et al., 1980; Volkman et al., 1980; Rechka and Maxwell, 1988a, b; Thiel et al., 1997) are biosynthesized by certain haptophytes (e.g., Marlowe et al., 1990). The individual stable carbon isotopic compositions of alkenones, in combination with other data, have been used to assess ancient \( p\text{CO}_2 \) levels (Jasper and Hayes, 1990; Jasper et al., 1994). CSIA \textsuperscript{13}C/\textsuperscript{12}C data for haptophytes are consistent with bonic anhydrase-catalyzed conversion of bicarbonate to CO\textsubscript{2} for cellular utilization by these organisms (Werne and Hollander, 2004). The ratio of long-chain alkenones and their stable carbon isotopic compositions are unaffected by zooplankton herbivory (Grice et al., 1998a). \( \delta^{13} \text{C} \) (ca. \(-36\%o\)) of abundant C\textsubscript{37} and C\textsubscript{38} \( n \)-alkanes in a Chinese hypersaline lacustrine (Eocene-Paleocene) sediment have been found to be significantly depleted in \( ^{13} \text{C} \) compared to phytoplankton markers pointing to a specific origin from alkenones in haptophytes (Grice et al., 1998a), ascribed either to different algal bloom periods of the haptophytes to other algae. D/H of long-chain alkenones have also been used to record freshwater flooding of the eastern Mediterranean during sapropel deposition over the last 10 Myr, with their \( \delta^D \) reflecting that of source water and sea surface salinity.

Ciliates

Unusual \( \delta^{13} \text{C} \) of the biomarker gammacerane can provide information on microbial food sources that has been reported. Thus, gammacerane of heterotrophic ciliates feeding on methanotrophic bacteria or archaea can be strongly depleted in \( ^{13} \text{C} \) (Werne et al., 2002), while gammacerane from ciliates that consume green sulfur bacteria may be enriched in \( ^{13} \text{C} \) relative to biomass of oxygenic phototrophic organisms (Sinninghe Damsté et al., 1995; Grice et al., 1998d; Santos Neto et al., 1998). Thus, while tetrahymanol occurs in a variety of different organisms, gammacerane derived from bacterivorous ciliates may be recognized by an unusual carbon isotopic composition (Sinninghe Damsté et al., 1995; Werne et al., 2002). Also the carbon isotopic composition of C\textsubscript{30} 17\( \alpha \) 21\( \beta \) (H) hopane has been found to be similar to gammacerane in certain samples (Grice et al., 1998d) consistent with the notion that C\textsubscript{30} hopane-3\( \beta \)-ol occurs together with tetrahymanol in certain bacterivorous marine sauticociliates (Harvey and McManus, 1991).

Terrestrial plants

Long-chain \( n \)-alkyl compounds (\( n \)-alkanoic acid, \( n \)-alkane, and \( n \)-alkanol) are key components of epicuticular waxes extracted from the leaves of higher plants (e.g., Eglinton and Hamilton, 1967). Such compounds are ubiquitous found in soils, sediments, atmospheric dust, petroleum, and coal samples. CSIA \textsuperscript{13}C/\textsuperscript{12}C of \( n \)-alkyl compounds have been determined in many geochemical studies (Freeman et al., 1990; Hayes et al., 1990), to establish vegetation inputs. For example, vegetation source assessments (C3 and C4) based on \( ^{13} \text{C}/\text{C}^ {12} \text{C} \) of \( n \)-alkyl compounds has been widely applied to soils (e.g., Lichtfouse, 1995; Lichtfouse, 1997; Lichtfouse, 1998; Conte et al., 2003; Wiesenberg et al., 2004; Quénéa et al., 2006), marine and terrestrial sediments (Ficken et al., 1998b; Huang et al., 2000; Zhao et al., 2000; Scheufuß et al., 2003; Zhao et al., 2003; Fekains et al., 2005; and Huang et al., 2006), and atmospheric dust particles (Simoneit, 1997; Huang et al., 2000; and Conte et al., 2003). More recently, CSIA D/H of \( n \)-alkyl compounds in geological samples has become an important proxy for paleohydrological cycles (Xie et al., 2000; Andersen et al., 2001; Sauer et al., 2001; Huang et al., 2002; Grice et al., 2003; Yang and Huang, 2003; Dawson et al., 2004; Sachse et al., 2004; Xie et al., 2004; Liu and Huang, 2005; Hou et al., 2006; Smith and Freeman, 2006; Sachse et al., 2006; Sessions, 2006; Pagani et al., 2006; Grice et al., 2008c; Zhou et al., 2010).

It is generally accepted that \( n \)-alkyl compounds show \( \delta^{13} \text{C} \) in the \(-35 \pm 5\%o\) range for C3 plants and \(-20 \pm 5\%o\) range for C4 plants. A significant difference in \( \delta^{13} \text{C} \) also exists between C3 angiosperm and gymnosperm, where gymnosperm biomass is \( ^{13} \text{C} \) depleted compared to gymnosperm biomass by ca. 5\%o (Chikaraishi et al., 2004b). \( \delta^D \) of \( n \)-alkyl compounds are quite similar for all types of higher plants, spanning a range of \(-250\) to \(-100\%o\). Terrestrial plants are more exposed to the external environment, and use water produced by the complex hydrological cycle. An additional source of isotopic fractionation associated with terrestrial plants is evapotranspiration, which leads to the evaporative loss of the light isotopic species of water on leaf surfaces, resulting in an enrichment of D in the remaining water (Dongmann et al., 1974; Liu and Huang, 2005; Smith and Freeman, 2006; Sachse et al., 2006). Several studies on biosynthetic isotopic fractionation during photosynthesis and production of \( n \)-alkyl compounds have been carried out (Sessions et al., 1999; Hayes, 2001; Grice et al., 2003; Chikaraishi et al., 2004a, b; Sessions, 2006; Chikaraishi and Naraoka, 2006). The small differences in \( \delta^D \) for various lipids reported between different plant types are mainly attributed to variations in physiology (e.g., Smith and Freeman, 2006). In general, terrestrially derived organic matter has been shown to display a wide variation in \( \delta^D \) (e.g., Schimmelmann et al., 2004; Xiong et al., 2005). While temperature, humidity, and latitude (Xie et al., 2000; Xie et al., 2004; Pagani et al., 2006) are the most important determining factors, the D/H of meteoric waters in the terrestrial hydrological cycle are also affected by altitude and continentality (e.g., Dawson et al., 2004). These effects are highly changeable, leading to the large isotopic variations observed in terrigenous organic matter. Schimmelmann et al. (2004) measured the \( \delta^D \) of the individual fractions of 75 terrestrially derived crude oils and found that the \( \delta^D \) values of saturated fractions covered a range from \(-245\) to \(-62\%o\). Xiong et al. (2005) determined the \( \delta^D \) of individual \( n \)-alkanes in terrestrial Chinese
source rocks from the Turpan and Liaohe Basins, which covered a range from −250 to −140%. Furthermore, the bulk δD of saturated hydrocarbon fractions of marine- evaporitic, lacustrine, and mixed oils reported by Santos Neto and Hayes (1999) showed that the lacustrine oils were enriched in D (by 10–26%) relative to other oils in the basin.

δ13C and δD of leaf waxes depends on understanding the relative contribution and isotopic signatures of angiosperm and conifer plants in the rock record, due to the variable isotopic fractionation of CO2 and water associated with differences in their stomatal structure (Smith et al., 2007). In a study by Pedentchouk et al. (2008) δ13C and δD compositions of n-C27 alkane data were consistent with a lower stomatal conductance for CO2 and water vapor in conifers relative to angiosperms.

Few studies have focused on δ13C of aromatic components derived from land plants. In torbanite deposits, alkylphenanthrenes were found to have similar δ13C to the higher-plant biomarker retene in the same samples (Grice et al., 2001) consistent with a land plant origin for these (e.g., Simonneit, 1997; Alexander et al., 1987, 1988). The proposed mechanism of formation involves rearrangement of phyllocladane precursors yielding 1-methylphenanthrene and retene (Simonneit, 1997; Alexander et al., 1987). The reported isotopic data for retene and alkylphenanthrenes are consistent with a 6p(H)-phyllocladane (ca. −23%) source also reported in Australian Gippsland Basin oils and close to the average for class I conifer resinites reported by Murray et al. (1998). 1,7-dimethylphenanthrene in the torbanites is thought to be derived from a different precursor, being slightly more depleted in 13C compared with retene possibly deriving from a pimarane type precursor. A multidisciplinary study using molecular and organic isotope geochemistry, petrology, and palynology have been used to establish palaeo-environmental conditions of fluvio-deltaic deposits from the Delambre-1 well, Western Australia (Triassic–Jurassic) (Grice et al., 2005b). The δ13C of retene ranging from −26.9 to −24.9% was also consistent with a source resinite from class I conifers (Murray et al., 1998) and was related to samples rich in Araucarian conifers.

δD analyses have also been extended to diterpenoid hydrocarbons from land plants in coal and carbonaceous mudstone samples from the Liaohoe Basin, China (Tuo et al., 2006). δD for a majority of the n-alkanes range from −150 to −220‰ Pristane is reported to be 34–69‰ depleted in D relative to phytane. Diterpenoid hydrocarbons were found to be also depleted in D relative to the n-alkanes indicating a different source for these compounds, although hydrogen exchange could not be fully excluded as a result of thermal maturity (see above). δ13C of terpenoid hydrocarbons (−24.5‰) in samples from the Middle Miocene second Lusatian lignite seam (Lubstów open cast mine, Poland) are attributed fossil wood fragments of gymnosperms (most probably a species of the coniferales families Taxodiaceae/ Cupressaceae) (Bechtle et al., 2007).

Archaea

Marine crenarchaeaota

Based on the carbon isotopic composition of kerogen and archaeal lipids, Kuyper et al. (2001) estimated that the dead biomass of marine Crenarchaeota and possibly other Archaea constitutes up to 80% of sedimentary organic matter deposited during the Cretaceous Oceanic Anoxic Event (OAEB event). The unusually heavy carbon isotopic composition (10‰ vs. PDB) of archaeal lipids suggests that the marine Crenarchaeota had a chemosynthetic metabolism.

Halophilic archaea

The C17 to C25 regular isoprenoids extracted from Dead Sea halite deposits (Miocene/Pliocene) were reported to be 7‰ enriched in 13C relative to lipids from phototrophic organisms (Grice et al., 1998d), consistent with the heterotrophic metabolism of Halobacteriales. In the Dead Sea today, Dunaliella sp. is the main primary producer and Halobacteriales feed off their biosynthetic products, such as glycerol (Oren, 1993). It is suggested that Dunaliella, capable of growing rapidly under hypersaline conditions, may be responsible for producing isotopically heavy products. The consumption of this isotopically heavy glycerol by Halobacteriales would in turn lead to a 13C enrichment of their biomass (Grice et al., 1998d and references therein).

Andersen et al. (2001) reported δD of n-alkanes and isoprenoids attributed to rapid climatic changes during the Messinian salinity crisis, their isotopic data showing that enrichment in D followed climatically driven hydrologic changes in response to intense evaporation.

Sources of methane

Methane can be biogenically produced from CO2 and H2, acetate, or methylated compounds such as trimethylamine and dimethylsulfide (Galimov, 2006 for a review). In marine sediments methane is produced almost entirely via CO2 reduction via microbes. In terrestrial settings (e.g., freshwater, marsh) acetate fermentation prevails. It has been well-established that methane produced in anaerobic settings is extremely depleted in 13C. δ13C of −60‰ upward are characteristic of microbial-produced methane (Games and Hayes, 1978). Cross plots of δ13C and δD measurements (Schoell, 1980; Galimov and Kvenvolden, 1983; Wofford et al., 1984; Whiticar et al., 1986) are used to establish (a) CO2 reduction (b) Acetate fermentation pathway. Isotopic shifts have been reported in methane evolved in both freshwater (δ13C ca. −55‰ to - ca. −90‰) and marine (δ13C ca. −40‰ to ca. −55‰) environments (Whiticar et al., 1986). As a result of such fractionations, organisms utilizing isotopically light methane are typically highly depleted in 13C (Summons et al., 1994).
Methanogenic archaea and methane oxidizing archaea

Due to their isotopically light carbon source, lipids of methanotrophic and methane-oxidizing archaea and euryarchaea are commonly characterized by very negative carbon isotopic values. Biomarkers of these organisms include, phytane, 2,6,10,15,19-pentamethylicosane (PMI), crocetane, archaeol, biphytane, hydroxyarchaeol, acyclic biphatic diacids, and various macrocyclic dihyptyl glycerol diethers (Birgel et al., 2006; Birgel and Peckmann, 2008). Biphytane has also been reported to be enriched in $\delta^{13}$C compared to phytoplankton biomarkers by about 4–5% in pelagic sediments and in the water column (Hoefs et al., 1997). This difference was ascribed to either (1) the use of dissolved inorganic carbon as carbon source, but with biosynthetic pathways within methanogens discriminating less against $\delta^{13}$C than phytoplankton, or (2) archaea obtaining their carbon source from low-molecular weight organic substrates (acetate/methylated amines) generated during decomposition of algal-derived particulate or dissolved organic matter. The methane produced by methanogens is isotopically light compared to their bulk biomass. $\delta^{13}$C of PMI in some black shales (Cretaceous) has been found to share a similar $\delta^{13}$C to phytane (Vinke et al., 1998). However, other presumed algal biomarkers were in fact lighter by about 10%, indicating that phytane is probably related to the same source as PMI (an archaeaell origin).

The anaerobic oxidation of methane (AOM) with sulfate is the most significant sink for methane in marine settings (Niemann and Elvert, 2008). This process is performed by a consortium of methanotrophic archaea and sulfate reducing bacteria (e.g., Hinrichs et al., 1999; Boetius et al., 2000; Orphan et al., 2001; Reitner et al., 2005; Birgel and Peckmann, 2008).

AOM accounts for an increase in alkaline conditions, causing subsequent precipitation of authigenic carbonates (Ritger et al., 1987; Aloisi et al., 2002). In modern seep environments, biomarkers and their $\delta^{13}$C have provided detailed information on microbial activity related to seeps. $^{13}$C-depleted archaeal biomarkers reported in limestones associated with ancient methane seeps have linked AOM back Jurassic times (Peckmann et al., 1999; Peckmann and Thiel, 2004; Birgel et al., 2006). Markers of sulfate-reducing bacteria (iso-C$_{15}$-fatty acids and anteiso-C$_{15}$-fatty acids) are characterized by very negative carbon isotopic values (Birgel and Peckmann, 2008).

Coolen et al. (2004) reported lipids of methanogenic archaea and $^{13}$C-depleted $\Delta^{8(14)}$ sterols supporting an active methane cycle in Ace Lake during the last 3,000 years; extant methanotrophs existed when it became a marine inlet.

Methane oxidizing bacteria

The isotopically light methane produced by methanogenic archaea (above) can be further fractionated by methanotrophic bacteria leading to biomass becoming depleted in $\delta^{13}$C (ca. $-90\%$) and their sedimentary hopanoid analogues are similarly highly depleted in $\delta^{13}$C (Freeman et al., 1990). The presence of $^{13}$C-depleted hopanoids in non-marine sediments has been related to bacterial methane cycling in the Messel Shale of Eocene age (ca. $-65\%$; Freeman et al., 1990), the Green River Formation (ca. $-80\%$; Collister et al., 1992) and in a recent lake sediment (ca. $-50\%$; Spooner et al., 1994).

Methanotrophic bacteria are probably the major source of $3\beta$-methylhopanoids in sedimentary rocks and oils, as these biomarkers may show a pronounced depletion in $\delta^{13}$C compared to co-occurring compounds (see Chapter Biomarkers (Organic, Compound-Specific Isotopes)) (Collister et al., 1992; Jahnke et al., 1999).

Apart from $^{13}$C depleted isoprenoids derived from methanotrophic archaea, $^{13}$C depleted triterpenoids ($3\beta$-methylated hopanoids, $\delta^{13}$C $-100\%$; lanostanes, $\delta^{13}$C $-80$ to $-70\%$) have also been reported from a number of ancient seep deposits (Birgel and Peckmann, 2008). These data are consistent with contributions of $^{13}$C depleted lipids from aerobic methanotrophic bacteria.

Cyanobacteria

Monomethyl and dimethyl branched alkanes, in particular 7-methylheptadecane, 8-methylheptadecane, and 7,11-dimethylheptadecane are known to be biosynthesized by cyanobacteria (e.g., Summons et al., 1998). For example, it has been reported by Summons et al. (1998) that cyanobacteria are able to synthesize normal or branched alkanes of different $\delta^{13}$C compositions depending upon the environmental conditions. Cyanobacteria also biosynthesize extended bacteriohopanetetrol derivatives. $\delta^{13}$C of extended hopanoids ($>C_{30}$) determined in sediments (Grice et al., 1996b, 1997; Schouten et al., 1997) generally have similar $\delta^{13}$C to algald-derived cholestan and/or phytane, indicating a possible origin from cyanobacteria inhabiting upper regions of a water column. However, Schoell et al. (1994) found $\delta^{13}$C differences between the cyanobacterial hopanoids ($\Delta^{29.5}$ to $-31.5\%$) and algald-derived steranes ($\Delta^{25}$%$) in the Middle Miocene which were interpreted as marking the interglacial/glacial transition from a well-mixed to a highly thermally stratified ocean. Schoell et al. (1994) attributed these isotopic differences to cyanobacteria living in deeper parts of the water column of a stratified oceanic environment utilizing a more depleted inorganic carbon source than the algae inhabiting the upper water column. Sinninghe Damsté et al. (2001) identified two novel glycolipids, docosanyl 3-$\alpha$-methyl-2-rhamnopyranoside and docosanly 3-$\alpha$-methylxylopyranoside in Ace Lake from Antarctica. $\delta^{13}$C measurements showed that the sugar moieties of these glycolipids were ca. 8–9% enriched relative to the alkyl chains. Since structurally similar glycolipids have been reported to occur in nitrogen-fixing cyanobacteria, a cyanobacterial origin is proposed for these glycolipids.
Based on empirical isotopic relationships between the tetrapyrrole nuclei of chlorophylls and phototrophic algae, it has been concluded that diazotrophic cyanobacteria were the dominant components of phytoplankton communities during the mid-Cretaceous oceanic anoxic events (OAES) in the western Tethys Sea (Kashiyama et al., 2007). N₂-fixation was an important major process in phytoplankton production during these OAEs.

Conclusion
Molecular carbon and hydrogen isotopic compositions of organic matter determined using irm-GCMS, provide significantly more useful information on palaeoenvironments than one gains from δ¹³C bulk data. The groundbreaking research into CSIA of biomarkers in geochemistry research has further led to applications in a multiple of microbiological, environmental, medical, geochemical, ecological or biochemical, and forensic research areas. Future areas of interest lie in radiocarbon measurements at the molecular level and the complexity of the N₂ fixation processes in our oceans.

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Cross-references

- **Algae (Eukaryotic)**
- **Anaerobic Oxidation of Methane with Sulfate**
- **Archaea**
- **Astrobiology**
- **Bacteria**
- **Biomarkers (Molecular Fossils)**
- **Cold Seeps**
- **Cyanobacteria**
- **Diatoms**
- **Isopotes and Geobiology**
- **Methane, Origin**
- **Methane Oxidation (Aerobic)**
- **Nitrogen**
- **Origins of the Metazoa**
- **Sulfate-Reducing Bacteria**
- **Sulfur Cycle**

**BIOMINING (MINERAL BIOLEACHING, MINERAL BIOOXIDATION)**

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**Summary**

Biomining is the use of autotrophic, acidophilic, iron-, and sulfur-oxidizing microorganisms to produce ferric iron and sulfuric acid. These chemicals are capable of oxidizing a variety of minerals containing insoluble metal sulfides such as FeS2, CuS, NiS, and ZnS into their soluble sulfate forms e.g., Fe2SO4, CuSO4, NiSO4, and ZnSO4, respectively, resulting in their extraction into water from which they may be more easily recovered. Metal leaching is carried out from mineral waste dumps, or more typically and especially from constructed heap or stirred tank reactors. The metals recovered commercially in the greatest quantities are copper and gold.

**Description of the terms bioleaching, biooxidation, and biomining**

The term bioleaching refers to the conversion of an insoluble metal (usually a metal sulfide, e.g., CuS, NiS, ZnS) into a soluble form (usually the metal sulfate, e.g., CuSO4, NiSO4, ZnSO4). As the metal is extracted into water, this process is called bioleaching. Because this process involves oxidation, it may also be termed biooxidation. However, the term biomining usually refers to processes in which the recovery of a metal is enhanced by microbial decomposition of the mineral, but the metal being recovered is not solubilized. An example is the recovery of gold from arsenopyrite ores where the gold remains in the mineral after biooxidation and is extracted by cyanide in a subsequent step. The term bioleaching is clearly inappropriate when referring to gold recovery (although arsenic, iron, and sulfur are bioleached from...
the mineral). Biomining is a general term that may be used to refer to both processes (Rawlings, 2002).

**Definition**
Biomining is a generic term that refers to a collection of biotechnological processes in which microorganisms are used in the recovery of metals from mineral ores and concentrates. The microbial processes involved are the same as those responsible for acid mine drainage, and where metals are recovered during the treatment of acid mine drainage such a process may be termed biomining. Although the bioadsorption of metals onto biological materials including microorganisms may be used in metal recovery, the term biomining has not been applied to bioadsorption processes.

Metals that are recovered in the greatest quantities in commercial processes are copper, gold, and cobalt. Illustrations of the number and scale of operations that employ microorganisms for metal recovery are to be found in Olsen et al. (2003) and Rawlings et al. (2003). Processes also exist for the treatment of nickel- and zinc-containing ores although recovery of these metals using “biomining” has not been commercialized. For several years uranium was recovered using related processes (McCready, 1988) and the technology has the potential for the recovery of several other types of metals. In general, to be amenable to biologically assisted leaching, the mineral should contain iron or a reduced form of sulfur.

Biomining processes use the action of microbes for one of two purposes (Rawlings, 2005). Either to convert insoluble metal sulfides (or oxides) to water soluble metal sulfates or as a pretreatment process to open up the structure of the mineral thereby permitting other chemicals to better penetrate the mineral and solubilize the desired metal. An example of the first type of process is the conversion of insoluble copper present in minerals such as covellite (CuS) or chalcocite (Cu₂S) to soluble copper sulfate. An example of the second is the removal of iron, arsenic, and sulfur from gold-bearing arsenopyrite so that the gold that remains in the mineral is more easily extracted by subsequent treatment with cyanide. Both are oxidation processes, but when the metal to be recovered is extracted into solution, the process is known as bioleaching, whereas when the metal remains in the mineral, bioleaching is an inappropriate term and the process should strictly be referred to as biooxidation. The term bioleaching is frequently used for both.

**Mechanisms of biologically assisted leaching**
Current understanding is that biooxidation is primarily a chemical process. Sand and coworkers have proposed that minerals can be divided into two broad categories, acid-insoluble minerals that are solubilized through oxidation by ferric iron (e.g., FeS₂, MoS₂, and WS₂) and acid-soluble minerals that are oxidized by the combined action of ferric iron and protons (e.g., ZnS, PbS, FeAsS, CuFeS₂, and MnS₂). The role of the microorganisms is to provide the ferric iron and sulfuric acid leaching agents. Leaching reactions have been presented in Schippers and Sand (1999) and Rohwerder et al. (2003) and are reviewed in Rawlings (2005).

Because bioleaching processes are chemistry-driven, if a mineral (such as chalcopyrite) is recalcitrant to biooxidation at 40°C, the solution to the problem lies primarily in the realm of chemistry rather than biology. For example, an increase in the temperature of the biooxidation process to 80°C will allow the chemical reactions to take place at a much faster rate. However, the biology needs to follow the chemistry, and microorganisms that are capable of iron and sulfur oxidation at 80°C are required to generate or regenerate the lixiviants (leaching chemicals).

Although it is possible to separate the chemistry of metal solubilization from the biology into two different processes, separation is unlikely to be as efficient as when both processes are carried out together. The reason for this is that the role of the microorganisms is considered to be more than the generation of the ferric iron and acid required for the chemistry of mineral biooxidation. Contact between the microbes and the mineral is important (though not essential) because the microorganisms produce an exopolysaccharide layer when attached to a mineral (Sand et al., 1995). This layer serves as a reaction space where mineral dissolution reactions take place more efficiently than in the bulk solution. Increased efficiency is partly because conditions within the exopolysaccharide layer (e.g., concentration of ferric iron, redox potential, and pH) are substantially different and more favorable for leaching compared with conditions in the bulk solution (Rawlings, 2005).

**Biomining processes**

**Heap reactors**
Commercial mineral bioleaching can take place using a low technology process such as the irrigation of waste ore dumps (Brierley, 1982). The efficiency of metal recovery may be improved by the construction and irrigation of specially designed heaps rather than by the irrigation of an existing, nonengineered dump (Brierley, 1982; Rawlings et al., 2003). During heap construction, agglomerated ore is piled onto an impermeable base and supplied with an efficient leach liquor distribution and collection system. Acidic leaching solution is percolated through the crushed ore, and microbes growing on the surface of the mineral in the heap produce the ferric iron and acid that carry out mineral dissolution and metal solubilization. Good aeration enhances the metal leaching reaction. Aeration can be passive, with air being drawn into the reactor as a result of the flow of liquid or may be active with air blown into the heap through piping installed near the bottom. Metal-containing leach solutions that drain from the heap are collected and sent for metal recovery. Heap reactors can be of enormous size (Rawlings et al., 2003), are
relatively cheap to construct and operate, and are therefore suited to the treatment of lower grade ores. Compared with tank reactors, the metal recovery efficiency of heap reactors tends to be low. Heap reactors are more difficult to aerate efficiently and undesirable gradients of pH, nutrients, and liquor channeling are difficult to manage. In addition, although initial rates of bioleaching can be improved by effective heap inoculation, it is difficult to achieve in practice and nondeliberate inoculation that occurs during irrigation may be the main method by which microorganisms are spread through a heap.

**Types of microorganisms**

Both heap and tank biooxidation processes contain a variety of microorganisms, with two different organisms usually dominating: an iron-oxidizer and a sulfur-oxidizer. A wide variety of these microorganisms may be present (Rawlings and Johnson, 2007). Irrespective of the type of process or temperature, these microbes have several features in common that make them suitable for their role in mineral dissolution. They grow autotrophically by fixing $\text{CO}_2$ from the atmosphere which makes it unnecessary to provide them with a fixed carbon source. Their energy is obtained by using either ferrous iron or reduced inorganic sulfur compounds (some use both) as electron donors. Oxygen is generally used as the electron acceptor. All are acidophiles and grow in low-pH environments ($\text{pH} 1.4–1.6$ is typical) and they tend to be tolerant of a wide range of metal ions (Dopson et al., 2003). These very modest nutritional requirements can generally be met by the aeration of the iron- and/or sulfur-containing mineral suspension in water or the irrigation of a heap. Small quantities of inorganic fertilizer may be added to ensure that nitrogen, phosphate, potassium, and trace element limitation does not occur.

The types of organisms found in heap and tank reactors that operate at different temperatures have been reviewed (Rawlings and Johnson, 2007). In general, bacteria belonging to the genera *Acidithiobacillus* and *Leptospirillum* tend to dominate the processes up to $40^\circ\text{C}$, with those belonging to the genera *Acidithio-

**Bibliography**


Cross-references
Acid Rock Drainage
Archaea
Bacteria
Copper
Extracellular Polymeric Substances (EPS)
Extreme Environments
Fe(II)-Oxidizing Prokaryotes
Gold
Microbial Biomineralization
Ores, Microbial Precipitation and Oxidation
Sulfide Mineral Oxidation
Sulfur Cycle

BIOPROTECTION
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Synonyms
Bioconservation; Bioconsolidation; Biopreservation; Biorestoration

Definition
Active or passive microbially induced or mediated consolidation, cleaning, and/or protection of (stone) artworks affected by chemical, physical, and/or biological weathering phenomena. Bioprotection includes the microbially mediated removal (cleaning) of deleterious compounds (sulfates, nitrates, and/or organics), and the in situ cementation (consolidation) of ornamental materials via microbially induced biomineralization. Bioprotection also occurs naturally on rock surfaces where microorganisms aid in the formation of cementing phases such as oxalates and carbonates, and biofilms.

Introduction
The term bioprotection has been applied to a range of processes and applications involving microorganisms. They include microbe-induced defense response in plant–soil interactions, plant/crop treatment, food processing, and toxic metal decontamination (Glazer and Nikaido, 2007), prevention of wood decay (Dawson-Andoh and Morrell, 1992), inhibition of steel corrosion (Volkland et al., 2000), natural protection of rock outcrops (Carter and Viles, 2005) and building stones (Di Bonaventura et al., 1999), as well as cultural heritage conservation (Ciferri et al., 2000; Rodriguez-Navarro et al., 2003; Ramirez et al., 2005; Webster and May, 2006). Here, the term bioprotection refers both to the use of microorganisms (mainly bacteria) for the conservation of stone artworks, and to the microbially mediated formation of natural protection layers on rock outcrops and stone surfaces. Such meanings are the most commonly used and accepted in geobiology, as they involve the interaction between the biosphere and the geosphere.

Although “carved in stone” is meant to be durable or eternal, ornamental stones are subjected to a range of physical, chemical, and biological weathering phenomena that lead to their eventual crumbling and disintegration. Such weathering processes have been exacerbated by anthropogenic emission of pollutants since the Industrial Revolution (Winkler, 1994). As a consequence, stone monuments and statuary are at risk worldwide.

Granites, sandstones, and carbonate rocks – limestone, dolostone, and marble – are among the most common stones used in monuments and statuary. Carbonate stones, made up of calcite (CaCO3) and/or dolomite (CaMg(CO3)2), are particularly prone to chemical weathering in polluted (urban) environments, as exemplified by the decay of the Parthenon or the Taj Mahal. Wet (acid rain) and dry deposition of atmospheric pollutants contribute to the leaching of these sparingly soluble carbonates and the formation of sulfate – gypsum (CaSO4·2H2O) – crusts, also named “black crusts” (Figure 1). Carbonate stones are also prone to physical–mechanical weathering associated to salt crystallization, insolation weathering, frost damage, and hydric expansion/contraction (Winkler, 1994; Gauri and Bandyopadhyay, 1999; Rodriguez-Navarro et al., 2003). Biodeterioration is also a major

Bioprotection, Figure 1 Black crusts developed on a limestone gargoyle at the Royal Chapel, Granada (Spain).
contributor to the decay of stones in general, and carbonate stones in particular (McNamara and Mitchell, 2005).

Conservation of decayed stoneworks typically involves cleaning (by mechanical or chemical means) and/or protection/consolidation. Organic (e.g., acrylic or epoxy resins), organo-inorganic (e.g., ethyl silicates), and inorganic (e.g., \( \text{Ba(OH)}_2 \) and \( \text{Ca(OH)}_2 \) solutions) protection/consolidation treatments have been applied in the past to alt or mitigate stone decay (Lazzarini and Tabasso, 1986; Price, 1996). However, none of these treatments have been proven to be satisfactory (see Rodriguez-Navarro et al., 2003 and references therein). The limitations and drawbacks of these “classic” conservation treatments have prompted the search for new methods to clean, protect and consolidate weathered ornamental stones. One promising line of research has focused on microbial bioprotection as a new means for the restoration and consolidation of ornamental stone.

**Microbial-stone interactions: biodeterioration versus bioprotection**

It has been known for decades that microbes actively contribute to the degradation of ornamental stone (McNamara and Mitchell, 2005). For instance, the metabolic activity of nitrifying bacteria (\( \text{Nitrosomonas} \) sp. and \( \text{Nitrobacter} \) sp.) results in the production of nitric acid, which in turn causes dissolution of stone and formation of deleterious nitrate salts (Fernandez, 2006). Sulfur-oxidizing bacteria such as \( \text{Thiobacillus} \) sp. produce sulfuric acid and contribute to the development of sulfate crusts (Warscheid and Braams, 2000). Biofilms can exacerbate microbial biodeterioration as they provide a shelter for the aforementioned bacteria, and act as a reservoir for nutrient storage (McNamara and Mitchell, 2005). Wetting and drying of the biofilm matrix may lead to swelling/shrinking of the extracellular polymeric substances (EPS), causing mechanical damage to the host stone (Warscheid and Braams, 2000).

Although microbes are often the problem, they can also be the solution (Rinaldi, 2006). For instance, the anaerobic sulfate-reducing bacteria \( \text{Desulfovibrio desulfuricans} \) and \( \text{D. vulgaris} \) have been shown to be effective for the removal of gypsum crusts (Webster and May, 2006; Cappitelli et al., 2007). Similarly, nitrate salts can be eliminated through the application of nitrate-reducing bacterial cultures (Fernandez, 2006), while \( \text{Pseudomonas stutzeri} \) has been successfully applied to eliminate organic residues from frescoes (Ranalli et al., 2005).

There is growing evidence that several microorganisms contribute to the natural bioprotection of rock outcrops (Carter and Viles, 2005) as well as ornamental stone (Di Bonaventura et al., 1999) via the formation of biofilms and cemented surface layers. Some bacterial communities can promote the production of protective calcium oxalates on building stone (Di Bonaventura et al., 1999), or inhibit calcite dissolution (Rinaldi, 2006). Numerous strains of bacteria isolated on different building stones are able to induce the precipitation of calcium carbonate (Urzi et al., 1999), an effect that may have aided in the natural protection of weathered ornamental stones. This latter ability has opened new ways for the effective protection and consolidation of decayed ornamental stones based on the in situ mineralization of bacterial carbonate cement (Rodriguez-Navarro et al., 2003; Webster and May, 2006; Jimenez-Lopez et al., 2007).

**Bacterially mediated biomineralization of carbonates**

The precipitation of carbonate minerals mediated or induced by both autotrophic and heterotrophic bacteria have attracted a substantial amount of research (see recent review by Wright and Oren, 2005). Bacterial precipitation of calcium carbonate has important implications in the past and present formation of carbonate rocks and sediments (Rodriguez-Navarro et al., 2003; Wright and Oren, 2005). It is known that cyanobacteria have aided in the formation of mineralized stromatolites since the Precambrian age (Konhauser, 2007), their photosynthetic activity thus contributing to atmosphere oxygen increase and CO\(_2\) budgeting by carbonate precipitation. While early work (until the 1980s) examined the ability of photosynthetic bacteria to precipitate calcium carbonates in marine environments, precipitation of carbonates induced by heterotrophic bacteria in other environments such as caves, soils, and monuments has become the subject of much research in recent decades (Boquet et al., 1973; Urzi et al., 1999).

Bacteria play both passive and active roles in carbonate nucleation (Mitchell and Ferris, 2006). In the first case, the metabolic activity of heterotrophic bacteria (e.g., ammonification of amino acids, urea degradation, and dissimilatory reduction of nitrates and sulfates), or photosynthetic HCO\(_3^-\) consumption and OH production by cyanobacteria, increases the alkalinity (high pH, and elevated CO\(_2\)) in the microenvironment surrounding the bacterial cells. This in turn increases supersaturation with respect to calcium carbonate if free Ca\(^{2+}\) is present, thus favoring calcification. Bacterial cell walls and/or sheets, as well as EPS, can actively aid in carbonate crystallization by providing heterogeneous nucleation sites (Ercole et al., 2007; Rodriguez-Navarro et al., 2007).

**Bioprotection of stone by bacterial biomineralization of carbonates**

Based on previous work on the natural ability of many heterotrophic bacteria to induce calcium carbonate precipitation, Adolphe et al. (1990) patented a method, CALCITE, for bacterial bioprotection of ornamental stones. The authors showed that calcinogenic bacteria, such as those typically found in soils (Boquet et al., 1973), were able to precipitate calcite on the surface of weathered limestone, thus contributing to their protection and consolidation. Le Métayer-Levrel et al. (1999) reported the
“successful” application of such a method on a sixteenth century limestone castle. Nonetheless, the authors used \textit{Bacillus cereus} cultures, which may pose a health risk. In addition, potentially harmful biofilm formation and only a few micrometers thick consolidated layer were observed. Tiano et al. (1999) evaluated the effectiveness of such a treatment for bioprotection of a highly porous limestone (Pietra de Lecce) using \textit{Micrococcus} sp. and \textit{B. subtilis}. They observed detrimental obstruction of pores (presumably by biofilm formation), and called attention to other potential drawbacks e.g., reaction of the stone and by-products of bacterial activity, and the formation of stain patches. Rodriguez-Navarro et al. (2003) showed that such limitations could be overcome by using different culture media and a non-pathogenic carbonate-producing heterotrophic soil bacteria, \textit{Myxococcus xanthus}. The treatment resulted in deep consolidation of a porous limestone (calcarenite) with new bacterial calcium carbonate cement, without detrimental pore plugging or extensive biofilm formation. \textit{M. xanthus} metabolic activity results in the release of \( \text{NH}_3 \), which leads to a pH rise in the medium surrounding the bacterial cells. This in turn favors carbonate supersaturation and results in CaCO\(_3\) precipitation on different calcareous stones such as limestone and marble (Figure 2). Carbonates typically form on the bacterial cell walls, where heterogeneous nucleation is facilitated, and grow as a coherent cement (often epitaxially) on the stone support. Rodriguez-Navarro et al. (2003) also showed that the new bacterial calcite cement was tougher and more resistant to dissolution than the original calcite in the stone. They interpreted these results considering that organic by-products of bacterial activity were incorporated into the bacterial calcite, which was in fact a (bio) composite material. Interestingly, Ben Chekroun et al. (2004) and Rodriguez-Navarro et al. (2007) showed that \textit{M. xanthus} induced the precipitation of either calcite or vaterite just by modifying the culture medium. These results demonstrate that polymorph selection in carbonate precipitation is not strain specific. On the other hand, recent research using \textit{B. subtilis} (Perito and Mastromei, 2003), \textit{B. sphaericus} and \textit{B. lentus} (Dick et al., 2006) shows that the range of microorganisms that can contribute to the bioprotection of ornamental stone via the precipitation of carbonates is very wide. In addition, it has been shown that bacteria such as \textit{M. xanthus} can precipitate a range of minerals, other than carbonates, just by changing the composition of the culture medium (Ben Chekroun et al., 2004). These results imply that an accurate selection of growth media and bacterial strains could yield improved protection and consolidation of ornamental stones with disparate composition and textures.

Bioprotection treatments based on bacterial biomineralization are not restricted to ornamental stone. Castanier et al. (1996) used \textit{Pseudomonas} sp. for calcite precipitation as a cementing phase in a biological mortar. Bang et al. (2001) have reported that polyurethane-immobilized \textit{B. pasteurii} (a carbonatogenic ureolitic bacteria) can fill the cracks in concrete. Whiffin et al. (2007) have shown that carbonate precipitation induced by \textit{Sporosarcina pasteurii} contributes to soil strengthening.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Bioprotection_Figure_2}
\caption{Bacterially mediated calcite biomineralization on Macael marble: (a) Severe damage on a Macael marble lion sculpture at the Lions Court, The Alhambra, Granada, Spain; (b) scanning electron microscopy photomicrographs of a Macael marble sample before and (c) after bacterial conservation treatment resulting in \textit{M. xanthus} mediated calcite cement formation.}
\end{figure}
Summary
Weathered ornamental stones typically contain a variety of endolithic and epilithic microbial communities (Urzi et al., 1999; McNamara and Mitchell, 2005). Application of bacterial treatments that include a nutritive medium may thus activate, among the microbial community contained in the stones, those bacteria that are able to induce the precipitation of calcium carbonate. In this respect, Jimenez-Lopez et al. (2007) have shown that the application of sterile nutritive media to porous limestone is able to activate carbonatogen bacteria. As a consequence of the growth of these bacteria, an alkalization occurs that results in calcium carbonate precipitation. The new precipitate is compatible with the substrate and consolidates the stone without pore plugging. Such effects are reinforced by the presence of M. xanthus. Therefore, a good candidate to in situ consolidate decayed porous limestone is the application of a sterile culture medium with specific characteristics that favor the development of calcogenic bacteria, while limiting the proliferation of detrimental microbiota (Jimenez-Lopez et al., 2007). Such an alternative to the direct application of life cells is highly promising. Finally, bacterial biomineralization of silicates as a means to consolidate non-carbonate stones such as granite and sandstone is another topic that should be further studied.

Bibliography
Biosignatures of cell components and metabolic activity

Fossil carbon molecules

All terrestrial life consists of assemblages of macromolecules based on carbon (with water as the universal solvent, Brack, 2002) and the different components of the cell, i.e., cytoplasm, chromosomes, chloroplast, and cell walls and envelopes, etc., are characterized by molecules of different composition and structure. Molecular degradation starts with the death of the organism and may also be aided by the activity of other microorganisms, e.g., the heterotrophs, who obtain their energy and carbon from the breakdown of organic substances. The chemical changes that take place during degradation (diagenesis) are dependent on the redox state and water chemistry of the environment. Degradation of macromolecules to form “fossil” molecules results in the gradual loss of the functional groups. Specific characteristics of fossil molecules that suggest a biological origin include (1) enantiomeric excess (chirality), (2) diastereoisomeric preference, (3) structural isomer preference, (4) repeating constitutional subunits or atomic ratios, (5) systematic isotopic ordering at molecular and intramolecular levels, and (6) uneven distribution patterns or clusters, such as C-number, concentration, or δ^{13}C, of structurally related compounds (Summons et al., 2008).

Preservation of fossil molecules

Fossil organic molecules or biomarkers are best preserved in anaerobic environments where they are protected from abiotic oxidation or aerobic microbial degradation. Subsequent burial, metamorphism, and heat events (e.g., hydrothermal/volcanic activity) affect the “maturity” or degradation state of the organic matter. This results in the formation of different types of kerogen characterized...
by H/C ratios ranging from >1.25 for Type I kerogen to <0.5 for type IV kerogen. The small molecular size of some of the degraded molecules means that they are readily mobile and can be transported into other formations with which they are not syngentic, thus causing contamination. The oldest known biomarkers occur in carbonaceous oil shales from the 2.7 Ga-old Fortescue Group in Australia where molecules such as hopanes and steranes, the degradation products of prokaryotes (specifically aerobic photosynthesizers, cyanobacteria) and eukaryotes, respectively, were interpreted as evidence for oxygenic photosynthetic activity by 2.7 Ga (Brocks et al., 1999) and, more importantly, the early rise of eukaryotes (Summons et al., 1999). These biomarkers have, however, recently given rise to controversy regarding their syngenicity (Eigenbrode and Freeman, 2006; Rasmussen et al., 2008).

Syngenetic organic molecules occur in even older sediments but the kerogens in the volcanic sediments in the 3.5–3.3 Ga-old Barberton (South Africa) and Pilbara (Australia) greenstone belts are so degraded by age and low-grade metamorphism (prehnite–pumpellyite to lowermost greenschist facies) that they no longer contain recognizable compositional biomarkers. The kerogens do, however, retain structural characteristics that are indicative of a biological origin (Westall et al., 2006a; Derenne et al., 2008). Evidence of syngenicity of the kerogens analyzed is provided by in situ Raman spectra showing D and G peaks indicative of a maturity consistent with the metamorphic grade of the rocks (Westall et al., 2006a), as well as covalent linking of the molecular structures in the kerogen matrix (Derenne et al., 2008).

Traces of cell metabolism and metabolites
The metabolic activity of microorganisms can result in elemental, isotopic, and mineral signatures that can be preserved in rocks.

Bioelements: Unusual concentrations of metals may be associated with microorganisms and their metabolic products. Adsorption of these elements may be either an active or a passive process. Active adsorption includes the chelation of metals to anionic functional groups in the cell walls to neutralize their negative charges, thus contributing to the stability of the cell wall. Microorganisms also require metals, such as Fe, Co, Ni, Cu, Mn, Mo, Se, V, and F, for certain enzymatic activities (Banfield et al., 2001). They are actively adsorbed onto the cell walls by ligands that are specific for a particular metal (Konhauser, 2007). Microorganisms can also concentrate toxic elements, such as U, Cu, Pb, Al, As, and Cd, as well as precious metals (U, Cu, Pd, Pt, and Au) that are bound to cells and S-layers (Pollmann et al., 2006).

Passive adsorption of metal ions to cell surfaces is an additional kind of metal sequestration (Ledin, 1999). The availability of functional molecules, such as carboxyl or phosphate groups, determines the amount of metals that can be sorbed to the cell surface. With their large quantities of peptidoglycan, Gram positive bacteria can readily scavenge metal ions. Gram negative bacteria, on the other hand, are characterized by only a thin layer of peptidoglycan sandwiched between an outer lipopolysaccharide layer and an inner phospholipid layer. The amount of metal that they can bind is an order of magnitude less than that of the Gram positive bacteria (e.g., Beveridge et al., 1982). Microbial EPS is also an important factor in the binding of metals owing to their highly hydrated character that allows the metal ions to freely migrate through the structure (Geesey and Jang, 1989). This characteristic means that microorganisms, such as encapsulated bacteria, are more likely to chelate metals than non-encapsulated varieties. Metal-replaced microorganisms are common and are among the oldest traces of life. Fossil microorganisms preserved as pyritized filaments occur in a 3.2 Ga-old volcanogenic
massive sulfide deposit (the equivalent of a fossilized black smoker) from the Pilbara in Australia (Rasmussen, 2000). A younger example consists of ZnS-replaced filaments in Triassic calcareous rocks in the southern Alps (Kucha et al., 2005).

Concentrations of other biologically important elements, such as P and S, are considered to be significant biosignatures. For example, the combination of graphite associated with apatite inclusions (apatite can be a biomineral, cf. Table 2) and a δ13C of −26‰ in a highly metamorphosed rock from the 3.85 Ga-old Akilia province in W. Greenland was interpreted as a biosignature by Mojzsis et al. (1996). However, van Zuilen et al. (2002) demonstrated that abiological processes linked to alteration of the rocks by hydrothermal circulation could have produced carbon via the Fischer–Tropsch process with a similar isotopic signature. On the other hand, phosphatized microorganisms are well-known from the rock record. The spectacular preservation of soft body parts of animals, birds, amphibians, and fish from the Eocene Messel oil shales in Germany is due to phosphatized microbial biofilms that coated the dead organisms (Liebig et al., 1996).

High concentrations of sulfur can be found in kerogen owing to the process of sulfurization during which sulfur, liberated from degrading organic matter by the activity of sulfur-reducing bacteria, is bound to the organic macromolecules. Thus, the identification of the S-molecule thiophene in the organic cell walls of microfossils from the 800 My-old Draken Formation of Svarlbard (Lemelle et al., 2008) and even in 2.7 Ga-old kerogen associated with stromatolites from the Tumbiana Formation in the Pilbara (Lepot et al., 2008) has been linked to the degradation of organic matter by sulfur-reducing bacteria.

**Biosignatures in Rocks, Table 2**

<table>
<thead>
<tr>
<th>Metal oxides</th>
<th>Fe/Mn hydroxides and oxyde-hydroxides (or oxyhydroxides), e.g., ferrihydrate (ferric hydroxide), Fe3+2O3·0.5(H2O), goethite, FeO(OH), schwertmannite, Fe3+16O16(OH)12(SO4)2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amorphous silica</td>
<td>Opal, SiO2·n(H2O), e.g., opal-A and opal-A/CT</td>
</tr>
<tr>
<td>Carbonates</td>
<td>Ca-carbonates, e.g., calcite, Ca(CO3), magnesian calcite, High-/Low-Mg Ca(CO3), aragonite, CaCO3, dolomite, CaMg(CO3)2</td>
</tr>
<tr>
<td>Phosphates</td>
<td>Fe-phosphates, e.g., vivianite, Fe3+3(PO4)2 8H2O</td>
</tr>
<tr>
<td>Sulfates</td>
<td>Barite, BaSO4</td>
</tr>
<tr>
<td>Sulfides</td>
<td>Chalcopyrite, CuFe3-2S2</td>
</tr>
<tr>
<td>Silicates</td>
<td>Clay minerals, e.g., phillipsite, (Ca,Na,K)1–2(Si,Al)6(O,OH)2 6(H2O), chamosite, (Fe2+,Mg,Fe3+)3Al(Si3Al)O10(OH)2 2(H2O), smectite (Na,Ca)Al4(Si,Al)2O10(OH)4 2(H2O)</td>
</tr>
</tbody>
</table>

Iron hydroxides (ferrihydrate) are among the most common microbially induced deposits and may be formed passively, or by the activities of chemoheterotrophic, photoautotrophic, or chemolithoautotrophic (e.g., *Gallionella*) microorganisms. The ferric hydroxides may act as precursors for Fe oxides, such as hematite. Manganese nodules are an example of microbially induced mineral precipitates.
iron-manganese oxides that can form volumetrically important deposits in the deep-sea and lacustrine environments (Mero, 1962).

Amorphous silica commonly nucleates passively onto the surfaces of microorganisms in hot spring environments (Figure 1a) (e.g., Cady and Farmer, 1996) and precipitates from saturated solutions in other environments as well. For example, Monty et al. (1991) and Westall (1994) documented silicified microorganisms in the pore space of 15–10 My-old deep-sea sediments. At the other end of the geological record, silica-saturated seawaters on the early Earth led to the silicification of early Archaean microbes and the preservation of the oldest known traces of life (Figure 1b) (Walsh, 1992; Westall et al., 2001, 2006a, b; Westall and Southam, 2006).

Perhaps the most geologically significant microbially induced mineral deposits are those related to carbonates. The carbonate minerals are produced by processes including mineralization of microbial surfaces, chemical precipitation from supersaturated solutions, and physical erosion of preexisting carbonates (Riding, 2000). Microbial carbonates occur in a wide variety of environments ranging from deep-water cold seeps (Figure 2) (e.g., Cavalazzi et al., 2007), shallow-water platforms (Figure 3a) (e.g., Monty, 1995), lacustrine environments (Figure 3b and c) (e.g., Laval et al., 2000) to carbonate (travertine) springs (e.g., Chafetz and Folk, 1984).

Biologically controlled mineralization occurs when microorganisms exert direct control on the nucleation and growth of the mineral (Mann, 1988). The resulting minerals are characterized by their highly ordered crystalline structure, size, and morphology. Chains of single-domain magnetite crystals are formed in this way by magnetotactic bacteria (e.g., Bazylinski et al., 1995) and Chang and Kirschvink (1989) record chains of magnetite crystals in the 1.9 Ga-old Gunflint Formation (Canada) that are similar in size and shape to those produced by recent bacteria. Indeed, the presence of chemically pure magnetite crystals with a specific size/shape range in an ancient Martian meteorite, ALH84001, is one of the key lines of evidence for life on Mars by 3.9 Ga (McKay et al., 1996; Thomas-Kerpta et al., 2001, 2002; Friedmann et al., 2001). However, the supposedly biogenic magnetites form only a fraction (<30%) of a larger population that includes magnetites of clearly abiological origin and it is questioned whether this assemblage is purely fortuitous. Moreover, similar magnetites have been produced in the laboratory by abiogenic processes (Golden et al., 2004).

The presence of microbial metabolites plus the physical presence of the organic components can have an enormous effect on the elemental composition and dissolution of minerals, as well as the shape and size of the crystals. Microbial dissolution of nonorganic substrates is a common process, especially in oligotrophic environments where access to essential elements is necessary for survival. Microbial dissolution of silicate minerals, such as feldspars and mafic minerals, contributes strongly to crustal weathering. Microbial colonies and biofilms attach to a mineral surface and obtain their nutrients by physical disaggregation of the minerals, as well as by dissolution through the production of organic acids (Konhauser et al., 1994; Konhauser, 2007). Etching patterns produced in the presence of actively metabolizing cells show clear differences to those produced by inorganic acids (Figure 4) (Welch et al., 2002). Microbial etching of volcanic glass (e.g., Thorseth et al., 1995) has recently received much attention because certain tunnel-like structures in ~3.5 Ga-old pillow lava rims from the Barberton and Pilbara greenstone belts have been interpreted as microbial corrosion biosignatures (Furnes et al., 2004; Banerjee et al., 2006).

Compositional variability can occur in minerals and rocks as a result of microbial metabolic activity. The distribution of elements (major, minor, and trace) in the
surfaces of minerals colonized by microorganisms may be different to that of the bulk mineral because the microorganisms leach out those elements that are important for their metabolic activities. For instance, sulfur can be leached from sulfides by sulfur-oxidizing bacteria (McGuire et al., 2001) and phosphorus from P-containing minerals is leached in P-poor environments (Taunton et al., 2000a, b). Also, unusual concentrations of trace elements may be associated with mineral-entombed microorganisms since the microbes preferentially concentrate elements that are necessary for their metabolic functions, such as Co, Ni, Cu, Mn, Mo, Se, and V, (Banfield et al., 2001).

The incorporation of organic compounds within a mineral structure may be indicative of biologically induced precipitation as, for instance, when microorganisms are permeated and entombed by minerals (Westall et al., 1995, 2000). Indeed, association of organic molecules with microfossils is a key complementary biosignature for traces of early life on Earth (Westall and Southam, 2006).

Whereas biologically controlled mineral growth produces specific crystalline shapes, biologically induced mineralization produces minerals with non-perfect habits (Banfield et al., 2001) due to alteration of the stability of the crystal surface in the presence of organics (Mann et al., 1993; Albeck et al., 1996). However, the presence of organic matter does not always have an influence on the morphology of a mineral precipitate. For instance, Orange et al. (2009) note that silica precipitated in nutrient media with and without cells had exactly the same structure, the only difference being the visible incorporation of EPS in the medium with cells.
A number of studies have noted a distinct difference in the size of minerals precipitated in the presence of microorganisms and those precipitated abiologically with the former being smaller than the latter. Douglas et al. (2008), for example, document smaller biofilm-induced calcite and Mn-oxyhydroxide mineral precipitates compared to abiogenic gypsum precipitates in an unusual microbial mat from a Death Valley pool.

Isotopic fractionation: Until recently, the preference of microorganisms for the lighter stable isotope of carbon $^{12}$C over the heavier one, $^{13}$C was considered to be one of the most important biosignatures in rocks. Indeed, Schidlowski (1988) had shown that these signatures can be traced back to rocks 3.8 Ga-old from the Isua province in Greenland. Recent studies, however, have demonstrated that $^{12}$C/$^{13}$C ratios similar to those produced by living organisms occur in carbon produced by abiotic hydrothermal/metasomatic Fischer–Tropsch synthesis (e.g., van Zuilen et al., 2002). Contamination of the rock by younger organisms can also produce an erroneous biosignature. Westall and Folk (2003), for instance, identified Holocene fossilized endolithic microorganisms in the Isua banded iron formation that was used for Schidlowski’s (1988) isotopic studies.

Biosignatures in Rocks, Figure 4 Experimental dissolution of apatite (a) in an inorganic acid and (b) in the presence of actively metabolizing cells. Arrow in (b) indicates a cellular body. Scale bars = 2 μm. (Images reprinted with permission from Banfield et al., 2001).

Biosignatures of structural components

Cells, colonies, biofilms, mats, EPS

Microorganisms generally occur as assemblages (colonies) of cells that may be monospecific, but, more often than not are pluri species. Colonies are often linked by a film of polymers known as extracellular polymeric substances (EPS). Colonies coating a substrate in a two-dimensional manner are termed biofilms. Microbial mats are complex structures formed by layers of biofilms and may be microscopic to macroscopic in size, sometimes reaching dimensions of several square kilometers, for instance, on the floors of lagoons (e.g., Margulis et al., 1980). Although typically associated with the activity of photosynthetic microorganisms in shallow-water environments, microbial mats may also be constructed by non-photosynthetic microorganisms, such as the sulfur-oxidizers *Beggiatoa*, in deep-water habitats on the flanks of deep-sea hydrothermal vents and at hydrocarbon seeps (e.g., Kelley et al., 2005).

The structural components, cells, colonies, biofilms, mats, and EPS, may be preserved in rocks in a number of ways. Cells may leave organic walled impressions, mineral-coated or impregnated structures, or empty casts in a mineral precipitate. Biofilms and mats may also be preserved as organic impressions in sediments or mineralized structures.

Preservation of microbial structures

Organic walled structures: Cells walls can be preserved as organic impressions in fine-grained, anaerobic sediments (Javaux et al., 2001). The fixation of metals, such as Fe, on cell envelopes may retard lysis, thus contributing to the potential for preservation of cell walls (Ferris et al., 1988). While structures such as pollen spores are large enough to be visible in the rocks using light microscopy, individual microbial cells are often difficult to identify in situ in sediments because of their small size. Study of these organic remains is derived from palynological methods in which the detrital silicates encasing the cells are eliminated by acid-digestion of the rock. A major problem with this method is that microorganisms commonly colonize fractures in rocks, thus introducing younger contamination (e.g., Westall and Folk, 2003; Walsh and Westall, 2008). Such contamination led Pflug (1979) to identify eukaryotic yeast cells in a 3.8 Ga-old rock from Isua as in situ microfossils. This type of problem can be overcome by verifying the maturity of the kerogen by high-resolution TEM and Raman spectroscopy and/or by demonstrating chemical bonding between the organic molecules and the mineral matrix that indicates the syngenicity of the kerogen (Marshall et al., 2005; Derenne et al., 2008).

Mineralized microbial structures: The most common form of preservation of microbial structures is mineral fossilization. In this process, minerals bind to the organic surfaces of the cells/EPS in a passive reaction. The surfaces of microorganisms and EPS are very reactive, containing
ionized ligands to which mineral nuclei can attach themselves. The sorption reactions may take place via cation bridging, hydrogen bonding with functional groups such as carboxyls or hydroxyls, or by direct electrostatic fixation of the mineral to the functional groups (Lalonde et al., 2005). The attached nuclei then attract further nuclei, resulting in encrustation or permeation of the organic structure. The microbial surfaces and exopolymers therefore act as “mineralizing templates” (Konhauser, 2007). Depending upon the availability of the minerals in solution, the microorganisms may be completely entombed in a mineral precipitate. The type of mineral precipitated depends on the composition of the fluids and the redox conditions in the immediate environment of the microorganism. Many mineral phases can bind to microbial cell walls (Konhauser, 2007), including silica (Figure 1), carbonates (Ca, MgCa, Fe, Mn), metal oxides/hydroxides (Fe/Mn and magnetite), sulfates (Ca, Sr, Ba, Fe), sulfides (Fe, Ni, Pb, Zn, CuFe), phosphates (Ca), clays, and zeolites.

As noted above, cell wall composition and structure plays an important role in the fixation of metals because of the differential distribution of functional groups in different cell components. Mineral nucleation is similarly affected. Thus, Gram positive bacteria with their thick outer layer of peptidoglycan and the abundance of functional groups can be encased by a thick mineral crust, whereas the Gram negative bacteria with their thin layer of peptidoglycan sandwiched between an outer lipopolysaccharide layer and an inner phospholipid layer only fixed a thin, delicate crust (Westall, 1997). The latter author concluded that Gram negative cells therefore had less chance of being preserved in the rock record than Gram positive ones.

The cell walls of Archaea differ in their composition and structure to those of Bacteria and are also more variable. Crenarchaeota are characterized by a layer of paracrystalline proteins, the S-layer, which is loosely associated with the plasma membrane (NB: S-layers are also associated with the plasma membrane of many species of Bacteria). The Euryarchaeota exhibit great variability in the composition of their cell walls, including a peptidoglycan-like component, pseudomurein. Experimental silicification of an Archaea having an S-layer, the hyperthermophile Pyrococcus abyssi showed that it produced a delicate silica crust, similar to that forming around Gram negative bacteria (Orange et al., 2009).

What is the fate of the organic components in a mineralized cell? Experimental fossilization shows that the macromolecules of the cell envelope may remain trapped in the mineral crust (Westall et al., 1995). The cytoplasmic space, on the other hand, may be empty after lysis, or, if lysis did not occur, it may contain balls of degraded macromolecules that can also nucleate minerals (in the process producing false nuclei, Westall et al., 1995). As noted above, the organic components of fossilized microbial structures may be preserved in anaerobic environments but they can be completely oxidized in oxidizing environments. For example, the silicified remains of cyanobacteria in hot spring deposits from Yellowstone no longer contain any organic molecules (Cady and Farmer, 1996).

In order to be preserved in the rock record, the mineral-coated/permeated microbial structure needs to become encased in a mineral cement or by fine-grained sediments. Here, further diagenetic changes may take place, including changes in mineralogy (e.g., transformation of oxyhydroxides to oxides), replacement (complete or partial) of one mineral by another (e.g., silicification of carbonate mineralized remains), or dissolution. The final mineral or sediment-encased microbial fossils may exhibit different morphological preservation modes.

Artifacts: Abiological mineral precipitates can be notoriously confused with fossilized microorganisms. Many minerals may form simple spherical, oval, elongated, and even twisted morphologies. Silica spheres may occur in aggregates that look surprisingly like dividing microorganisms (e.g., Monty et al., 1991). Jones (2004) found that spherical silica aggregates nucleated onto a mucus thread in a siliceous hot spring forming filamentous structures that could possibly be mistaken for silica encrusted filaments. García-Ruiz et al. (2003) even showed that artificial mixed silica/organic precipitates could produce microbial-like morphologies (bacteriomorphs). However, these precipitates were not produced from naturally occurring solutions.

Examples of mineralized microbial structures: Microbial fossils occur throughout almost the whole of the rock record. The oldest known microfossils are found in silicified shallow-water volcanic sediments from the 3.5–3.3 Ga Barberton and Pilbara greenstone belts in which filaments, coccolidal and rod-shaped silicified microorganisms and associated EPS have been documented (Figure 1b) (Walsh, 1992; Westall et al., 2001, 2006a, b). [Older sediments from the 3.8 Ga-old Isua greenstone belt in Greenland are too highly metamorphosed to contain identifiable, syngenic microfossils.] The very small size of the early Archaean microbial structures means that sophisticated instrumentation having high spatial and analytical resolution is necessary to observe and analyze them (Westall and Southam, 2006). Moreover, given the problem of establishing the biogenicity and syngenicity of microbial remains in these ancient sediments, multiple lines of evidence are required, including morphological, chemical, and isotopic biosignatures. In this way, Westall et al. (2006a, b) documented multispecies colonies of probable chemolithotrophic microorganisms within 3.446 Ga-old mudflat sediments from the Pilbara and microbial mats on the surfaces of littoral sediments in a 3.334 Ga-old formation from Barberton. The small crystal lattice of silica results in excellent preservation of fine textural detail, such as cell surface wrinkles (Westall et al., 2006a). Silicified (permineralized) microfossils occur throughout geological time (Knoll, 1985). Proterozoic formations contain extremely well-preserved cyanobacterial...
fossils, for instance (Green et al., 1989), and silicified microfossils have been documented from numerous Phanerzoic hot spring environments (Jones et al., 2001; Jones, 2004).

The oldest calcified microfossils are relatively poorly preserved benthic cyanobacteria from the 2.6 Ga Campbellrand Subgroup in South Africa (Kazmierczak and Altermann, 2002). Well-preserved calcified microfossils occur in younger Proterozoic formations, such as the Draken Formation, Svarlbard (Knoll et al., 1993).

Microfossils preserved by other minerals, such as pyrite, occur through much of the geological record, the oldest such fossils occurring in the above-mentioned 3.2 Ga-old massive sulfide deposits from the Pilbara (Rasmussen, 2000) and younger fossils occurring in the Proterozoic, e.g., pyritized filaments in stromatolites from the Altyn Formation of Montana (White, 1974), or pyritized biofilms coating soft body parts (Canfeld and Raiswell, 1991). Phosphatized microfossils are known from the Phanerozoic, for example, phosphate coatings on cyanobacteria and/or sediments (MacIntyre, 1985; Shapiro, 2000). Encrustations, concretions, and coatings are generally microbial products that contain a record (morphological, geochemical, and/or isotopic) in their sedimentary phases of the biochemical processes involved in their genesis (Raiswell and Fisher, 2000; Kiriakoulakis et al., 2000). For instance, Fe-Mn coatings and pyritiferous carbonate nodules are considered to be microbial fabrics (Peckmann and Thiel, 2004).

Microbialites and microbial fabrics in rocks

The physical expressions of microorganisms and the products of their enzymatic and metabolic activities result in microbialite structures and textures that are characterized by a particular internal structure or texture. They include a wide range of macroscopic to microscopic phenomena, such as stromatolites, thrombolites, oncolites, biolaminates, and crusts (Figure 3). Macro- to micro-scale fabrics and textures related to the presence of microorganisms also include MISS (the MISS of Noffke et al., 2003), and microfossils (Burne and Moore, 1987; Monty et al., 1995; Riding and Awramik, 2000). Microbialites and microbial fabrics are found in rocks throughout the geological record with the oldest stromatolites occurring in 3.5–3.3 Ga-old formations in the Pilbara of Australia and in Barberton, South Africa (Lowe, 1980; Walter, 1983; Byerly et al., 1986; Hofmann et al., 1999; Allwood et al., 2006). The biogenic origin of the early Archaean stromatolites has been challenged by Lowe (1994) and Lindsay et al. (2005) but Riding (2008) suggests that some of the Precambrian stromatolites may be mixtures of microbial and abiogenic, stromatolite-like fabrics. The large and diverse stromatolites characteristic of the Proterozoic declined in importance throughout the latest Precambrian to Cambrian times and, from the early Cambrian onwards, microbialite structures are dominated by microbial mounds formed by non-stromatolitic calcifying microbes (e.g., cyanobacteria, Monty, 1995). Microbial mounds are well-developed throughout the geological record spanning from the Proterozoic to the Recent in both shallow- and deep-water settings and are considered to be a significant expression of the history of Earth’s microbial life (Figure 2) (Monty, 1995). Both stromatolites and microbial mounds are characterized by the activity of microbes which calcify, trap, and bind sediments, forming delicate and persistent microstructures rich in micrite (microcrystalline calcite) and organomicrite. Microbiologically generated mounds and microbialites formed in shallow-water environments mainly involve photosynthetic/phototrophic and heterotrophic microbes. In deeper waters, however, chemosynthetic microbes are associated with hydrocarbon seeps and hydrothermal-related mound deposits (Peckmann and Thiel, 2004). Deep-water stromatolitic fabrics and crusts have been described from hydrocarbon seeps on the Aleutian accretionary margin as a product of methane-consuming Archaea (Greinert et al., 2002).

The most common microbially induced fabrics from carbonate rocks are clogged and peloidal fabrics (Burne and Moore, 1987; Shapiro, 2000; Flugel, 2004). These fabrics consist of spherical-to-elliptical microscopic aggregates of microcrystalline clots or peloids (Figure 5a) (e.g., calcite, aragonite), cemented by carbonate and/or sediments (MacIntyre, 1985; Shapiro, 2000). Fenestral and stromatolitic fabrics from organic-rich sedimentary deposits (Figure 5b), such as microbial mounds or geothermal springs (e.g., travertine), are microbiological in origin (Flugel, 2004). Encrustations, concretions, and coatings are generally microbial products that contain a record (morphological, geochemical, and/or isotopic) in their sedimentary phases of the biochemical processes involved in their genesis (Raiswell and Fisher, 2000; Kiriakoulakis et al., 2000). For instance, Fe-Mn coatings and pyritiferous carbonate nodules are considered to be microbial fabrics (Peckmann and Thiel, 2004).

Microbially induced sedimentary structures (MISS) are formed by the rhythmic alternation of photosynthetic microbial mats and sediments in mudflat environments (Noffke, 2008). MISS are widespread in the geological record and have also been identified in sediments 3.2 Ga-old from South Africa (Noffke et al., 2003).

Banded iron formations (BIFs) occur throughout the rock record from 3.8 Ga onwards (BIFs occur in the Isua greenstone belt in Greenland). The influence of microorganisms on the formation of these laminated accumulations of iron oxides and chert is the subject of much controversy. Photochemical oxidation of ferric iron of hydrothermal origin is one explanation (Cairns-Smith, 1978) but other biological controls have also been suggested, such as oxidation by oxygenic photosynthesizers (Cloud, 1965), or simple sorption of Fe$^{2+}$ onto microbial cell surfaces to catalyze a basically abiological precipitation process (Konhauser, 2000), or the precipitation of Fe oxides by Fe oxidizing microorganisms (Posth et al., 2008).

Relevance of biosignatures and their astrobiological implications

We have seen above that a number of the biosignatures described can be imitated by abiological processes
resulting in significant controversy. These are serious limitations when searching for the first traces of life on Earth or for biosignatures on other planets. How can nonliving molecules be distinguished from very primitive living cells—and in the fossilized form? A carbon isotope signature on its own is not sufficient because it could be either abiogenic or biogenic in origin. A collection of simple-shaped minerals, even joined together with compromised boundaries, could represent abiogenic precipitates or possibly a fossilized microbial colony. Identification of biosignatures in ancient terrestrial or extraterrestrial materials therefore needs to be based on a combination of structural, chemical, and isotopic traces that are related to the three main characteristics of living organisms: their physical structures, the chemical components of the structures, and their metabolic or living processes (Westall and Southam, 2006; Westall, 2008). Another aspect to take into account is the fact that living organisms by their very existence and metabolism interact with and influence their immediate environment.

On a biotic world where the context of structure, fabric, and composition are well-constrained, certain characteristics, such as element enrichment, mineral composition, shape, and size can be used as complementary evidence for the existence of living organisms. Can these characteristics be used on a world where the existence of life forms is uncertain, for instance on the primitive Earth or Mars? Prebiotic molecules of mostly extraterrestrial origin, characterized by a wide range in composition and structure, including simple amino acids, polycyclic acidic hydrocarbons (PAHs), thiophenes, fullerenes etc. (Cronin, 1998), abounded on these worlds (Maurette et al., 2000). Could prebiotic organic macromolecules scavenge elements from the environment in the same way as biomolecules and, if so, would some elements be preferentially fixed? These macromolecules have very few of the functional groups that characterize biogenic molecules, and it is therefore unlikely that they will be able to induce significant metal fixation. Thus, element concentration and distribution in potentially biogenic materials (organic or mineralized organics) could be considered as a useful complementary biosignature when associated with other types of biosignatures. Could prebiotic organic molecules induce mineral precipitation? Again this is unlikely since biominerals precipitate because of changes in the environment caused by the excretion of metabolic waste products, or enzymatic mediated changes in the redox state, or the presence of a charged cell surface that attracts mineral nucleation, conditions that are not met by prebiotic molecules. On the other hand, the physical presence of prebiotic molecular matter in an environment where minerals are being precipitated abiologically from supersaturated solutions (saline ponds, hot springs, the early, silica-saturated oceans) could eventually have an influence on the size and shape of minerals because of alteration in the stability of the crystal surfaces (cf. Mann et al., 1993; Albeck et al., 1996).

Once the biogenicity and syngenicity of the different biosignatures in a rock have been established, how are they to be interpreted? What do they tell us about the community of microorganisms that lived in a particular environment? Are all the original microorganisms in the community preserved? This is particularly relevant because not all microorganisms can be fossilized. For instance, Orange et al. (2009) noted that, although two species of thermophilic Archaea, Pyrococcus abyssi and Methanocaldococcus janaschii had similar cell wall structures and compositions, the former could be experimentally silicified whereas the latter naturally lysed before mineral nuclei could fix to the cell walls.

Another aspect of the study of biosignatures is the importance of observation and analysis at the scale of the microorganisms (Westall and Southam, 2006; Westall, 2008, 2009). Many fossilized microorganisms are very small and cannot readily be distinguished from abiogenic...
mineral accumulations at the optical microscope scale. They need to be studied using higher-resolution electronic microscopes. The question of scale is also pertinent to analysis of chemical or isotopic signatures. What information about the microbial community is provided by isotope measurements made on kerogen extracted from the bulk rock when the rock may contain traces of different microbial communities at the micrometer scale? The microbial communities (colonies, biofilms, and mats) may, themselves, consist of multiple species, thus, resulting in additional mixture of the isotopic signature. To complicate matters even further, specific components of an individual cell may have different isotopic signatures. For example, van de Meer et al. (2000) note that the carbon isotope composition of the lipids of Chloroflexus aurantiacus differ from the bulk isotopic composition of the cells. This means that isotope measurements made even in situ (e.g., with a spot size of ~50 µm, van ZuiLEN et al., 2007) need to be interpreted with caution.

Conclusion

While it is clear that the study of biosignatures in rocks is not without difficulties, these can be overcome by combining signatures of the different aspects of living organisms, i.e., structural, chemical, and isotopic traces, as well as evidence for the interaction of living organisms with the environment. Further investigation of the microbial communities that produced the biosignatures needs to be undertaken at the microbial scale in order to obtain relevant information about the types of organisms and their metabolic activities. This is a challenge for spatial and analytical resolution but it is this level of detail that is necessary for disentangling the bulk biosignatures from those produced by the individual microorganisms and for better understanding the complexity of past communities.

Bibliography


BLACK SHALES


Cross-references
Archaean
Astrobiology
Bacteria
Biogenic Films and Fossilization
Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification
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**BIOSILICIFICATION**

The biological formation of opal-like amorphous hydrated silica. This phenomenon occurs on a globally vast scale in a wide variety of organisms, including protists, radiolarian, foraminifera, sponges, mollusces, brachiopods, copepods, ascidians, diatoms, and higher plants. See entries “Silica Biomineralization, Sponges”, “Microbial Silicification – Bacteria (or Passive)” and “Microbial Bio-mineralization” for further reading.

**BLACK SHALES**

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**Definition**

Most modern and ancient aquatic (marine, brackish, and lacustrine) environments were sufficiently oxygenated to permit colonization by numerous benthic, planktic, and nektic organisms. Due to excess oxygen the biologically produced organic matter is rapidly degraded. The sediments formed thereby exhibit only very low amounts of organic matter.

In contrast, very high rates of primary production and/ or stagnant water bodies result in black shale sedimentation, which is associated with oxygen depletion or even lack of oxygen within the substrate and to some extent also within the water column. Degradation of organic matter is incomplete, partly by anaerobic bacteria, causing strong enrichment of organic matter in sediments. Black shales therefore commonly exhibit excellent preservation of organic molecules and mineralized soft tissues and hard parts of organisms.

The importance of black shales for scientists and the society are twofold. Black shales serve as source rocks for fossil fuels (oil and gas), which cover most of the energy needs of modern societies, and due to excellent preservation they provide particular sites for palaeontological studies.

**Sedimentary characteristics of black shales**

Black shales sediments in common are dark gray to black in color and are characterized by microlamination, which may vary from distinct to blurred. The lamination is caused by quiet depositional conditions and the scarcity or lack of bioturbation. In many cases, the lamina were formed by alignments of fecal pellets (Figure 1a), which rained down in great numbers from surface waters. The
diameters of fecal pellets exceed the average annual sedimentation rate by far, and cause indistinct to blurred types of lamination. Commonly, it is assumed that seasonal variation in sedimentation (clastic input vs. primary production) may cause microlamination. However, the low sedimentation rates in general practically hamper a clear proof of varves.

Black shales typically consist of four main components (e.g., Röhl et al., 2001). Siliciclasts, mainly clay to silt sized particles form the major component of the rocks (50 to >90 wt.%). They derive from fluvial or aeolian input from hinterland areas. Carbonates originate either as skeletal remains of phytoplankton organisms (e.g., coccoliths, Figure 1b), or they are precipitated during early diagenesis at or close to the sediment surface (Frimmel et al., 2004). Their content varies remarkably. The proportion of organic matter may vary between 0.5 and 50 wt.% of the whole rock. It mainly derived from surface water primary production, but detritus from land plants and bacterial organic matter may also contribute. Black shales with a content of organic matter below 10% are termed as bituminous mudstones, and above 10% as oil shales. Pyrite (FeS₂) in black shales occurs as finely dispersed tiny framboids (Figure 1d), which were formed as synsedimentary precipitates due to bacterially driven iron- and sulfate-reduction. In case of excess organic matter, larger pyrite aggregates may be formed during diagenesis. The proportion of pyrite reaches 10–20 wt.% maximum.

**History of organic matter preservation**

During the early history of the Earth and life (Hadean and Archaean periods), virtually no free oxygen existed in the ocean and atmosphere. After oxygenic photosynthesis was established and the formerly reduced compounds had been oxidized, free oxygen started to accumulate in the ocean and the atmosphere ca. 2.2 billion years ago (e.g., Catling and Claire, 2005; Rouxel et al., 2005). The first eukaryotic organisms with highly resistant organic cell walls (acritarchs and/or primitive green algae) evolved during late early Proterozoic times (e.g., Samuelsson et al., 1999; Arouri et al., 2000). Their remains escaped rapid degradation and formed a major sink for organic carbon. Large quantities of organic matter had been embedded within marine sediments, leading to the first black shale formation (e.g., Banerjee et al., 2006). At least since Phanerozoic times, the atmosphere showed roughly the present-day oxygen levels (Catling and Claire, 2005; Berner, 2006) and aerated conditions persisted in the oceans. However, certain climatic and geographic factors promoted sluggish circulations with reduced turn over rates, thus preventing mixing of the water column. Related environments are commonly
Oceanographic models for oxygen depletion and black shale formation

Certain combinations of physical and biotic factors cause oxygen depletion within marine areas, and have been described in various models from modern and ancient environments. In the following, a brief overview on the various models and fossil examples are given. Fossil examples in many cases however, were described in more than one of these models.

Silled basins

Land-locked silled basins with a positive water balance and a shallow and narrow connection to the open sea are characterized by a low salinity surface water outflow and a high salinity deeper water inflow (estuarine circulation). The difference in density between low and high salinity waters results in a permanent halocline and subsequently, thermocline. As convection and influx of oxygen to lower parts of the water column are prohibited, anoxic conditions develop below the halocline. The Baltic Sea and the Black Sea (e.g., Bahr et al., 2005, 2006) represent modern examples. Proposed fossil examples are e.g., the Permian Kupferschiefer (e.g., Bechtel and Püttmann, 1997) and Toarcian black shales in Central Europe (e.g., Röhl et al., 2001; van de Schootbrugge et al., 2005).

Upwelling systems

Today, upwelling is known from western continental margins in subtropical areas. Trade winds cause an offshore flow of surface waters and force oxygen-poor and nutrient-rich bottom waters to ascend. The mixing with oxygen-rich surface waters results in a very high plankton productivity and export production to the sea floor. Decomposition consumes all available oxygen, resulting in stagnant conditions and enrichment of organic matter in and on the sediment. Well-known modern to Pleistocene examples are the coastal areas off Peru and off Namibia (e.g., Wefer et al., 1996; Berger and Wefer, 2002; Hebbeln et al., 2002).

Oceanic anoxic events (OAEs)

Oceanic anoxic events (OAEs) were characterized by oxygen depletion in intermediate waters in major parts or even complete ocean basins. They were explained by a number of factors, namely (1) intensified deep water formation in high latitudes, (2) origin of warm and saline bottom waters at low latitudes, (3) enhanced fresh water runoff from continents associated with high rates of nutrient delivery, and (4) increased primary production associated with extreme greenhouse conditions. These factors all favor the development of low oxygen to anoxic conditions with increased organic matter preservation. A modern to Pleistocene example is the Arabian Sea in the low-latitude Indian Ocean (e.g., Schmiedl and Mackensen, 2006). Numerous OAEs occurred during the Earth’s history, of which the Cretaceous OAEs are the most widely known. They are explained by high organic carbon burial related to global warming and greenhouse periods (e.g., Erbacher et al., 2001; Herrle et al., 2003). Many of these events were probably regional occurrences within restricted basins. However, there is increasing evidence for an open ocean and maybe global extension of the OAE 2 at the Cenomanian/Turonian boundary (e.g., Erbacher et al., 2005).

Eutrophic shelf

Many modern shelf environments (e.g., North Sea and Adriatic Sea) are characterized by man-made eutrophication that stimulates primary production with subsequent oxygen consumption and accumulation of organic matter (Dethlefsen and von Westernhagen, 1983; Kollmann and Stachowitsch, 2000). Fossil examples refer to a number of epicontinental seas particularly during the periods of greenhouse climate (e.g., Kimmeridge clay in western Europe (Oschmann, 1990; Müller, 1991), Toarcian black shales (van de Schootbrugge et al., 2005)).

Tidal mud flats and shallow lagunal environments

Most of the land-derived organic matter is deposited in near-shore environments, particularly in tidal mud flats. Additionally, land-derived nutrients stimulate primary production and subsequent oxygen depletion, thus promoting the enrichment of organic matter in the sediment (e.g., Wilms et al., 2006; Rink et al., 2007). Well-known fossil examples refer to the middle and late Triassic tidal and shallow subtidal mud flat areas from the northern Tethyan margin, e.g., at Mt. St. Giorgio, Switzerland (Röhl et al., 2002) and Seefeld, Austria (Hopf et al., 2001).

Black shales as ecosystems

Black shales and modern oxygen-depleted environments show various geochemical and ecological characteristics indicative of increasingly harsh life conditions (Table 1). Rhoads and Morse (1971) distinguished aerobic, dysaerobic, and anaerobic biofacies within marine environments. This classification has subsequently been used in many studies of modern and ancient oxygen-controlled environments (e.g., Tyson and Pearson, 1991; Wignall, 1994). Observations from modern environments show a remarkable seasonal oxygen fluctuation (Oschmann, 1994, 2000). Therefore, a modified scheme of classification was introduced, where the term dysaerobic was replaced by poikiloaerobic to address seasonal variation in oxygen supply.

The proportions of organic matter, pyrite, and biomarkers increase from the aerobic toward the anaerobic biofacies. In the same direction, the diversity of eukaryotes diminishes. Particularly, deep burrowing organisms
are affected by the rise of the redox boundary and try to settle close to the sediment surface (Oschmann, 1994; Schmiedl and Mackensen, 2006). Additionally, the proportion of organisms with benthic (lecithotrophic) larval development decreases with increasing oxygen depletion. If the redox boundary finally migrates out of the substrate, eukaryotic life is excluded. Dwarfs with body size remarkably smaller than normal and juveniles occur within the dysaerobic/poikiloaerobic facies, whereas microbial mats and partly also larval shells can be found within the anaerobic facies. The sporadic occurrence of the latter indicates failed larval spatfalls during unfavorable conditions.

**Conclusions**

1. Black shale sediments mainly form in marine to brackish waters under low oxic to anoxic conditions.
2. They are characterized by remarkably enriched proportions of organic matter and pyrite. The organic matter derives from marine primary production, terrestrial input, and bacterial derived compounds.
3. Black shales date back to late Proterozoic times and are widespread during Phanerozoic age.
4. Origination of black shales is explained by a number of models. A common aspect of most of these models is the formation of a density stratified water column, which prevents oxygenation of deeper waters.
5. The degree of oxygen depletion in benthic ecosystems during black shale deposition can be reconstructed by a number of geochemical and ecological features.

Bibliography


The breakup of Rodinia: introduction

Although it is widely accepted that Rodinia broke apart during the latter half of the Neoproterozoic era (1,000–542 Ma) and that some fragments reassembled by the early Cambrian (ca. 530 Ma) to form Gondwanaland (Hoffman, 1991), our knowledge of the timing of the breakup events and the processes involved is still sketchy.

Record of Rodinia breakup

The breakup of Rodinia was recorded both geologically and geophysically. The most important geological records of the breakup process are globally widespread continental rift deposits and related magmatism, and records of the geological transition from continental rifting to passive margin deposits where rifting succeeded to form new oceans. One of the best matches of rifting records between continents is that between southeast Australia and South China, where episodes of rift-related magmatism at ca. 825 Ma, 800 Ma, 780 Ma, and 750 Ma have been well documented (Preiss, 2000; Li et al., 2003) (Figure 2). Similar records are emerging from other continents.

Radiating dyke swarms and remnants of large igneous provinces (LIPs) have also been regarded as important geological records of the breakup of Rodinia (Figure 2c and d; see discussion below under Mechanism for the breakup).

The most important geophysical constraint on the breakup of Rodinia is provided by paleomagnetism. Whereas, an individual pole of a given age for a continent constrains its paleolatitude at that time but not its paleolongitude, comparable apparent polar wander paths (APWPs) for different continents can indicate a unique paleogeographic fit between them. In the latter case, when two matched APWPs start to diverge, it implies that the two continents started to move independently (i.e., a breakup between them occurred). Unfortunately, such an ideal situation has rarely been identified for the Rodinia time because of the limited data currently available.

Timing of events

The ages of continental rifting events can be easily dated because of the availability of datable minerals such as zircon in the rift-related magmatic rocks, and it is now widely accepted that continental rifting in the lead-up to Rodinia breakup started at ca. 825 Ma or earlier (see summary in Li et al., 2008; Figure 2). However, the timing of the breakup events is still controversial, and different parts of the supercontinent seem to have broken apart at different times.

The stratigraphic record in southeast Australia indicates a rift-drift transition between the Sturtian glacial deposits (with a poor age constraint of ca. 750–690 Ma) and the overlying sag-phase deposits (Powell et al., 1994) that were recently dated at between 657 ± 5 and 643 ± 2 Ma (Re-Os ages, see Kendall et al., 2006). These age
Breakup of Rodinia, Figure 1 Three of the alternative Rodinia reconstructions: (a) Hoffman (1991), (b) Pisarevsky et al. (2003), and (c) model adapted by IGCP440 for the Geodynamic Map of Rodinia. (Li et al., 2008.)
constraints seem to agree with those for south China and western Laurentia. Although rifting occurred at ca. 750 Ma along the eastern margins of Laurentia, the breakup there appears to have occurred during ca. 615–570 Ma (Cawood and Pisarevsky, 2006). These younger events are possibly recognizable in global tectonic subsidence curves (Bond et al., 1984).

Such geological evidence for the breakup of Rodinia is sometimes at variance with paleomagnetic constraints. Paleomagnetic data suggests that India was either adjacent to western Australia (Torsvik et al., 2001) or had already broken away from Rodinia at 750 Ma (Figure 3b). South China must also have broken away from Australia and Laurentia by 750 Ma if it occupied a central position in Rodinia as shown in Figures 1c and 3a (Wingate and Giddings, 2000; Li et al., 2008). Alternatively, Rodinia may have remained intact at ca. 750 Ma if a different configuration occurred (see Fig. 5 of Li et al., 2004).

**Mechanism for the breakup**
The most commonly invoked mechanism for the breakup of Rodinia is the activity of mantle plumes or of a mantle superplume (superswell) beneath the supercontinent,
Breakup of Rodinia, Figure 3  Cartoons illustrating the breakup process of Rodinia. (After Li et al., 2008.)
which thinned and thermally weakened the lithosphere resulting in widespread continental rifting and eventually breakup (Figure 3a and b).

Evidence for plume/superplume activity during the time of Rodinia include remnants of LIP and radiating dyke swarms (Figure 2c), syn-magmatic, continental-scale doming indicating the arrival of a plume-head (Li et al., 1999), large-scale anorogenic but often dominantly felsic melting that requires a pan-Rodinia introcontinental heat source (Li et al., 2003), and abnormally high-temperature (>150°C) mantle melting (Wang et al., 2007). Mechanisms proposed for the formation of such a superplume (or superswell) include thermal shielding by the supercontinent (e.g., Anderson, 1994), gravitational push-up effects on the hotter lower mantle by circum-Rodinia avalanching slabs (Li et al., 2004, 2008), return flow of whole-mantle convection (Zhong et al., 2007), and partial melt of recycled mid-ocean ridge basalt near the core-mantle boundary under Rodinia (Maruyama et al., 2007).

Summary
The breakup of Rodinia was a diachronous and protracted process. Widespread continental rifting started at ca. 825 Ma when Rodinia spanned from the equator to high latitudes (Figure 3a), but the breakup, initially between Australia/East Antarctica, Laurentia, and possibly South China did not occur until ca. 750 Ma (Figure 3b) or later when the supercontinent was centered on the equator. Gondwanaland was already assembling before some of the West Gondwanan continents broke away from Laurentia at ca. 600 Ma (Figure 3c), and by the early Cambrian (530 Ma) the assembly was complete (Figure 3d). The breakup of Rodinia is believed by some to have been related to the formation of a mantle superplume beneath the supercontinent.

Bibliography

Cross-references
Gondwanaland, Formation
Snowball Earth
CALCAREOUS ALGAE

The term "calcareous algae" refers to various kinds of benthic and planktonic algae whose thalli contain biochemically precipitated calcium carbonate (CaCO₃) as skeletal material (Wray, 1977; Braga and Riding, 2005). Precipitation of CaCO₃ (as calcite and/or aragonite) may occur within or on the algal bodies. The term may also include mechanically accreted deposits of calcium carbonate caused by algae, usually as an interaction of biological and physical processes. Calcareous algae are a highly artificial group that constitutes calcifying members of the Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae) and is sometimes also used for Cyanobacteria. At present, calcareous algae are one of the most important reef builders (see "Carbonate Environments"). For a detailed reading, please refer to "Algae (Eukaryotic)."

Bibliography


CALCIFIED CYANOBACTERIA

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Definition

Cyanobacteria are alga-like bacteria that can perform oxygenic photosynthesis and nitrogen fixation (Whitton and Potts, 2000; Herrero and Flores, 2008). They have a long history and are diverse and widespread in marine, freshwater, and terrestrial environments at the present-day, where they are key primary producers in both microbial mat and planktic ecosystems. As the initiators of plastids, they have played a fundamental role in algal and plant evolution (Raven, 2002). Cyanobacteria occupy benthic substrates and can also drift in the water column. Their photosynthetic uptake of inorganic carbon can stimulate CaCO₃ precipitation. This calcification can produce filamentous microfossils in benthic mats that are preserved as stromatolites and thrombolites, and can also cause water column precipitation of carbonate mud that settles to lake and sea floors.

Introduction

However, cyanobacterial calcification is not obligatory and is directly dependent on environmental conditions. This accounts for apparent discrepancies between the geological ranges of organic-walled and calcified cyanobacterial fossils. Calcified cyanobacteria have not been recognized in marine sediments older than ~1200 Ma ago (Kah and Riding, 2007), whereas there is evidence that cyanobacteria appeared in the late Archaean or Palaeoproterozoic, in the range ~2900–2150 Ma ago (Cavalier-Smith et al., 2006; Hofmann, 1976). This mid-Proterozoic appearance of sheath-calcified cyanobacteria is thought to reflect the development of CO₂-concentrating
mechanisms (CCMs) as atmospheric CO₂ levels declined (Riding, 2006). Calcified cyanobacterial fossils remained conspicuous components of marine stromatolites and thrombolites through much of the Neoproterozoic, Palaeozoic, and Mesozoic, but became vanishingly scarce in the Cenozoic, including present-day seas (Riding, 1982), probably due to decline in seawater saturation for CaCO₃ minerals (Riding, 1993, p. 514; 2000, p. 200; Kempe and Kazmierczak, 1994). Cyanobacterial calcification is thus a good example of "induced," as opposed to "controlled" biocalcification. Its close environmental dependence can be used to interpret changes in past conditions such as carbonate saturation state and the availability of inorganic carbon for photosynthesis. In addition to their sedimentological importance, calcified cyanobacteria can therefore assist in the reconstruction of seawater chemistry and atmospheric composition over long geological time scales.

Controls on calcification

The ability of cyanobacteria to grow and reproduce whether they are calcified or uncalcified illustrates the non-obligate nature of their calcification (Pentecost and Riding, 1986). Two factors that directly influence cyanobacterial calcification are the saturation state of ambient waters and the mechanism of photosynthetic uptake of inorganic carbon (Riding, 2006).

Carbonate saturation state: Cyanobacterial calcification requires waters in which CaCO₃ precipitation is thermodynamically favored (Pentecost, 1981; Kempe and Kazmierczak, 1994; Merz-Preiß and Riding, 1999). It has been very common in marine environments at times since the Neoproterozoic, but these episodes are markedly episodic and can be interspersed by long periods when cyanobacterial calcification is scarce (Riding, 1992). Cyanobacterial sheath calcification has not been confidently recognized in present-day marine environments, and is rare to absent throughout the Cenozoic (Riding, 1982; Arp et al., 2001). In contrast, cyanobacterial calcification is locally well-developed in present-day calcareous streams and lakes, and is often significantly involved in the formation of tufa and oncoids (Golubic, 1973; Pedley, 1990; Pentecost, 2005). In streams, calcification reflects increased saturation state that results from warming and degassing of spring waters, especially in turbulent zones, together with the stimulus of photosynthetic carbon removal (Merz-Preiß and Riding, 1999; Bissett et al., 2008). In lakes, precipitation is stimulated by seasonal warming as well as the activity of phytoplankton blooms that include cyanobacteria (Kelts et al., 1978; Thompson and Ferris, 1990). These present-day environments are vulnerable to pollutants such as agricultural fertilizers. Cyanobacterial calcification has declined over the past century in temperate climate hardwater streams and lakes of Europe and North America, largely in response to these anthropogenic changes (Pentecost, 2005, pp. 283–287), among which phosphate inhibition of CaCO₃ precipitation (Raistrick, 1949) may be important (Hägele et al., 2006).

CO₂-concentrating mechanisms: Photosynthetic carbon uptake can directly influence cyanobacterial calcification. Diffusive entry of CO₂ into the cell may not significantly affect ambient pH, but active bicarbonate uptake increases pH near the cell (Miller and Colman, 1980) that promotes calcification (Thompson and Ferris, 1990). Bicarbonate (HCO₃⁻) is actively transported into the cell and intracellularly converted to CO₂ for photosynthesis. These processes lead to increased pH in the immediate vicinity of the cell. Where ambient waters are sufficiently saturated for CaCO₃ minerals then this localized pH increase can trigger the nucleation of CaCO₃ crystals or near the cell surface in the enveloping mucilaginous sheath (Figures 1 and 2):

\[ 2\text{HCO}_3^- + \text{Ca}^{2+} \rightarrow \text{CH}_2\text{O} + \text{CaCO}_3 + \text{O}_2 \]

Active bicarbonate uptake and its conversion within the cell to CO₂ by carbonic anhydrase are adaptations to reduced availability of CO₂. They constitute CCMs (Kaplan and Reinhold, 1999). CCM induction can be triggered by localized carbon limitation, e.g., within microbial mats or phytoplankton blooms, and also by fall in global atmospheric CO₂ levels. Modeled estimates suggest that atmospheric CO₂ has fluctuated widely during the Phanerozoic, up to levels that are 25 or more times higher than present atmospheric level (PAL) (Berner and Kothavala, 2001). CCMs are well-developed in cyanobacteria (Kaplan et al., 1980; Giordano et al., 2005) and experiments suggest that they are induced when ambient CO₂ falls below a critical threshold that is roughly equivalent to 10 PAL (Badger et al., 2002). It therefore seems likely that CCM induction plays a significant role in cyanobacterial calcification (Thompson and Ferris, 1990; Merz, 1992), especially at times in the geological past when CO₂ levels have been below 10 PAL (Riding, 2006, p. 302).

Sites of calcification

Calcification in cyanobacteria is close, but external, to the cell. CaCO₃ crystals nucleate either within the protective mucilaginous sheath, or on or close to the cell surface (Thompson and Ferris, 1990; Merz, 1992). Sheath impregnation by crystals (Figure 1) can produce coherent tubular and shrub-like calcified structures that preserve the sheath morphology and can be preserved as microfossil for hundreds of millions of years (Riding, 1991). In contrast, if isolated crystals form near the cell surface, they do not form a preservable shape but can be released as allochthonous particles (“whitings”) (Figure 2). These can accumulate as masses of micron-size carbonate mud sediment on lake and sea floors and can also survive as ancient geological deposits. However, they are not known to possess features that distinguish them as
Calcified Cyanobacteria, Figure 1  Model of in vivo sheath calcification in a filamentous cyanobacterium related to CO₂-concentrating mechanism (CCM)-enhanced photosynthesis (based on information in Riding, 2006, Fig. 3). The CCMs involve active carbon transport into the cell and conversions that liberate OH⁻ ions. Calcification is stimulated by photosynthetic carbon uptake and OH⁻ release which elevates sheath pH. If ambient carbonate saturation is already elevated, with raised pH, extracellular HCO₃⁻ converts into CO₃²⁻, further increasing saturation state that promotes CaCO₃ nucleation in the sheath.

Calcified Cyanobacteria, Figure 2  Model of in vivo sheath calcification in a picoplanktic coccoid cyanobacterium related to CCM-enhanced photosynthesis (based on information in Riding, 2006, Fig. 3). The CaCO₃ nucleation (blue diamonds) occurs on and near the cell surface. The crystals can ultimately be sedimented to the lake or sea floor as carbonate mud.

cyanobacterially induced precipitates, and cannot at present be differentiated from carbonate mud of other origins.

Sheath calcification: The protective mucilaginous sheath that envelops benthic calcified cyanobacteria provides a diffusion limited site that enhances the pH rise resulting from carbon uptake (Figures 3 and 4). The sheath is a structured form of EPS (Drews and Weckesser, 1982) providing support, stability, and protection against physical damage, dehydration, and grazers (Dudman, 1977). It can contain the pigment scytonemin that acts as a barrier to ultraviolet radiation (Garcia-Pichel and Castenholz, 1991; Proteau et al., 1993; Dillon and Castenholz, 1999), binds nutrients and metals (Yee et al., 2004), and facilitates gliding motility (Stal, 1995, p. 4; Hoiczyk, 1998).

Sheath calcification can be partial or complete, and can be limited to the sheath interior (sheath impregnation) or form an external crust (sheath encrustation) (Riding, 1977). For example, it can occur as isolated crystals (Pentecost, 1987, Fig. 1d), form a crystalline network (e.g., Friedmann, 1979, Fig. 9), or create a relatively solid tube of closely juxtaposed crystals (e.g., Couté and Bury, 1988, pl. 2).

Although saturation state with respect to carbonate minerals and CCM induction appear to be key controls on cyanobacterial sheath calcification over geological timescales (Riding, 2006), differences in degree of calcification between different species/strains of cyanobacteria in the same environment also indicate taxonomic specificity for calcification (Golubic, 1973; Merz, 1992; Défarge et al., 1994). Further research is required to elucidate the extent to which such specificity may reflect differences in sheath structure and sheath development, CCM induction, or other factors.
In addition to in vivo sheath calcification, postmortem sheath degradation by heterotrophic bacteria can result in partial external calcification (Chafetz and Buczynski, 1992), although in culture experiments this may in part be related to the growth medium used (Arp et al., 2002). This incomplete and irregular calcification of decomposing sheaths contrasts with sheath impregnation that produces well-defined tubiform fossils such as *Girvanella* in which wall-thickness remains constant in individual specimens (Riding, 1977, 2006).

**Biogenic whiting precipitation:** In contrast with relatively large and distinct calcified fossils, such as *Girvanella*, that result from sheath calcification, CaCO₃ precipitates associated with coccoid cyanobacterial blooms are most noticeable as “whitings”. These are ephemeral milk-white patches in freshwater calcareous lakes and shallow tropical seas formed by dense masses of suspended small CaCO₃ crystals (Cloud, 1962). Whiting is a descriptive term, and all whitings are not necessarily biogenic. In addition to the triggering effect of phytoplankton photosynthesis, they could reflect essentially abiogenic CaCO₃ crystal nucleation in the water column, and also – in very shallow water – bottom mud re-suspended by currents or fish (Shinn et al., 1989).

Biogenic cyanobacterial whitings are documented by studies of seasonal blooms of unicellular picoplanktonic (cell size in range 0.2–2 µm) cyanobacteria, such as *Synechococcus*. These benefit from efficient CCMs (Badger and Price, 2003) where dissolved inorganic carbon (DIC) availability is limited, as under bloom conditions (Rost et al., 2003). Together with diatoms and other planktic algae, *Synechococcus* is implicated in stimulating biogenic whiting precipitation in present-day freshwater calcareous oligotrophic lakes (Thompson and Ferris, 1990; Dittrich et al., 2004; Lee et al., 2004). Since a sheath is lacking in picoplankton such as *Synechococcus*, calcification is instead localized on a paracrystalline surface layer that provides a binding site (Thompson, 2000, p. 253). This surface layer can be shed, producing biogenic whiting crystals that are deposited from suspension, either individually or as poorly structured aggregates along with organic cells, on lake beds.

In addition to lakes, picoplanktic cyanobacteria are widespread in the open ocean and in nearshore marine environments. They form blooms in Florida Bay, and marine strains of *Synechococcus* calcify under experimental conditions (Lee et al., 2004). There has been much debate concerning whether marine whitings in shallow tropical seas, such as the Bahama Banks, have a similar origin to those in temperate calcareous lakes, and could therefore potentially account for abundant lime mud production on ancient carbonate platforms. If marine whitings on the Bahama Banks are water column precipitates, then they would be important sources of carbonate mud and could be triggered by planktic cyanobacteria (Robbins and Blackwelder, 1992; Robbins et al., 1997). However, CaCO₃ precipitation in freshwater lakes is favored by low pH buffering, whereas in present-day seawater buffering limits pH fluctuation, thereby reducing the response to photosynthetic removal of CO₂ and HCO₃⁻. Several studies have suggested that Great Bahama Bank whitings are not due to water column precipitation (Broecker and Takahashi, 1966; Morse et al., 1984; Broecker et al., 2000). For example, whiting CaCO₃ has a ¹³C/C ratio that differs from that of inorganic carbon in the whiting water, but is similar to that of the seafloor sediment. In addition, the saturation state of Bahama Bank waters appears to be too low for pseudohomogeneous precipitation of CaCO₃.

**Calcified Cyanobacteria, Figure 3** *Girvanella*, calcified cyanobacterial sheath, early mid-Ordovician, Lunnan, Tarim, China. Width of view 1 mm. Thin-section courtesy Jia-Song Fan.

**Calcified Cyanobacteria, Figure 4** Thin-section of present-day oncoid microfabric showing calcified shrub-like sheath surrounding space left by the strand of cells (trichome, arrowed). The cyanobacterium is thought to be the oscillatoriacean *Schizothrix calcicola*, and the calcified sheath closely resembles the microfossil *Angulocellularia* (also *Angusticellularia*), which is locally common in Cambro-Ordovician reefs (Riding and Voronova, 1982). Squaw Island, Canandaigua Lake, New York State, USA.
Fossil calcified cyanobacteria

Calcified cyanobacterial fossils have long been noticed in limestones, although they have often been confused with other organisms. Sheath calcified cyanobacteria are morphologically simple fossils, mainly with the appearance of shrub-like dendritic masses (Figure 4) and tangle or erect, sometimes radially arranged, tubes (Figure 3). Densely micritic branched filaments such as Epiphyton, and chambered clusters such as Renalcis have also often been regarded as possible calcified cyanobacteria. At the same time, because of their general lack of distinguishing features, many of these fossils have often also been compared with calcified green and red algae, and also with foraminifers and sponges. For example, when Nicholson and Etheridge (1878) named Girvanella they compared it with foraminifers, and it has subsequently variously been regarded as a calcareous sponge (Seely, 1885), green alga (Rothpletz, 1891), and red alga (Korde, 1973). Bornemann (1886) recognized its cyanobacterial nature, and Pollock (1918) identified it as a calcified sheath. Individual calcified cyanobacteria are normally microscopic, but they commonly form much larger aggregates that are significant components of stromatolites, oolites, and chambered clusters such as Girvanella and Ortonella when he placed them in a new group, the Porostromata. Dendritic shrubs and filaments: Angusticellularia (also named Angulocellularia) described from the Cambrian of Siberia by Vologdin (1962) is a submillimetric vertically orientated and irregularly dendritic shrub-like mass of dense micrite (Figure 4). In contrast, narrow branched micritic filaments, usually not tubiform but in some cases with light-colored transverse bands, comprise a group that includes Epiphyton, originally described from the Cambrian of Sardinia by Bornemann (1886), and among others, Epiphytonoides, Gordonophyton, and Tubomorphophyton. Hollow chambers: Renalcis, described from the Cambrian of southern Siberia by Vologdin (1932), is a hollow thick-walled chambered microfossil that typically forms irregular submillimetric clusters. Broadly similar fossils, such as Ezhella and Shuguria, are distinguished by wall thickness and number of chambers. Some form millimetric dendritic growths. Chabakovia filaments consist of thin-walled chambers. In contrast, Gemma and Tarthina have few chambers with very thick walls. There is considerable mingling of characters among some of these generally simply organized fossils that can make them difficult to distinguish within these broad groups (Pratt, 1984; Riding and Voronova, 1985; Woo et al., 2008). Dense filaments and chambered forms are much more problematic, whereas tubiform and shrub-like forms can often be compared with present-day sheath calcified cyanobacteria. These include sedimentologically important Cambrian and late Devonian fossils such as Epiphyton and Renalcis that have some resemblances to cyanobacteria but are not identical to modern examples, and are generally referred to as calcimicrobes. Some Epiphyton closely resemble red algae (Luchinina and Terleev, 2008).

Proterozoic record and secular controls

The possibilities that marine carbonate saturation state and changes in atmospheric CO2 level could be reflected in the geological record of cyanobacterial calcified microfossils and carbonate mud (Riding, 2006, p. 309) focuses attention on the mid-Proterozoic interval when calcified sheaths first appeared (Kah and Riding, 2007) and carbonate mud became abundant (Sherman et al., 2000, p. 290) (Figure 5).

CCM induction

Badger et al. (2002) suggested that cyanobacterial CCMs developed in the late Palaeozoic as CO2 declined and O2 increased. However, both modeling and palaeosol proxies (Kasting, 1987; Sheldon, 2006) suggest that similar decline in CO2 occurred in the mid-Proterozoic. It can therefore be suggested that the Mesoproterozoic appearance of calcified sheath microfossils reflects development of CCMs as CO2 fell below a critical threshold that may...
have been $\sim 10 \times$ PAL (Badger et al., 2002; Riding, 2006; Kah and Riding, 2007). Under high early Proterozoic CO$_2$ levels, cyanobacterial photosynthesis may have relied on CO$_2$ diffusion that may not have significantly altered pH near the cell surface. Falling CO$_2$ levels would have encouraged active carbon uptake to maintain photosynthesis and this, in turn, promoted calcification (Figures 1 and 5).

**Carbonate mud:** Carbonate mud appears to have been scarce in the late Archaean (Sumner and Grotzinger, 2004), but it increased in abundance during the Proterozoic (Kah and Grotzinger, 1992) and was widespread in the Neoproterozoic (Herrington and Fairchild, 1989). Much of it is thought to have been produced by whitings stimulated by phytoplankton photosynthesis (Grotzinger, 1989, 1990). A significant increase in carbonate mud occurred $\sim 1400–1300$ Ma ago (Sherman et al., 2000, p. 290), prior to the appearance of sheath calcification (Kah and Riding, 2007). This “water column factory” transformed carbonate platform sedimentation by creating extensive micrite-rich subtidal deposits (Sherman et al., 2000, p. 290). The timing of this increase in carbonate mud therefore broadly coincided with the first appearance of *Girvanella*-like and other sheath-calcified fossils (see below), and may be linked to CCM development in cyanobacteria, but in this case to planktic forms, possibly similar to present-day *Synechococcus* (Riding, 2006). Muddy carbonate platforms were the deposits in which distinctive molar tooth facies developed (James et al., 1998; Sherman et al., 2000, p. 290). It can therefore be speculated that mid-late Proterozoic increase in micrites and molar tooth facies, together with *Girvanella* and thrombolites, all reflect decline in atmospheric CO$_2$ that stimulated CCM induction and calcification in cyanobacteria (Riding, 2006) (Figure 5).

**Cyanobacterial sheath calcification:** The first appearance of calcified cyanobacteria in the mid-Proterozoic is interpreted to reflect the first development of CCMs in photosynthetic organisms (Riding, 2006) – marked a radical change from stromatolitic to thrombolitic fabrics. Mesoproterozoic examples reported from the ~1200 Ma Society Cliffs Fm are currently the oldest known *Girvanella*-like calcified filaments, and are associated with micritic bush-like structures also interpreted as calcified cyanobacteria (Kah and Riding, 2007). The presence of filamentous fabrics had long been recognized in late Proterozoic and early Palaeozoic stromatolites. Microstructure with filament moulds was termed Canaliphorida and Filiformita by Komar (1976, 1989, see Bertrand-Sarfati et al., 1994, p. 182, Fig. 18). Aitken (1989, pp. 15–16) described “dendriform” and “lamelliform elements” as important components of Little Dal reefs in the Mackenzie Mountains. He regarded both as stromatolites with “unusual” or “unique” characteristics: thin-walled tubes and *Renalcis*-like objects in dendriform element, and a reticulate “ladder-rung” arrangement that “may be formed by a meshwork of tubes” in lamelliform element. He remarked that these fabrics “are not typically
stromatolitic” and that “sediment trapping may not have been the dominant process in their formation” (p. 15). Dendriform and lamelliform elements look quite similar in two of his illustrations (Figs. 10 and 13). Subsequently, Turner et al. (1993, 2000a, Fig. 10b, 2000b) compared the tubules with Girvanella and noted that the lamelliform fabric consists of alternating dark layers of “calcimicrobial filaments” and lighter “more cement-rich” areas. Little Dal “hollow tubules with micritic walls” are figured by Batten et al. (2004, Fig. 9b). Knoll and Semikhatov (1998, p. 410, Figs. 3 and 4) described well-preserved “filmy or platy” microstructure in early Neoproterozoic Baicalia lacera stromatolites from the Chernaya Rechka Fm, Igarka, Siberia. They found it to be associated with “a distinctly filamentous microstructure” in which “laminae comprising densely interwoven to scattered, vertically, or subhorizontally oriented filaments are interspersed with layers of spongy or dense microspar.” They interpreted the 8–10 µm tubes as “sheaths of LPP-type (Lyngbya, Phormidium, Plectonema) cyanobacteria and preserved as drusy microspar encrustations” (Knoll and Semikhatov, 1998, p. 411). Similar Baicalia lacera fabrics in the ~1 Ga Burovaya Formation of west-central Siberia locally contain calcified tubes resembling Siphonophycus (Petrov and Semikhatov, 2001, p. 270). The lamelliform element shown by Turner et al. (2000a, Fig. 10b) resembles the distinctive “filmy” microstructure of similar age Baicalia lacera (Petrov and Semikhatov, 2001, Figs. 5b and 6a) which also has steeply dipping laminae and, as noted above, quite possibly filamentous microstructure too. In these examples, layers of filamentous fabric are generally interleaved with lighter, sparry, layers. If the sparry layers were lacking, the deposit would be indistinguishable from “skeletalstromatolite” (Riding, 1977) and “porostromate stromatolite” (Monty, 1981). In addition to the Little Dal and Chernaya Rechka examples, calcified filaments reminiscent of Girvanella are relatively widespread elsewhere in the Neoproterozoic, e.g., in the ~750–700 Ma Draken Fm, ~725–675 Ma Svanbergfjellet Fm, and ~700 Ma Upper Eleonore Bay Supergroup, Greenland (refs in Knoll and Semikhatov, 1998, p. 413). Throughout the Neoproterozoic, these thrombolites with calcified microbial fabrics (Kennard and James, 1986) are intimately associated with stromatolites but typically occur in deeper subtidal environments (Aitken, 1967).

Marine carbonate saturation

Inception of cyanobacterial calcification would have required overall seawater carbonate saturation to be relatively high. Grotzinger (1990) has argued that seawater saturation state progressively reduced during the Proterozoic and that this could account for concomitant decline in stromatolite abundance. Nonetheless, the widespread continued development of microbial carbonates into the Neoproterozoic, together with calcified sheaths themselves, indicates that saturation state was not low. This may have changed during widespread glaciations that occurred in the late Proterozoic ~700–570 Ma ago (Walter et al., 2000). Sheath-calcified cyanobacteria appear to have been scarce during “Snowball” glaciations. Lower temperature and pCO₂ levels would have decreased seawater saturation state, hindering microbial calcification generally. Shields (2002) attributed decline in Molar Tooth facies ~750 Ma ago to reduction in carbonate saturation. Since cooling would also have favored diffusive entry of CO₂ into cells (Raven et al., 2002) it could have slowed CCM development, further reducing cyanobacterial calcification. As Earth emerged from these snowball glaciations, global warming and O₂ rise could have reactivated CCM development, and rising temperature, calcium, and pCO₂ levels would have increased seawater saturation state, stimulating calcification (Riding, 2006). Calcified cyanobacteria became relatively diverse

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**Calcified Cyanobacteria, Figure 6** Early Cambrian (Botomian) dendrolite-thrombolite reef, ~70 m thick, near Tafraoute, Anti-Atlas Mountains, Morocco. Figures in foreground for scale.
in the earliest Cambrian (Nemakit-Daldynian) (Riding and Voronova, 1984) and were important components of Cambrian reefs (Riding, 2000; Rowland and Shapiro, 2002).

Calcimicrobial reefs and the Cambrian radiation
Calcified microfossils such as *Epiphyton*, *Renalcis*, and *Angusticellularia* are major components of Cambrian reefs (Figures 6 and 7) and similar forms reappeared in the late Devonian. The widespread development of microbial reefs in the early Cambrian may have directly contributed to marine invertebrate diversification. Reefs constructed by calcified microbes appeared in the latest Neoproterozoic, ~550 Ma ago (Grotzinger et al., 2000) and were common in Siberia, Altai Sayan, and Mongolia during the Nemakit-Daldynian (543–526 Ma ago) (Riding, 2002). In the Tommotian, 526–522 Ma ago, this reef-building association was augmented by archaeocyath sponges, with calcified microbes generally being volumetrically dominant (Rowland and Shapiro, 2002), and the reefs became large and cavernous. The exceptional biodiversity that characterizes reefs in general is largely due to the process of reef bioconstruction itself (Cocito, 2004). Rapid accretion of framework with synoptic relief and internal growth cavities creates substrates and spaces that differ in incident light, water movement, and sedimentation, as well as accessibility. This increased habitat size and partitioning generates heterogeneity that promotes biodiversity. For example, Cambrian trilobites show peak diversity in reefal environments (Westrop and Adrain, 2001), and comparison of level-bottom and reef communities in general shows that early Cambrian reefs were centers of diversity (Burzin et al., 2001, Fig. 10.2) with number of reef-building species comparable to that of late Neogene fossil reefs (Kiessling, 2002, Fig. 20). The expansion of these globally distributed reef communities corresponded with rapid diversification of the shallow marine biota, especially during the Tommotian-early Botomian (~526–517 Ma ago) (Zhuravlev 2001, Fig. 8.1), suggesting a direct link between microbial carbonate development and metazoan evolution.

Phanerozoic record and secular controls
Cyanobacterial sheath calcification: Following their Mesoproterozoic inception, sheath-calcified cyanobacteria remained generally common in shallow-water marine carbonates during much of the Paleozoic and until the late Jurassic (Arp et al., 2001) (Figure 8b). The abundance of sheath-calcified cyanobacteria during the early Paleozoic (when CO₂ levels are thought to have been high, see Berner and Kothavala, 2001), suggests that CCMs continued to be induced even when pCO₂ substantially exceeded 10 PAL. This may reflect calcification in habitats subject to carbon limitation that induced CCMs, such as microbial mats and reefs (Riding, 2006). Nonetheless, enhancement of CCMs by overall decline in atmospheric CO₂ could be reflected by increased calcified-sheath abundance in the Mississippian, ~335 Ma ago (Riding, 2006) (Figure 5). However, it seems that this increase in calcified-sheath abundance promoted by falling CO₂ was itself terminated by subsequent steep decline in seawater saturation state ~325 Ma ago, before the end of the Mississippian (Figure 8a).

Broad similarities between calcified-sheath abundance and carbonate saturation are suggested by peaks in the late Cambrian (~500 Ma), late Silurian (~420 Ma), late Devonian (~370 Ma), late Triassic (~220 Ma), and late

![Calcified Cyanobacteria, Figure 7](image) Early Cambrian calcimicrobial reef framework, showing clusters of mainly pendant filaments, early cement and geopetal cavity fill. Near Taroudannt, Anti-Atlas Mountains, Morocco. Width of view ~35 cm.
Jurassic (~150 Ma) (Figure 8), supporting the view that saturation state has significantly influenced the degree of cyanobacterial sheath calcification over geological time (Riding and Liang, 2005).

Reduced abundance of sheath-calcified cyanobacteria ~120–80 Ma ago contrasts with calculated saturation state which is elevated during this interval. This anomaly is thought to reflect reduction in actual (as opposed to calculated) saturation state due to burial of CaCO₃ precipitated as calcified skeletons by plankton such as coccolithophore algae and globigerine foraminifers (Riding and Liang, 2005). Such biological removal of calcium is not included in the input used to calculate the saturation values shown. This effect of planktic calcifiers on the seawater system may have continued into the Palaeogene. During the past 50 Ma, from the early Eocene onward calculated saturation has declined steeply in response to low values of Ca²⁺ ions and pCO₂, and this is consistent with scarcity of marine calcified cyanobacteria. Consequently, although low pCO₂ values during the Cenozoic (see Berner and Kothavala, 2001) are expected to induce cyanobacterial CCMs, the absence of sheath calcification in marine cyanobacteria presumably reflects the overriding effect of low carbonate saturation.

**Calcified Cyanobacteria, Figure 8** Comparison of (a) Marine saturation ratio (Ω) for calcite, calculated from estimates of seawater and atmospheric composition, and (b) abundance of marine sheath-calcified cyanobacteria (Arp et al. 2001, Fig. 3d) (from Riding and Liang, 2005, Fig. 5). Obliquely ruled areas indicate five intervals approximately 500, 420, 370, 220, and 150 millions of years (Myr) ago where peaks of calculated saturation ratio broadly coincide with increased abundances of calcified cyanobacteria. This correspondence supports the view that cyanobacterial calcification has been increased at times of generally elevated seawater saturation for CaCO₃ minerals. The horizontally ruled area indicates the interval ~120–80 Myr ago in which high calculated saturation contrasts with low abundance of calcified cyanobacteria. This anomaly could reflect lowered Cretaceous carbonate saturation due to widespread deposition of pelagic carbonate by coccolithophore algae and globigerine foraminifers, since biological removal of CaCO₃ is not incorporated into the calculation of saturation values.

**Biogenic whittings**: It can be inferred that, following the development of CCMs in the mid-Proterozoic, picoplanktic cyanobacterial activity could have been an important source of marine carbonate mud whenever inorganic carbon could be a limiting resource, as in plankton blooms (Rost et al., 2003), even when overall atmospheric CO₂ levels were high. If this is correct, then the principal factor determining biogenic whiting occurrence would have been carbonate saturation state. Calculations suggest that saturation was relatively elevated during much of the early-mid Palaeozoic and parts of the Mesozoic (Riding and Liang, 2005; and Figure 8a).

The origins of micrite are notoriously difficult to interpret even in present-day seas, and it is known that calcified green algae can also be significant producers (Stockman and Ginsburg, 1967). How can the possible contribution of biogenic whittings therefore be assessed in the geological past? One line of reasoning is to assume that sheath-calcified cyanobacteria reflect generally similar conditions to those that favor picoplanktic whiting precipitation. The geological record of sheath-calcified fossils such as *Girvanella* is relatively well-documented, and they show marked fluctuations in abundance (Arp et al., 2001, Fig. 3d) (Figure 8b). It could therefore be predicted that picoplanktic mud should show a similar pattern of
abundance, with peaks for example in the late Cambrian, Devonian-Mississippian, and late Jurassic. It should be possible to test whether carbonate mud was relatively abundant at these times. It is also possible that biogenic whittings – and sheath calcification – should have been further intensified whenever pCO2 levels fell to levels near or below ~10 PAL, as for example ~350 Ma ago (Figure 5).

Cenozoic decline in seawater carbonate saturation, suggested by calculations based on modeled values (Riding and Liang, 2005, Figs. 5a and 8a), would be expected to have reduced biogenic whiting precipitation. Certainly, sheath calcification is scarce to absent during most of the past 100 Ma (Arp et al., 2001, Fig. 3d) (Figure 8b). In this context, it is not surprising that there is uncertainty whether present-day marine whittings on the Bahaman Banks are biogenic or mainly reflect sediment resuspension (Broecker et al., 2000). From a geobiological perspective, however, even if present-day marine conditions do not greatly favor phytoplankton-stimulated precipitation of whittings, these may have been very important at times in the geologic past environment when carbonate saturation state was sufficiently high. If this is correct, biogenic whittings could account for a substantial part of the carbonate mud that accumulated on shallow shelves between the late Mesoproterozoic and Mesozoic.

Summary
Calcified cyanobacteria are easily overlooked fossils, yet their long history coupled with their sensitivity to environmental controls on their calcification, make them potentially significant indices of changes in seawater chemistry and atmospheric CO2 level throughout the Proterozoic and Phanerozoic. Locally they are also sedimentologically important reef components, and they have played a major role in the formation of calcimicrobial thrombolites that are especially widespread in the Neoproterozoic and early Palaeozoic. At the present day their vanishing scarcity in marine environments is thought to reflect relatively low seawater carbonate saturation that has persisted throughout the Cenozoic. However, their local abundance in oncoids and tufas of freshwater calcareous streams and lakes, and their involvement in lacustrine whittings, are reminders of the ability of cyanobacteria to promote intense CaCO3 deposition when conditions that include ambient saturation state, are favorable.

Bibliography


Calcite precipitation, microbially induced

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Definition
Microbially induced calcite precipitation describes the formation of calcium carbonate minerals from a solution due to the presence of microbial cells, biosynthetic products, or metabolic activity.

Calcium carbonate precipitation
The most basic requirement for the precipitation of calcium carbonate (CaCO₃) minerals, calcite, and aragonite is that the product of the concentrations of calcium [Ca²⁺] and carbonate ions [CO₃²⁻] exceeds the solubility product of calcite (Equation 1) and aragonite, respectively.

\[ [\text{Ca}^{2+}][\text{CO}_3^{2-}] > 10^{-8.35} \] (1)

The solubility of carbonate minerals depends on the temperature and pressure, decreasing with increasing temperatures and increasing with the increasing pressure. When a solution is in equilibrium with carbon dioxide, [CO₃²⁻] is determined by pH. In solutions that are undersaturated or not highly saturated, such as modern seawater, the biological activity can strongly control the precipitation of CaCO₃. Biological processes exert considerably less control on the precipitation of CaCO₃ in environments characterized by high temperature, high pH, and high evaporation and degassing rates such as soda lakes, hot springs, caves, and freshwater tufas.

Microbial processes that promote the precipitation of CaCO₃
Culture-dependent and culture-independent studies have shown that microbes (Bacteria and Archaea) can induce extracellular precipitation of calcium carbonate minerals by:

(a) Increasing the local pH and the concentration of carbonate ion
(b) Promoting the nucleation of calcium carbonate minerals and removing the kinetic inhibitors of CaCO₃ precipitation.

Increase in local pH and the concentration of carbonate ion
Because the concentration of carbonate ion increases with the increasing pH, the precipitation of calcium carbonate minerals will also increase with the increasing pH. Many microbial metabolic processes can increase the pH and/or the concentration of carbonate ions: sulfate reduction in marine sediments (Dupraz and Visscher, 2005; Van Lith et al., 2003; Visscher et al., 2000), oxygenic...
photosynthesis in freshwater streams and lakes (Merz-Preiß and Riding, 1999; Thompson et al., 1997), anaerobic methane oxidation coupled with sulfate reduction in marine sediments (Reitner et al., 2005), denitrification, degradation of amino-acids and small organic acids (Chekroun et al., 2004), and degradation of calcium oxalate in soils (Braissant et al., 2002). Whether microbes are able to significantly increase local pH and [CO$_3^{2-}$] will largely depend on the buffering of the medium by dissolved inorganic carbon. Microbial activity in poorly buffered solutions such as lakes and streams is more likely to influence the saturation state of CaCO$_3$ (Merz-Preiß and Riding, 1999) than in well-buffered solutions such as soda lakes, hot springs, and solutions analogous to the early soda oceans (Arp et al., 1999a, b; Bosak and Newman, 2005).

Precipitation of calcium carbonate minerals also can be inhibited by the adsorption of various metabolites such as phosphate, sulfate, and organic moieties onto the faces of the growing crystals (Bosak and Newman, 2005; Liu and DeYoreo, 2004; Plant and House, 2002; Teng et al., 1998). Microbial growth and metabolic processes that lower the concentration of these ions in the solution can promote the precipitation of minerals even when they do not significantly influence pH and [CO$_3^{2-}$] (Bosak and Newman, 2005; Kawaguchi and Decho, 2002).

Nucleation of calcium carbonate minerals on organic surfaces

Minerals can nucleate either homogeneously on the surface of preexisting carbonate grains, or heterogeneously on organic or other surfaces. When the external calcium carbonate sediment is sparse or absent, nucleation is the most energetically expensive step in the precipitation of calcium carbonate minerals. Organic matter can lower the free energy barrier required to form the initial nuclei, thus promoting mineral formation. Nucleation of CaCO$_3$ on organic surfaces (i.e., heterogeneous nucleation) occurs when negatively charged surface groups such as carboxylate, phosphate, and sulfate complex bind calcium. Although this process can initially inhibit the precipitation of minerals, once the local concentrations of calcium and carbonate has exceeded the binding capacity of the organic matrix, or when the matrix is partially degraded, mineral nucleation occurs preferentially on the organic template (Arp et al., 2003; Braissant et al., 2003; Decho et al., 2005). Heterogeneous nucleation can increase the number of crystal nuclei even when the bulk medium is supersaturated with calcium carbonate by more than an order of magnitude (Arp et al., 1999b; Bosak and Newman, 2003).

Heterogeneous nucleation of CaCO$_3$ can take place on the cell surfaces and envelopes, or within the exopolymeric matrix surrounding microbial cells. Examples of the former are the calcification of the sheaths surrounding cyanobacterial cells (Merz-Preiß and Zankl, 1993) and the whiting events caused by the precipitation of calcite on the outer S-layer of some planktonic cyanobacteria (Thompson et al., 1997). These processes are most commonly observed in freshwater environments and require actively photosynthesizing cyanobacteria (Thompson et al., 1997). Cyanobacteria encrusted by CaCO$_3$ are considerably less abundant in modern marine calcium carbonates (Arp et al., 2001). Similarly, the relationship between marine whittings and cyanobacterial presence and activity has not been demonstrated conclusively (Thompson, 2000). Instead, in marine photic zones, carbonate minerals precipitate mainly within the extracellular exopolymeric matrix in areas of heterotrophic degradation (Chafetz and Buczynski, 1992; Dupraz and Visscher, 2005; Gautret et al., 2006; Van Lith et al., 2003; Visscher et al., 2000). In addition to the nucleation on exopolymeric substances or cell surfaces, CaCO$_3$ also preferentially nucleates around submicron vesicles that are secreted by sulfate-reducing bacteria (Aloisi et al., 2006). The nucleation of CaCO$_3$ on these vesicles may be a common mechanism in the environment, because outer-membrane vesicles are secreted by many Gram-negative bacteria (Beveridge, 1999; Kuehn and Kesty, 2005).

Recognition of microbially induced CaCO$_3$ precipitates in the rock record

Even when microbes do not induce measurable changes in the chemical composition of the bulk solution, they can alter the pH and carbonate chemistry, remove kinetic inhibitors, and promote mineral nucleation and precipitation at a microscale (Figure 1). When minerals precipitate, surrounded by microbial cells and microbial aggregates (biofilms) (Figure 1), they can preserve remnants of organic matter, such as acidic amino acids and microbial casts, long after the original cells have been degraded (Bosak et al., 2004a; Sprachta et al., 2001). Furthermore, CaCO$_3$ precipitated in the presence of organic compounds often has distinct shapes (Bosak and Newman, 2005; Braissant et al., 2003; Chafetz and Buczynski, 1992; Teng et al., 1998), although similar shapes can also be produced abiotically.

Biological influence on the morphology of carbonate crystals and structures is most prominent in skeleton-building organisms. Macroscopic and microscopic eukaryotic organisms such as calcareous algae, protists, corals, and mollusks precipitate hard body parts. Many of these organisms precipitate CaCO$_3$ by genetically encoded mechanisms that result in shells, skeletons, and carbonate grains with species-specific shapes. In contrast to these products of biologically controlled calcification, the shapes of microbially induced carbonate minerals can be distinguished from abiotically precipitated grains rarely (Golubic et al., 2000). Consequently, the potential identification of biosignatures of microbially induced carbonates in modern and past sedimentary carbonates can be controversial (Grotzinger and Knoll, 1999).

Recognition of microbially induced carbonates is also impeded by the difficulty in distinguishing between CaCO$_3$ structures that grew in the presence of microbes
or organic matter from structures that grew because of microbial activity. Some examples of carbonate grains and structures with often ambiguous origin include peloids, ooids (Figure 2), whitings, fossil stromatolites, and nanobacteria. Recent developments in analytical techniques such as X-ray microscopy have enabled the detection of small quantities of fossil organic matrices in fossil carbonates at the microscale, thereby establishing that some fossil carbonates grew in the presence of microbes (Benzerara et al., 2006; Lepot et al., 2008). Despite the inclusion of organic matter, the role of biology in forming these structures remains unknown. As an alternative approach, experimental and quantitative models of the morphogenesis of sedimentary structures at the macroscale and microscale have been developed in an attempt to understand which fossil structures and shapes require biological processes (Batchelor et al., 2004; Bosak et al., 2004b; Dupraz et al., 2006; Grotzinger and Rothman, 1996; Reid et al., 2000; Suess and Futterer, 1972).

**Summary**

The potential of microbes to induce precipitation of calcium carbonate has sparked scientific interest for almost a century. The calcium carbonate (CaCO₃) minerals, calcite and aragonite, often precipitate in association with microbial cells and biofilms in marine and terrestrial sedimentary environments such as reefs, lakes, streams, beaches, soils, hot springs, and caves. Being ubiquitous and relatively insoluble, these minerals have helped preserve bacterial and archaeal cells and biofilms throughout geologic time (Figure 1). Microbes promote the precipitation of calcium carbonate metabolically, by increasing the pH and the alkalinity of their surroundings, or by the secretion of substances that facilitate the mineral nucleation. The recognition of microbially influenced calcium carbonate precipitates in the rock record is often challenging because abiotic carbonate precipitation can produce crystals with similar shapes and chemical signatures. Current research in the field of microbial carbonates tackles this challenge by conducting culture-dependent and culture-independent studies of microbial mineral precipitation, employing analytical techniques that can detect...
small quantities of organic matter in fossil carbonates, and by developing theoretical models that explain the shape of macroscopic carbonate sedimentary structures. Microbially induced precipitation of carbonate minerals is likely to garner even more interest in the future with a primary focus on carbon sequestration, the production of biomimetic materials, and the conservation of ornamental stone.

Bibliography


Calcium (Ca) has the atomic number 20 and is an alkaline earth metal with an atomic mass of 40.978 amu. The important role of Ca in biogeochemical processes is based on its chemical versatility, which is related to its highly adaptable coordination geometry, its divalent charge, modest binding energies, fast reaction kinetics, and its inertness in redox reactions (Williams, 1974). Calcium is a soft grey alkaline earth metal, and is the fifth most abundant element in the Earth’s crust and the seventh most abundant element in the ocean. It is essential for living organisms, particularly in cell physiology, shell formation, and calcification and hence, is usually the most common metal in many animals.

Calcium isotopes and their application in Biogeochemistry

There are seven Ca isotopes present in nature of which three isotopes (42Ca (0.646%), 43Ca (0.135%) through 44Ca (2.086%)) are stable, one isotope (40Ca (69.941%)) is radiogenic, one isotope (41Ca) is cosmogenic, and two more isotopes (46Ca (0.004%) and 48Ca (0.187%)) are radioactive having extremely long half-lives (40Ca: 2.8 × 1015 years; 41Ca: 4.1 × 1019 years). The cosmogenic isotope 41Ca is radioactive (half live of 103,000 years) and is produced by neutron activation of 40Ca in the upper soil column. 41Ca has received much attention in stellar studies because it decays to 41K, a critical indicator of solar system anomalies. 40Ca is the radiogenic daughter product of 40K decay, along with 40Ar. While K–Ar dating has been used extensively in the geological sciences, the prevalence of 40Ca in nature has impeded its use in dating. Techniques using mass spectrometry and a double spike isotope dilution have been used for K–Ca age dating (Nägler and Villa, 2000).

Natural Ca isotope fractionation has, among other iso-otope systems such as oxygen (d18O) and carbon (d13C) fractionation, been applied for the study of calcification processes and the so-called vital effect (c.f. Gussone et al., 2003; Lemarchand et al., 2005). Latter effect describes the deviation of the expected inorganic isotope fractionation value or element partitioning coefficient (e.g., Sr/Ca, Mg/Ca, etc.) from the measured one in calcifying organisms such as coccolithophores, foraminifer, corals, and other calcifying organisms.

The more or less species-dependent Ca isotope fractionation of marine calcifying organisms as a function of the adjacent seawater temperature can be applied for the reconstruction of the dynamic of Ca concentrations throughout the Earth’s history (c.f. Griffith et al., 2008; Farkas et al., 2007; Heuser et al., 2005; Fantle and DePaolo, 2005; De La Rocha and DePaolo, 2000) and for the reconstruction of past sea surface temperatures (c.f. Nägler et al., 2000; Hippler et al., 2006).
one million year. A number of scientific studies show that continental and oceanic weathering, mid-ocean ridge hydrothermal activity, and carbonate sedimentation are the major factors controlling the marine Ca cycle (Hart, 1973; Thompson, 1983; Zhu & Macdougall, 1998; Berner, 2004). Besides continental weathering and riverine influx, comprising about 70% of the Ca flux to the ocean, submarine magmatism and associated hydrothermal activity at the mid-ocean ridges as well as seafloor weathering are also recognized to be major sources for the marine Ca budget (Berner & Berner, 1996). A number of studies indicated that Ca is released from the oceanic crust during water–rock reactions ranging from high-temperature hydrothermal interactions to seafloor weathering at low temperatures below about 150°C (Berner, 2004; Amini et al., 2008). Ca-bearing mineral phases such as aragonite, calcite, anhydrite, and subordinately hydrous Ca-silicates (e.g., prehnite, zeolites) are formed during different stages of ocean crust alteration.

Marine calcification

The dynamic of Ca in the ocean is of particular interest because of the formation of biogenic and nonbiogenic calcium carbonate (CaCO₃), known as the process of calcification, and its interference and close linkage with the global carbon cycle (c.f. De La Rocha & DePaolo, 2000; Heuser et al., 2005). In particular, the formation of calcareous skeletons by marine planktonic organisms and their subsequent sinking to depth generate a continuous rain of CaCO₃ to the deep ocean and the underlying sediments. This is important in regulating marine carbon cycling and ocean–atmosphere CO₂ exchange. The present rise in atmospheric CO₂ levels causes a significant decrease (acidification) in surface ocean pH and a major change of carbonate chemistry. Laboratory experiments have shown that such changes slow down calcification in corals and coralline macroalgae. Over geological time scales, the Ca balance in the ocean is believed to be directly related to pCO₂ variations in the atmosphere, and it thus may interfere with climate changes on geological time scales (e.g., Demicco et al., 2003; Fantle and DePaolo, 2005).

Summary

Future progress in instrumental analytic will allow high-resolution measurement of Ca elemental and isotope partitioning in inorganic and biological systems, allowing to trace elemental pathways from a bulk solution to the site of calcification.

Bibliography


Cross-references

Animal Biocalcification, Evolution
Calcified Cyanobacteria
Calcite Precipitation, Microbially Induced
Carbonate Environments
Carbonates
Divalent Earth Alkaline Cations in Seawater
Dolomite, Microbial
Isotope Fractionation (Metal)
Isotopes and Geobiology
Cap carbonates are unusual deposits of dolostones and limestones, often enriched in barite, that sharply overlie Neoproterozoic glacial deposits. They are from 2 to 50 m thick and occur on shallow platforms, shelves, and slopes worldwide, even in regions otherwise lacking in carbonate strata.

Glaciation events during the late Neoproterozoic (Cryogenian–Ediacaran) are widespread on Earth, reflecting up to four global ice ages (“Snowball Earth Hypothesis”) between 730 and 580 Ma. Numerous publications dealing with these events exist (for example, see Chapter by Hoffman P.F., Snowball Earth, this volume; Shields, 2005; Jiang et al., 2003). Some of them exhibit characteristic, microcrystalline cap dolostones, and also limestones, which is unique and reflects a climate paradox. Particularly the Marinoan glaciation ca. 635 Ma ago is peculiar, because it provides evidence for low altitude glaciation at equatorial latitudes. Three models have been proposed to explain an increase in alkalinity inducing these global carbonate formation events:

1. Overturn of an anoxic deep ocean;
2. Enormous rates of chemical weathering in the hinterland after glaciation (Snowball Earth Hypothesis);
3. Destabilization of methane clathrates and increase of alkalinity caused by anaerobic oxidation of methane (AOM).

However, none of the hypotheses sufficiently explains the consistent sequence of precipitation events observed within cap carbonate successions. The formation of massive dolostones is very problematic and hardly understood. Massive microbial sulfate reduction may explain this event. Barite precipitation is also very common. The source of sulfate is most probably the weathered hinterland. Caused by seawater oversaturated with aragonite, diagenetically very early large aragonite crystals were formed. It is suggested that cap carbonates are primarily formed by a microbially mediated precipitation. Possibly, the highly oxygenated shallow marine environments of the postglacial Neoproterozoic oceans provided excellent conditions for the evolutionary development of new microbial communities, phototrophic organisms, and of course, the rapid development of Metazoa (Figures 1 and 2).

Bibliography

Cross-references
Anaerobic Oxidation of Methane with Sulfate
Animal Biocalcification, Evolution
Calcite Precipitation, Microbially Induced
Calcium Biogeochemistry
Carbonate Environments
Cold Seeps
Dolomite, Microbial
Microbialites, Stromatolites, and Thrombolites
Permafrost Microbiology
Snowball Earth
**Definition**

Organic carbon (OC) refers to any carbon that occurs in compounds of ultimately biological origin that can be oxidized.

**Introduction**

Carbon is made up of organic and inorganic fractions, and the biochemical and geochemical cycling of these forms through the Earth processes is referred to as the global carbon cycle. The global carbon cycle is closely linked to other global cycles such as oxygen, nitrogen, and sulfur because of the important role these elements play in biological processes. Organic carbon (OC) is present in both living and nonliving systems and ultimately fuels all of the Earth’s biogeochemical processes (Summons, 1993); therefore, understanding the formation, cycling, and preservation of OC is essential to understanding the cycling of all bioelements between living and nonliving reservoirs, the formation of economically important petroleum and coal deposits, and predicting how human activity may change the natural balance of these systems.

Much of the OC created during photosynthesis or by chemosynthetic autotrophs is decomposed by heterotrophic organisms. Heterotrophic processes occur in the water column of marine and aquatic environments, as well as in sediments, soils, and rocks. Much of the decomposition of organic matter (OM) occurs under low temperature (<50°C) and pressure during diagenesis. Diagenesis occurs primarily through biological processes (microbes and larger organisms that live in surface sediments) and over depths ranging from microns to hundreds of meters. The OC preserved following diagenesis is subject to further alteration by the processes of catagenesis and metagenesis, which occur at higher temperatures and pressures and lead to the formation of petroleum and natural gas.

**Reservoirs**

The cycling of OC involves fluxes to and from the major OC reservoirs (Figure 1). Sedimentary rocks are the largest OC reserve, making up approximately 99.5% of the

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**Carbon (Organic, Cycling), Figure 1** The global organic carbon (OC) cycle, adapted from Hedges (1992). The OC cycle involves fluxes to and from the major surficial and deep reservoirs. Reservoirs are denoted by capital letters, with values in blue. Fluxes due to physical processes (italicized) and primary production (not italicized) are denoted with arrows. Flux terms are presented in lower case font, with values given in red. Information presented in this figure is based on published values from Schlesinger (1991), Longhurst (1995), Hedges et al. (1997), Kvenvolden (2002), Richey (2004), IPCC (2007).
total global OC. Despite the relative size of this reservoir, OC only represents one-fifth of the total sedimentary carbon, since inorganic carbon is the dominant fraction (Hedges, 1992). Sedimentary OC occurs primarily as kerogen, coal beds, and petroleum reservoirs, which ultimately come from biogenic processes or sources. Kerogen is an amorphous, highly insoluble particulate OM that is thought to form by two pathways – selective preservation and geopolymerization, both of which occur under low temperature and pressure conditions (Killops and Killops, 2005). Selective preservation involves the formation of kerogen from biomacromolecules that are resistant to microbial degradation. Geopolymerization occurs through a random series of condensation and polymerization reactions involving humic substances. Petroleum is defined as naturally occurring fluid (liquid and gases) that evolves from kerogen. Petroleum is formed primarily from the remains of microbes (algae and bacteria), although some types of petroleum are formed from higher plants. In contrast, coal is a solid and is formed from the remains of higher plants. The most common type of coals, humic coals, is formed through the processes of peatification and coalification, which involve both biological and geochemical processes (Killops and Killops, 2005). The OC in ancient sedimentary rocks provides these economically important fossil fuels as well as a molecular record of life, which has allowed the construction of evolutionary trees and an understanding of the geologic timing of past events and the response of natural systems to tectonic, environmental, and biological change (Brocks and Pearson, 2005). Historically, sedimentary OC affects biologically mediated cycles on geologic time scales, although humans have recently accelerated this cycle through fossil fuel combustion (7.5 × 10^15 g C year^-1; IPCC, 2007).

The active (surficial) pools of OC include soil humus, land and marine plant tissue, seawater dissolved OC (DOC, <0.5 μm diameter), and surface marine sediments and gas hydrates (Kvenvolden, 2002; Hedges, 1992). Dead and living particulate OC (>0.5 μm) are also present in freshwater and seawater pools; however, the DOC pool dominates. With the exception of plant tissue and gas hydrates, most of the active pools of OC are poorly characterized. Terrestrial plant tissue can be further classified as woody biomass (~75%), nonwoody biomass (~15%), and litter (~10%) (Olson et al., 1985). Gas hydrates contain mostly methane and some ethane molecules, and may serve as a large reservoir for OC, although global estimates of the size of this sink are still uncertain (see Kvenvolden, 2002). Soil OM is made up of plant remains (detritus), soil microbes, and humic substances.

### Production and Fluxes

The major fluxes between organic pools are driven by marine and terrestrial primary production. Photosynthesis, the harvesting of light energy to convert inorganic carbon to reduced (organic) forms in the tissue of plants, is the primary mode of primary production. However, chemosynthesis by microbial organisms, which uses chemical energy rather than sunlight, is also important. Photosynthesis is carried out by prokaryotes such as cyanobacteria as well as eukaryotic algae and higher plants. The fixation of carbon by plants over the Earth’s history accounts for the oxygen in our present atmosphere, thus the cycles of carbon and oxygen are closely linked over geologic time (Hedges, 2002; Hedges and Keil, 1995). In fact, to maintain current atmospheric oxygen levels, rapid cycling of carbon in the surficial pools is required. These pools exchange on relatively short timescales (10^2–10^4 years). In contrast, the OC that remains in the sedimentary system cycles slowly (~10^8 year; Schlesinger, 1991). Sedimentary reservoirs only become exchangeable through geologic uplift, followed by oxidative weathering of OC by exposure to the atmosphere.

Net global primary production (NPP) on land (45–65 × 10^15 g C year^-1) is dominated by more labile nonwoody plant tissues such as leaves, grasses, and herbaceous annuals (Schlesinger, 1991). The highest gross terrestrial production (82 × 10^15 g C year^-1) and storage as biomass (56%) occur in the tropics, while polar regions contribute the least (8 × 10^13 g C year^-1, 0.5%; Taube, 1992). Within these regions, forests are the most productive continental biomes and deserts and tundra are the least (Taube, 1992), reflecting global patterns of temperature and precipitation. Marine primary productivity (~50 × 10^15 g C year^-1) is dominated by open water (pelagic) phytoplankton productivity because open water constitutes approximately 75% of the total ocean area (Ducklow and McCallister, 2005; Longhurst, 1995). However, when normalized to area, coastal and upwelling areas together account for nearly two thirds of the total oceanic productivity because a greater supply of nutrients is available to support phytoplankton in these areas (Longhurst, 1995). Other primary producers along the coasts (i.e., seagrass, marsh plants, macroalgae, mangroves) are locally important sources of OC.

Delivery of OC (as both dissolved and particulate fractions) from the land to coastal marine environments by rivers accounts for 0.4 × 10^15 g C year^-1 (Meybeck, 1982; Ittekot, 1988). However, there remains considerable uncertainty in our ability to adequately quantify carbon exchange from land to the coastal ocean and in our understanding of the processes influencing the fate of terrigenous carbon in coastal sediments (Berner, 1982; Sarmiento and Sundquist, 1992; Hedges and Keil, 1995; Schlünz and Schneider, 2000). Generally, the total annual riverine OC load to the ocean is proportional to the total annual river flow, which is dominated by a small set of rivers that transport the bulk of the fresh water from land to sea (Schlesinger, 1991). However, seasonal and annual/interannual variations in hydrology as well as large events such as storms and hurricanes influence the input, dispersion, and cycling of sediment OM in coastal environments, thus making it difficult to trace terrigenous OM.
In addition, recent molecular and isotopic evidence suggests that OC in seawater and marine sediments is marine, rather than terrestrial, in nature, which suggests that further remineralization of terrestrial material must occur in rivers prior to delivery to the coastal ocean (Schlünz and Schneider, 2000; Richey, 2004; Mayorga et al., 2005) or in the marine environment (Hedges et al., 1997). This is an area requiring further research. Eolian (air) flux from the land to the sea approximates $0.1 \times 10^{15}$ g C year$^{-1}$, although this estimate remains uncertain due to methodological difficulties (Romankevich, 1984; Zafiriou et al., 1985).

**Decomposition and preservation**

Only $\sim0.1\%$ of global NPP is ultimately preserved within sediments; however, it is this OC that will eventually become part of the sedimentary record and may be transformed into fossil fuels. The remaining 99.9% of global NPP is remineralized to inorganic carbon, nutrients, and water. Remineralization represents a sink for oxygen, linking the global cycles of OC and oxygen. Microfauna, bacteria and fungi (on land) are responsible for the breakdown of OM. Bacterial and fungal decomposition of OM involves a series of steps; macromolecules must first be broken down using extracellular enzymes into dissolved polymers, which are then broken down into low molecular weight monomers (Tissot and Welte, 1984).

The rates and extent of decomposition are influenced by environmental, biological, and physical factors. First, there must be sufficient supply of labile OM to be degraded. The depth-integrated rate of OM remineralization in marine sediments correlates closely with carbon flux to the sediment surface (Henrichs, 1993). Interestingly, the fraction of OC that is not remineralized is also highly correlated with the carbon flux to the sediment surface. Thus, the efficiency of OM decomposition appears to be lower in systems that receive the highest input of OC compared to systems with lower accumulation rates (Henrichs and Reeburgh, 1983). Second, like most metabolic processes, decomposition rates are proportional to temperature, resulting in global patterns of decomposition rates (e.g., higher degradation rates in the tropics than in tundra systems) (Schlesinger, 1991). Oxygen is also a controlling factor for decomposition. Recent evidence suggests that oxygen exposure time, rather than oxygen concentration, may be an important determinant of the extent to which OM is decomposed (Hartnett et al., 1998). OM decomposition occurs under both oxic and anoxic conditions; however, the rates and bacterial communities involved in the processes differ. Oxygen is used preferentially as the terminal electron acceptor during decomposition and results in the most efficient mineralization (i.e., the highest energy yield). Once oxygen is depleted, bacteria utilize other electron acceptors such as sulfate and nitrate. Aerobic decomposition is facilitated by microbes as well as higher organisms with a range in enzyme capabilities. Anaerobic decomposition is carried out exclusively by microorganisms, and complete remineralization is typically dependent on a consortium of cooperating organisms. Further, anaerobic decomposition processes differ in terrestrial and marine environments because sulfate is abundantly present in marine systems but limited in terrestrial systems. As a result, sulfate reduction is the dominant anaerobic pathway for oxidizing OM in marine systems, while fermentation and methanogenesis dominate in freshwater systems (Henrichs, 1993). The chemical and structural makeup of OM also plays a role in its susceptibility to decomposition (e.g., number of double bonds and functional group composition). For example, nitrogen-rich compounds such as proteins and polynucleotides are usually decomposed preferentially to carbon-rich structural compounds (Wakeham and Canuel, 2006). Additionally, the architecture of the OM may be an important control on its decomposition. Architecture refers to both physical structure (e.g., conformation) as well as OM associations with organic and inorganic matrices (see below).

Decomposition and preservation of OC are closely linked processes, since only OC that is not decomposed is available for preservation, but there are additional factors that may enhance preservation (Burdige, 2007; Wakeham and Canuel, 2006). For instance, the amount of OC that reaches the sediment surface and has the potential to be buried depends in part on productivity in the overlying photic zone and water depth. Sufficiently high productivity is essential for OC to reach the sediment with the potential for preservation (Killlops and Killlops, 2005). In addition, physical or chemical protection of OM plays a key role in controlling preservation. Protection by binding to inorganic ions, clays, and organic residues decreases the surface area of OM that is exposed to bacteria for degradation. This protection or encapsulation of OM explains why compounds that appear to be labile, or readily available for decomposition, have been found in sedimentary OC deposits. Lastly, the amount of time that OM is exposed to oxic conditions determines the extent to which oxygen-sensitive compounds, compounds that specifically require oxygen for degradation, are preserved (Burdige, 2007).

Preservation of OC occurs predominantly in anoxic marine sediments. More than 80% of preserved OC is located in deltaic-shelf environments associated with fine grain sediments (Berger, 1982) that support the physical or chemical protection model, since these sediments provide the most potential surface area with which OM can bind. This has likely been true for millions of years, as current petroleum deposits are found in areas that were formerly coastal, fine grained sediments. Major depositional environments include lacustrine environments (open, closed lakes), peat swamps and coal, and continental margins, enclosed or silled basins, and the Black Sea (Killlops and Killlops, 2005).
Conclusion
The global OC cycle involves exchange of OC between surface and subsurface (buried) reservoirs on time scales of less than one year to millions of years. These fluxes are driven by the production and cycling of OC through biological, physical, and geological processes. The global OC cycle is intimately linked with other global cycles and is sensitive to human-induced perturbations. Humans may have reduced total NPP by up to 40% due to wildfires and pollution, which may be the largest sink for primary production to support a single species in the history of life on the Earth (Vitousek et al., 1986). Fossil fuel combustion and destruction of forests (especially tropical) have decreased the global pool of OC and increased atmospheric carbon dioxide, which has been linked to global climate change. Global atmospheric concentrations of CO₂, methane (CH₄), and nitrous oxide (N₂O) have increased markedly as a result of human activities since pre-industrial times (i.e., 1,750). In 2005, concentrations of atmospheric CO₂ (379 ppm) and CH₄ (1,774 ppb) exceeded the natural range over the last 650,000 years (IPCC, 2007). Understanding the changes in the size and composition of the OC reservoir through space and time will help us to better predict the changes in its global cycle as well as the associated cycles of other biologically important elements.

Bibliography


Cross-references
Aerobic Metabolism
Algae (Eukaryotic)
Anaerobic Oxidation of Methane with Sulfate
Anaerobic Transformation Processes, Microbiology
Archaea
Bacteria
Carbon (Organic, Degradation)
Methane Oxidation (Aerobic)
Methane, Origin
Microbial Degradation
Nitrogen
Soils
Sulfate-Reducing Bacteria
Sulfur Cycle

CARBON (ORGANIC, DEGRADATION)

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Synonyms
Carbon biodegradation; Carbon remineralization

Definition
Organic carbon (OC). In geochemistry, carbon that occurs in materials of ultimately biological origin and that can be oxidized. Organic matter (OM) is the material that contains organic carbon (OC).

Degradation. Transformations of OM within the range of temperatures, pressures and environmental conditions found in or near the Earth surface environments. If biological activity is confirmed to cause the transformations, it is termed biodegradation.

Remineralization. Degradation of OM that results in net conversion of OC to inorganic (oxidized) carbon species such as CO2 or HCO3–.

Introduction
The Earth exists in a near balance between photosynthesis and respiration. Photosynthesis, by which inorganic carbon is chemically reduced to biologically-available OC using light energy, generates an estimated 107 Pg C year–1 of net new production each year (1 Pg = 1015 g), nearly evenly distributed among terrestrial (57 Pg C year–1) and marine (50 Pg C year–1) environments (Field et al., 2007; Sabine et al., 2004). Of this biomass, over 90 Pg C is remineralized each year to inorganic carbon through aerobic respiration on land and in the oceans, leaving 17 Pg year–1 to be redistributed among different pools of nonliving OM (Field et al., 2007; Sabine et al., 2004). These pools include soils, aquatic and marine sediments, and nonliving OM in the world’s oceans. Degradation of organic carbon in these pools balances the crustal carbon cycle, such that total global respiration plus net OC remineralization are believed to match global rates of photosynthesis (Berner, 2003). Maintaining or slowly shifting this balance has been a critical component of the coupled atmosphere–ocean climate system throughout the Earth’s history, and has served to maintain an equable amount of atmospheric oxygen (O2) for the past 600 million years (Berner, 1989, 2003).

Degradation of OC in soils
Soils contain complex mixtures of weathered rocks, new minerals formed within soils, and OM. The world’s soils contain an estimated 3,200 Pg C (Sabine et al., 2004 and references therein). Much of this OM derives from terrestrial vegetation. As dead vegetation ages within soils, it is degraded by a combination of chemical reactions and biological processes. Soluble components of decaying vegetation may be leached out of soils and relocated by groundwater movement. Organic acids and chelating agents released by plant roots further facilitate the dissolution and hydrolysis of vegetation. Soil macrofauna, especially detritivores such as earthworms, serve to physically mix soil horizons from different depths, moving OM-rich surface leaf litter down to more water- and mineral-rich horizons and reducing the OC and nutrient content of surface soils (Bohlen et al., 2004). Detritivores also accelerate the formation and disintegration of soil carbon aggregates, resulting in overall larger aggregates of fresh (less-degraded) OM mixed to greater depth within soils (Blair et al., 1995; Cortez, 1998). This activity increases soil porosity and permeability, allowing access to oxygen to greater depth in soils to support aerobic respiration processes.

Throughout in the soils, microbial processes both positively and negatively influence soil OM degradation. Fungal hyphae and other microbial exopolymers bind soil carbon and mineral particles together into aggregates. These exopolymers add to the stock of soil OC contributed by terrestrial vegetation, and support mechanisms of physical protection of OM against further degradation; aggregate-bound OM is much less susceptible to degradation than unassociated OM (Baldoock and Skjemstad, 2000). Other microorganisms participate in the degradation of soil OM as they obtain energy and cellular carbon through various metabolic processes. While aerobic respiration is confined to shallow depths of the soil where O2 can penetrate, anaerobic processes extend throughout the soil profile, from O2-free microenvironments within surface soils to deeper O2-free soils and weathered regolith.

Soil OC degradation is limited by the availability and reactivity of both OM and electron acceptors that fuel anaerobic respiration. Sulfate is not an abundant
electron acceptor in most soils, while ferric iron and nitrate can be. Soil OM is also susceptible to degradation other than respiration. Fermentative degradation of soil OM breaks down terrestrial inputs and biopolymers into small molecules including low molecular weight organic acids, alcohols, and sugar monomers. Methanogenesis too is an important mechanism of OC degradation in soils, consuming fermentation products of such as methanol, H₂, and acetate, sustaining the fermentative breakdown of soil OM.

Many components of soil OM are fairly susceptible to degradation and do not persist in soil environments. These include carbohydrate biopolymers (e.g., cellulose, chitin, and other polysaccharides), proteins, and nucleic acids. Other components such as lipids, lignin, and black carbon are much more resistant to degradation, and can persist for millennia as intact compounds or transitional degradation products in soils (Derenne and Largeau, 2001). Lipids commonly found preserved in soils include highly aliphatic waxes, free hydrocarbons such as n-alkanes and isoprenoids, and biopolymers such as cutan and suberan. Lignin is a structural biopolymer in plant cell walls that derives from condensation of phenolic precursors. Black carbon comprises a broad suite of aromatic and graphitized organic materials that form during incomplete combustion of terrestrial biomass or fossil fuels; these materials are believed to be extremely resistant to degradation (Schmidt and Noack, 2000).

During degradation in soils, soil OM components are remineralized at rates depending on a complex combination of intrinsic reactivity (polymeric material being more resistant than individual monomers), physical protection, (inside mineral aggregates or encapsulated within more resistant biopolymers) and lability (proteins, nucleic acids, and carbohydrates being more reactive than lipids and lignin).

Degradation of OC in the oceans

OC occurs in both dissolved and particulate forms in the oceans. Approximately 50 Pg C year⁻¹ derives from marine primary production driven by phytoplankton (Field et al., 2007; Sabine et al., 2004 and references therein). Rivers deliver an estimated 0.5 Pg of OC to the oceans each year. Primary production is confined to the photic zone of the world ocean, where light and nutrients combine in sufficient abundance to support photosynthetic activity. Within and immediately below the photic zone is a zone of intense heterotrophic activity, in which grazing zooplankton and aerobic heterotrophic bacteria consume particulate OM (Lee et al., 2004). The heterotrophic activity in near-surface waters has several impacts on OC degradation in the oceans. One is that >90% of primary production is effectively remineralized in the upper 200 m of the marine water column, leaving only a small fraction of surface particulate OM for export to the deep ocean (Suess, 1980; Honjo et al., 2008). Second is that mineral ballasting (association of OM with biogenic minerals produced by plankton or with minerals delivered from continents to the surface ocean by winds and river) increases the sinking rate of OM through the water column, reducing the time available for degradation in the ocean (Lee et al., 2004; Armstrong et al., 2002). Aggregation of particulates in surface waters results in increased particle size and sinking velocity, further accelerating the export of less-degraded particulate OM to a depth (Sheridan et al., 2002). Lastly, heterotrophic grazing of surface particulates results in net evolution of the composition of sinking particulates, as more labile or easily degraded components are consumed (Lee et al., 2004; Wakeham et al., 1997).

In living biomass of the photic zone, OM that can be identified at the molecular level comprises >80% of total OC. Between 200 and 1,000 m water depth, molecularly uncharacterized material comes to dominate total OM, such that much of the material delivered to seafloor sediments cannot be assigned to typical compound classes such as lipids, carbohydrates, and amino acids (Lee et al., 2004). Some of this relative increase in uncharacterized material may result from selective preservation of photic zone precursors, while some may be added to sinking particulates as exopolymeric material generated by heterotrophic bacteria that feed on sinking particulate matter. There are also changes observed in the molecularly characterizable fraction of sinking particulate OM. Lipids resistant to degradation, such as long-chain saturated fatty acids and alkenones, are substantially enriched in relative abundance compared with initial photic zone biomass, while pigments, many carbohydrates, and polyunsaturated fatty acids are readily lost from particulates within or below the photic zone (Lee et al., 2004).

This background picture of OC degradation is conformed by the role of mineral surfaces. Minerals, whether delivered to surface waters from the continents by winds and rivers, or produced by plankton in the water column, make up the bulk of sinking particulate mass. OM can be sorbed to mineral particles, and with their greater density, OM-associated particulates can sink rapidly through the water column, helping to export relatively undegraded OC beyond the photic zone. In this way, terrestrially derived OM, which may be protected from degradation by association with detrital minerals or OM macromolecules, can be delivered to deep sea sediments. More generally, marine OM may be protected from degradation by adsorption in mesopores of sinking mineral particles, or OM mineral aggregates taken together may resist degradation, where OM is located in interstices between the mineral grains (Hedges et al., 2001). Clearly, degradation of OM within the marine water column reflects a complex interplay of inherent resistance of various organic materials across a continuum of lability, coupled with mechanisms of both speeding the rate of sinking through the water column and increasing protection from degradation by the association of OM with biogenic and terrigenous minerals.

This discussion of heterotrophic degradation of OC applies to an oxygenated water column. In several locations around the globe, large proportions of the water column are
anoxic. These include the Black Sea, the Cariaco Basin, and seasonally in the Arabian Sea, eastern tropical North Pacific and California Borderlands Basins, among others. Lack of oxygen effectively shuts down aerobic respiration in these regions, allowing the roles of anaerobic microbial processes to dominate OC degradation. In these anoxic basins, the extents of OM degradation as a fraction of primary production are lower than in open ocean sites, leading to higher sediment OC contents. Although the rates of degradation under oxic and anoxic conditions may be similar, the overall proportion of total OM that resists degradation may be greater under anoxic conditions (Van Mooy et al., 2002).

Degradation of OC in marine sediments

Efficient heterotrophic processes in the water column mean that only a small fraction (<1%) of primary production reaches the sea floor. Of this, most is degraded within the upper meters of sediment, leading to OC burial of approximately 160 Tg C year\(^{-1}\) (Berner, 1989; Hedges and Keil, 1995). Clearly, a large fraction of ocean carbon inputs (~50 Pg C year\(^{-1}\)) is degraded in both the water column and shallow sediments.

There is a positive relationship between sediment accumulation rate (either cm year\(^{-1}\) or g\(_{\text{carbon}}\) cm\(^{-2}\) year\(^{-1}\)) and OC burial efficiency, where burial efficiency is the fraction of OC ultimately preserved at depth in sediment relative to OC delivered to the sediment-water interface (Burdige, 2007; Aller, 1998; Canfield, 1989). At very low sedimentation rates, the sediment layers are buried very slowly, and aerobic processes have ample time to remineralize the OM that is delivered to these sediments, thereby explaining the low total OC contents of many low sedimentation rate marine settings. Closer to shore as sedimentation rates increase, rapid burial means that anoxic conditions dominate at shallower sediment depths, and anaerobic degradation processes become more important. Nonetheless, sediments underlying oxygen-deficient waters do not uniformly exhibit high carbon burial efficiency, indicating that oxygen alone is not the primary driver controlling OM degradation rates (Burdige, 2007). Burial efficiencies among a range of marine sediment types range between 10% and 20%. Thus, very little primary production escapes degradation processes to become incorporated into the sedimentary record.

Controls on OC degradation in sediment are similar to those operating in soils and in the marine water column: selective degradation of inherently resistant components, physical protection through association with mineral or organic polymeric surfaces, and availability of electron acceptors and/or appropriate substrates for nonrespiration degradation processes such as fermentation and methanogenesis (Burdige, 2007). Oxygen does not limit OC degradation. Most marine sediments are anoxic within centimeters of the sediment–water interface. Denitrification, sulfate-, iron- and manganese-reduction, fermentation, and methanogenesis are all active components of OM degradation. Oxygen exposure time is nonetheless a useful proxy for understanding the extent of OC degradation from one environment to the other. Longer cumulative oxygen exposure times result in more OC degradation in sediment and overall lower OM burial efficiency (Aller, 1998; Hartnett et al., 1998).

Most OC is buried in shallow, continental margin sediments. These environments have been targets of numerous investigations over the years, and have provided insights into OC degradation processes in the upper few meters of buried sediments (see Martens et al., 1992). Deeply buried marine sediment such as from the Woodlark Basin and Peru Margin in turn demonstrate microbial activity hundreds of meters deep in sediments, indicating OC degradation can persist at slow rates for geologic spans of time (D’Hondt et al., 2004; Wellsbury et al., 2002).

Degradation of OC in ancient sedimentary rocks

The single largest pool of OC on the Earth (15 million Pg C) is represented by OM contained in ancient sedimentary rocks, a mass that dwarfs all other pools of OC combined (Field et al., 2007; Sabine et al., 2004). Traditionally, OC in sedimentary rocks has been viewed as resistant to degradation other than high temperature oil and gas generation or graphitization. Degradation can occur where sedimentary rocks are exposed at the Earth’s surface through erosion and soil formation (Petsch et al., 2000, 2001). One unexpected theme emerging from this work is that OC degradation during rock weathering is not completely efficient. Highly aged OC is detected in river particulates from watersheds draining sedimentary lithologies (Longworth et al., 2007; Goni et al., 2005; Blair et al., 2003; Leithold et al., 2006).

However, very little of the total pool of sedimentary OM is exposed in the Earth surface environments; most remains deeply buried in the Earth’s crust for millions of years. The persistence of OC in sedimentary rocks for long expanses of geologic time, and its preservation up to the present day, would suggest that degradation processes must largely cease after the burial of OM in sediments. Yet, microbial degradation of deeply buried OM has been described in a number of subsurface environments. These include clear signatures of hydrocarbon biodegradation in many petroleum systems (Head et al., 2003, 2006; Jones et al., 2008) and OM-rich sedimentary rocks (Formolo et al., 2008), coupled with molecular biological analyses that support an interpretation of an active subsurface biosphere involved in OC degradation (Waldron et al., 2007; Jones et al., 2008). These subsurface OC degradation processes are particularly valued for their role in natural gas generation in petroleum systems and other sedimentary basins.

Summary

OC degradation represents the suite of processes by which OM is transformed and remineralized, mainly through biological activity, within soils, aquatic systems,
sediments, and sedimentary rocks. The diverse forms and compositions of natural OM result in a wide range of rates of degradation, as some components are inherently resistant to degradation while others are labile and are thus quickly consumed following biosynthesis. The inherent resistance of some components of OM results in selective preservation in soils and sediments. This selective preservation is attenuated by mechanisms of physical protection, either by association with mineral surface or encapsulation of labile components within more recalcitrant organic matrices. In this way, nominally labile OC can be preserved for greater extents of time or transported across longer distances than would otherwise be the case. Future investigations of OC degradation will likely focus on (1) improved construction of carbon budgets for soils, the oceans, and sediments; (2) the mechanisms by which certain compound classes and organic materials remain resistant to biological degradation; (3) improved understanding of the association of organic materials with mineral surfaces, their selective adsorption/desorption behavior, and the physical scale of these relationships; (4) the roles that terrestrial inputs of OM to marine sediments and recycling of ancient OM during rock weathering play in controlling the overall reactivity and preservation of OC in sediments; and (5) improved understanding of couplings between OM composition and degradation processes active within deep subsurface sedimentary environments.

Bibliography
The carbon cycle is a biogeochemical cycle that describes the movement of carbon between its different compartments (reservoirs) on the Earth, namely biosphere, atmosphere, hydrosphere, pedosphere, and geosphere. Please refer to entries “Carbon (Organic, Cycling)” and “Carbon (Organic, Degradation).”

**CARBON ISOTOPES**

Please refer to “Isotopes and Geobiology” and “Biomarkers (Organic, Compound-Specific Isotopes).”

**CARBONATE ENVIRONMENTS**

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**Definition**

Carbonate environments occur both in the terrestrial and marine realms as well as in transitional zones between the land and the sea. The variety of environments spans from high-elevation continental lakes to the deep sea, from the equator to latitudes of about 60°, and they include dry and wet climate realms. The majority of carbonates are produced in the sea, currently in more or less similar proportions in neritic and pelagic settings. Carbonate production is to a large part a consequence of biological activity, either directly as in shell and skeleton formation or indirectly as a result of metabolic reactions, which trigger precipitation. The highest rates of carbonate production per unit time and space are found in tropical coral reefs and they probably reached in tropical coral reefs where several kilograms of calcium carbonate form on 1 m² during 1 year’s time.

Introduction

Modern carbonate sediments are largely a product of biologic activity, either enzymatically controlled like in calcium carbonate (CaCO₃) shell and skeleton formation of aquatic organisms or indirectly by the metabolic activity.
of organisms, which change water chemistry so that carbonate precipitation is enhanced (Milliman, 1974; Scholle et al., 1983; Scoffin, 1987; Tucker and Wright, 1990; Flügel, 2004; Schlager, 2005). The former refers to common carbonate producers such as corals, mollusks, calcareous algae, brachiopods, bryozoa, echinoderms, sponges, foraminifera, or coccolithophorids. The latter includes microbes and algae, which are of great importance in the formation of microbialites (stromatolites and thrombolites), tufa, in the initiation of carbonate precipitation, e.g., by removing carbon dioxide (CO₂) during photosynthesis, or as photosymbionts in reef corals, larger benthic foraminifera, giant clams, and sponges (Figure 1).

Possible exceptions of biologic production of carbonate include some carbonate muds and non-skeletal carbonate particles such as ooids, peloids, and aggregate grains that are currently forming in the Bahama Banks, the Persian Gulf, restricted platforms, or certain lakes. Peloids include both cemented fecal pellets as well as micritized or recrystallized skeletal grains (Purdy, 1968). Recrystallization is a common pattern in carbonate sediments and rocks, which is a geologically fast process (Bathurst, 1971; Reid and Macintyre, 1998). Carbonate cements are another common phenomenon in which biogenic versus abiogenic precipitation is discussed (Schneidermann and Harris, 1985; Schlager, 2005). Classic examples of relatively fast and abundant cementation include tropical coral reef frameworks (Grammer et al., 1993) and beachrock (Gischler, 2007).

The majority of carbonate sediments by far are produced in the sea. Presently, about half of this carbonate originates in the pelagic realm and largely includes shells of planktonic and benthic foraminifera, pteropod mollusks, and coccolithophorids. The other half is produced in the neritic realm by corals, mollusks, calcareous algae, brachiopods, bryozoa, and echinoderms. It is estimated that this 50:50 ratio between pelagic and neritic carbonate sedimentation changed to 90:10 during Quaternary lowstands of sea level (Milliman and Droxler, 1996).

Today, carbonate minerals commonly formed in the sea are aragonite and high-magnesium calcite (calcite with 4–21% MgCO₃). After sodium (Na⁺), magnesium (Mg²⁺) (1,300 ppm) and calcium (Ca²⁺) (410 ppm) are the second and third most common cations in the ocean, respectively. Carbonate as HCO₃⁻ is the third most common anion (140 ppm) after chloride (Cl⁻) and sulphate (SO₄²⁻). Calcium carbonate is precipitated after the formula:

\[
Ca^{2+} + 2HCO_3^- \rightarrow \text{CaCO}_3 + H_2O + CO_2
\]

In freshwater environments, for comparison, where ion concentrations in general and the magnesium/calcium ratio in particular are much lower, low-magnesium calcite typically is precipitated. Subaerial exposure surfaces of limestone are characterized by dissolution, and form typical karst features.

Dolomite (MgCa(CO₃)₂) may form in limited quantity both in the ocean and in lakes. Preconditions for dolomite precipitation are a high Mg/Ca-ratio and elevated salinity. Modern examples of dolomite formation include the deep sea, certain lakes, and sabkhas, which are intertidal carbonate areas in an arid climate. The majority of dolomite presently exposed on the earth’s surface, however, was formed secondarily in the burial environment, by dolomitization of carbonate rocks from magnesium-rich solutions.

The analysis of non-skeletal grains such as ooids and cements throughout the marine geologic record has revealed that the mineralogy of precipitated carbonate has changed between aragonite/high-magnesium calcite and low-magnesium calcite (Sandberg, 1983). This observation, known as the Sandberg-Cycles, is a consequence of changing Mg/Ca-ratios in the ocean as controlled by plate tectonic activity. When lower spreading-rates, colder climate, and lower sea levels are experienced during the periods such as today’s or the late Paleozoic icehouse worlds, aragonite predominates over low-magnesium calcite (high Mg/Ca). When spreading-rates and sea level were high and the climate was warm, such as during Devonian or Cretaceous greenhouse worlds, low-magnesium calcite precipitation was favored (low Mg/Ca). Meanwhile, additional fossil and aquarium studies have shown that quite a number of carbonate-producing organisms also change their skeleton or shell mineralogy according to Mg/Ca-ratios of seawater (Stanley and Hardie, 1998). Investigations in Precambrian carbonate rocks have supported the contention that carbonate concentrations in the ocean also fluctuated during Earth history, thereby influencing carbonate deposition (Knoll et al., 1993). In the Proterozoic, carbonate concentrations were so high that in places cements were physicochemically precipitated on the seafloor. Carbonate concentrations decreased in the Cambrian when shell and skeleton production exploded. Carbonate concentrations presumably sank again in the Mesozoic when calcareous plankton such as planktonic foraminifera and coccolithophorids
evolved. The Soda Ocean model of Kempe and Degens
(1985) speculates that calcium concentrations in the ocean
also dramatically decreased with shell and skeleton evolu-
tion during the end of the Precambrian through the Camb-
rian. These authors interpreted shell and skeleton
formation as calcium expelling as a consequence of cell
poisoning.

In the following, highlights of typical carbonate envi-
ronments are discussed in a cross section going from the
terrestrial, coast, and shallow marine to deep marine set-
tings (Figure 2).

**Terrestrial environments**

**Springs**
Carbonate deposits are normally very rare in running
freshwater because of the low ion contents of the water.
Turbulence that causes CO₂ degassing may lead to
low-magnesium calcite precipitation. Also, pressure
release or cooling of hot waters at springs may bring about
calcite precipitation. The limestone product is usually
termed tufa, which forms under ambient temperatures
and may contain calcified remains of bacteria, algae, and
plants (Chafetz and Folk, 1984; Ford and Pedley, 1996).
Travertine, which forms in hot water (hydrothermally), is
dense, usually clearly laminated, and largely lacks algae
and plant remains. Typical morphologies of tufa deposits
include barrages, cascades, or terraces with pools. Ooids
may form in pools that hold water. Tufa deposits are
restricted to the late Cenozoic, which presumably is
a consequence of limited preservation potential (Figure 3).

**Lakes**
Lacustrine carbonates occur in both freshwater and saline
lake environments (Matter and Tucker, 1978; Hakanson

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**Carbonate Environments, Figure 2** Carbonate environments in a land-to-sea cross section. (Modified from Flügel, 2004.)

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**Carbonate Environments, Figure 3** Photo of sliced Quaternary tufa deposit on Casino building of Goethe University, Frankfurt,
Germany, with microbial lamination and calcified remains of algal thalli.
and Jansson, 1983; Anadon et al., 1991). Freshwater lakes in humid climates such as Lake Constance, Germany, Lake Zurich, Switzerland, or Green Lake in North America are usually perennial. Their size can be as small as <10 km² as in Green Lake to more than 500 km² as in the example of Lake Constance. They are characterized by fine-grained carbonate deposits, usually in an annually laminated fashion (Figure 4). Fine-grained siliciclastics are more common during winter. Low-magnesium calcite is precipitated preferentially during warm summer months, enhanced by photosynthetic activity of phytoplankton that withdraws CO₂. Other carbonate producers are usually ostracods, gastropods, pelecypods, and the charophytic alga Chara. The thalli of this alga may calcify and form characteristic tubes. Reproductive cells (oogonia) are calcified also. Diatoms occur as well. In the case of high carbonate content of the lake water, microbial activity may occur and produce stromatolites, thrombolites, and/or oncolites (Figure 5).

Saline lakes in arid climates such as the Great Salt Lake in North America or the Dead Sea have considerable sizes of 4,400 and 600 km², respectively. Saline lakes are often ephemeral. They are also characterized by abundant carbonate deposition. Controlled by the Mg/Ca-ratio, either low-magnesium calcite, high-magnesium calcite, aragonite, magnesite (MgCO₃), or dolomite is precipitated. Under high evaporation rates, sulphates and chlorides such as gypsum (CaSO₄ · 2H₂O) and halite (NaCl) may form. In the case of the Great Salt Lake, aragonitic ooids occur in abundance in shallow areas along shore. Also, microbial buildups and sheets are found, which are to large parts due to cyanobacterial activity. Along shore of saline lakes, caliche may be common due to fluctuating lake levels that expose larger areas. When springs are present, tufa or travertine is formed as sheets, mounds, and towers. The latter is characteristic of Mono Lake in California where spectacular towers of tufa are found.

Subaerial exposure

Subaerial exposure of limestone leads to dissolution by meteoric waters. Typical landforms that may have considerable relief are termed karst (James and Choquette, 1988). Landforms include dolines, towers, karren, or poljes. Dissolution may produce cavities and larger caves. Calcium carbonate is re-precipitated in limestone caves in the form of speleothems such as stalactites and stalagmites. Cave pearls or pisoids (“diagenetic ooids”) may form in ponds. Residues of dissolution together with organic material may later become soils on limestone such as laterite or terra rossa. In warm and/or arid regions, carbonate-rich soils form deposits that are called caliche or calcrete (Wright and Tucker, 1991). A caliche is characterized by patterns such as brecciation, clotted textures, root holes or rhizoids, and low-magnesium calcite cements, sometimes in a needle fiber texture (“whiskers”). Laminated soilstone crusts are a special case of calcrete that can resemble stromatolitic formations. Soilstone crusts form by precipitation of calcium carbonate from evaporating pore water. The identification of subaerial exposure surfaces in the fossil record is of great importance, e.g., for the reconstruction of fluctuations in sea level and for sequence stratigraphy (Figures 6 and 7).

Transitional environments

Beaches and shores

Carbonate beaches are usually formed by sand-sized particles that originate from the adjacent shallow seabed (Inden and Moore, 1983). Shells and skeletons of organisms as well as non-skeletal grains occur. Beach zones include the shoreface, the foreshore, and the backshore. The shoreface lies below the lower tide level and is characterized by cross-bedding. The foreshore is situated between

![Carbonate Environments, Figure 4](Image)
low- and high-tide levels. The backshore lies above the high-tide level. Sediments of the foreshore and backshore zones exhibit parallel lamination and beds dip gently toward the sea (Figure 8).

A striking feature in the tropical/subtropical realm, and common in latitudes up to 60°, is beachrock. Beachrock forms in the intertidal zone by rapid cementation of sedimentary particles by marine aragonite and high-magnesium calcite cements. The formation of beachrock is not fully understood, and is assigned to physicochemical and/or biological processes (Gischler, 2007). The strictly intertidal position of beachrock supports the contention that cements precipitate from evaporating pore water during low tide. Also, beachrock appears to be common in windward and rather exposed sites indicating that flushing of pores by water rich in calcium carbonate is of importance. Typical sediment structures of beachrock are seaward dipping beds. Keystone vugs are small cavities in beaches and beachrock that are produced when air escapes from the beach (Figure 9).

Behind the backshore, in the landward direction, dunes may occur that are a product of eolian deposition (Abegg et al., 2001). Carbonate eolianites are common behind carbonate beaches, and are found in abundance along the eastern coast of the Yucatan Peninsula, on the islands of the Bahamas and Bermuda, along the Trucial Coast (southern Persian Gulf), and on the Balearic Islands in the Mediterranean Sea. High angle cross-bedding as well as rhizoids and caliche, indications of the meteoric realm, are characteristic. Microcodium are small spherical bodies
of radiating calcite that forms among rhizoids in eolianites. Carbonate cements include meteoric types composed of low-magnesium calcite.

Tidal flats
As indicated by the name, tidal flats are broad and low-relief areas, which are usually flooded during high tide and subaerially exposed during low tide (Ginsburg, 1975). Classic modern carbonate-dominated examples include the humid tidal flats west of Andros Island on Great Bahama Bank (Shinn et al., 1969) and the arid examples on the southwestern side of the Persian Gulf (Kendall and Skipwith, 1969). Tidal flats are dissected by tidal channels, which are lined by levees. Levees usually enclose low-relief ponds. Beach ridges form the transition to the adjacent subtidal realm. In the case of humid tidal flats, algae such as Batophora and microbial mats as well as mangroves thrive along the channels and further inland in the so-called marsh. In the Bahamas, microbial mats are largely formed by the genera Scytonema and Schizothrix. Gastropods of the genus Batillaria occur in great abundance. Benthic foraminifera are present. In arid tidal flats or sabkhas, which transition into the desert on the landward side, microbial mats occur along channels. Also, burrows of crabs may be abundant along the channels. Nebkhas are small mounds that are produced by halophyte plants that baffle sediment around their roots (Figure 10).

Sediments on tidal flats are usually poorly sorted muds, silts, and sands. They are horizontally layered and are largely derived during storms from the adjacent seafloor, from precipitation, or from eolian transport. Fine-grained sediment is usually found in ponds. Mud cracks and wrinkled, dried-out, and curled microbial mats are a typical phenomenon. Sands make up levees and beach ridges. A characteristic sedimentary structure is the bird’s eyes structure, millimeter-sized fenestral pores that result from gas-bubble formation. In the fossil record, these structures are good indicators of the intertidal and shallow subtidal realm. As a consequence of evaporation on tidal flats, cemented carbonate crusts are formed that may contain dolomite. In the arid realm, gypsum and/or anhydrite is precipitated within the sediment and forms nodules, enterolithid folds, discs, and chicken-wire textures (Figure 11).

Marine environments

Carbonate shelves and platforms (Figure 12)

Restricted shelves and platforms
These carbonate environments are shallow (0–20 m) and characterized by partial or complete enclosure by islands, reefs, or shoals. Modern examples include Florida Bay (Ginsburg, 1956; Stockman et al., 1967), the Gulf of Batabano (Hoskins, 1964), Cuba, or Shark Bay, western Australia (Logan et al., 1974). The partial or complete enclosure of these platforms results in restricted circulation and in elevated water temperature and salinity. This in turn leads to low diversity biota of mollusks, benthic foraminifera, crustaceans, and echinoderms. Burrowing crustaceans (Callianassa) are responsible for abundant bioturbation (Figure 13). Tolerant corals such as Solenastrea and Siderastrea are occasionally found in Florida Bay. Sea grasses (Thalassia, Syringodium, Posidonia, and Cymodocea) are common, which act as bafflers of sediment. In Florida Bay, the codiacean algae Penicillus and Halimeda occur in great abundance. Dasycladacean algae such as Acetabularia are common. In Shark Bay, stromatolites thrive in intertidal and subtidal areas. Their abundance is classically explained by the exclusion of grazers due to the high salinities in the bay.

Sediments in Florida Bay are largely of skeletal origin. Dominant carbonate producers are mollusks, foraminifera, and the alga Penicillus, which is the most important source of aragonite mud (Stockman et al., 1967). Mud banks, elongated hillock-type accumulations of mud, are densely covered by sea grass (Bosence, 1995). Both skeletal (mollusk, foraminifera) and non-skeletal (peloidal) grain types are found in the Gulf of Batabano. Likewise, Shark Bay sediments include skeletal grains of mollusks and foraminifera as well as mud from the breakdown of Penicillus. Non-skeletal grains include ooids that are formed in shallow areas, peloids, and reworked lithoclasts from underlying Pleistocene limestone (Figures 14–16).

Open-rimmed shelves and platforms
Intensively studied examples of rimmed carbonate shelves and platforms include the Florida Reef Tract, the Belize shelf and barrier reef, Central America, and the Queensland shelf and Great Barrier Reef, NE Australia. These environments are largely characterized by normal marine
conditions. Water depths range from 0 to 200 m. The areas covered are several 1,000 km² to several 100,000 km² in the case of the Queensland shelf. In between the coast and platform margins, low and high islands as well as patch reefs and faroes (small circular lagoon reefs) of coral can be found.

Carbonate sediments on these rimmed platforms are almost exclusively of skeletal origin and facies belts more or less parallel to the coast (Ginsburg, 1956; Maxwell, 1968; Purdy and Gischler, 2003; Purdy et al., 2003). On the Belize and Queensland shelves, siliciclastics derived from the hinterland mix with carbonate sediment. As
a consequence, facies are siliciclastics-dominated near the coast. Marls are found in the middle shelf. Largely carbonate sediments occur at the outer platform and platform margins. Central shelf marls are rich in fragments of mollusks and foraminifera. Mud content is high. In Belize and NE Australia, pelagic biota such as pteropods and planktonic foraminifera, respectively, are found in these platform areas due to the high depths of the shelves. Shelf reefs and outer platforms and margins are predominated by mostly coarse-grained sediments rich in coral, coralline red algae, Halimeda, and benthic foraminifera (Figure 17).

Platform margins are several kilometers wide and water depths are between 0 and 50 m. Coral reefs are widespread with the common genera Acropora, Porites, Montastraea, Diploria, Platygrya, Pocillopora, and Millepora. The reef crest proper is at sea level. The shallow forereef slope is often characterized by spur-and-groove systems (Shinn et al., 1981) (Figure 18). In the case of Belize, a steep sometimes vertical slope occurs in the deep forereef. This bypass margin was investigated in detail by James and Ginsburg (1979). The Florida and Queensland margins with lower angles are examples of depositional margins (Read, 1985).

Isolated platforms

The largest known isolated carbonate platforms include the Bahamas in the Atlantic and Great Chagos Bank in the Indian Ocean, which each cover 10,000 to 100,000 km², respectively. In the Bahamas archipelago, Great Bahama Bank (Purdy, 1963; Ginsburg, 2001) is probably the best known example (Figure 19). Great Bahama Bank is a shallow, low-relief submarine bank, which is surrounded by deep water. Water depths on the bank only rarely exceed 6 m. The bank has very high and steep bypass margins, especially on the eastern side. The steepness is presumably the result of both biological construction and erosion. On the eastern side, a well-developed coral reef rim with abundant Acropora exists in shallow water. Otherwise, the margins of Great Bahama Bank are largely predominated by ooid sand shoals. These shoals are very shallow, high-energy environments where ooids form and are moving in the form of up to several kilometer-long rippled sand waves, more or less parallel to the bank margins. Joulters Cay ooid shoal in the northeastern corner of the bank is intensively studied (Harris, 1979). The western bank margin also has long and wide ooid shoals. Near the southern end of Tongue of the Ocean, ooid sand waves are oriented perpendicular to the bank margin. The eastern interior part of Great Bahama Bank is covered by the Pleistocene island of Andros, which gets as high as 30 m above present sea level. Most of the platform interior is covered by shallow water as mentioned above. Benthic marine animals such as gastropods (Strombus) and echinoids (Oreaster) are rather rare. Codiacian algae such as Penicillus and sea grass (Thalassia) are common. Salinities are above normal marine on the bank and may reach 45% close to Andros Island. Sediments are mostly non-skeletal and include peloidal muds as well as aggregate grain (“grapestone”) and peloidal sands (Purdy, 1963). Skeletal sands only occur around the eastern reefal margin and in a small band on the western margin.

The origin of carbonate mud on Great Bahama Bank is a matter of debate since several decades [see discussion in Carbonate Environments, Figure 9 Tropical beach with beachrock outcrop. Loggerhead Key, Dry Tortugas, South Florida.]
Gischler and Zingeler (2002). Mud largely consists of micron-sized aragonite needles. It potentially originates from codiacean algae such as *Penicillus*, from physico-chemical precipitation in the warm and saline surface waters of the bank, or from biologically (bacterially) induced precipitation of calcium carbonate. Commonly occurring clouds of suspensions of carbonate mud and water on the bank, known as “whitings,” have been described as expressions of spontaneous precipitation phenomena. However, there is also evidence that whitings are simply a consequence of bottom sediment stirring either by fish or by currents.

More than 2 m high subtidal stromatolites were discovered by Dill et al. (1986) on the Bahamas in normal marine seawater near the Exuma islands. Previously, only small microbial buildups were known from the Bahamas. These stromatolites develop within very strong currents and significant bottom sediment movement. Apparently sediment movement largely excludes grazers and triggers carbonate accumulation via trapping and binding.

Seismic profiling and deep drilling has revealed that the geologic history of Great Bahama Bank was not simply a process of aggradation (Ginsburg, 2001). A complex interplay of slow subsidence and sea-level change has
modified the bank during the Neogene in that shallow water banks repeatedly aggraded, backstepped, and prograded, thereby filling deep seaways and moving the western margin of Great Bahama Bank by 30 km to the west into the Straits of Florida.

Carbonate ramps
The term ramp was introduced to the carbonate literature for unrimmed carbonate shelves with a low-angle dip (<1°) toward the open sea (Burchette and Wright, 1992; Wright and Burchette, 1998). The classical modern example is the southwestern Persian Gulf. Other modern ramp examples include the western Florida margin or Campeche Bank, Mexico. Geometrically, there are homoclinal and distally steepened ramps (Figure 20). Depositional environments are usually divided into inner, middle, and outer ramps. The inner ramp environment is above fair-weather wave base (FWWB); the mid-ramp between FWWB, and the storm wave base (SWB); the outer ramp below SWB. In the southern Gulf, inner ramp environments consist of shoals and barrier facies with ooid-skeletal sands. Smaller reefs are found just seaward of the barriers. Back barrier sediments are usually fine-grained peloidal and mollusk muds and sands. Mollusk-rich silts and sands are common on the mid-ramp. Outer ramps are characterized by carbonate and argillaceous muds (“marl”) with abundant mollusks. In the northern Gulf, barriers are largely lacking. In contrast to the southern Gulf, which is tide-dominated, the northern Persian Gulf ramp is largely wave-dominated. On the inner ramp, quartz grains derived from the adjacent mainland desert may be present in addition to ooid shoals. Small coral patch reefs may also be found in the inner ramp zone. Mid-ramp and outer ramp deposits are also dominated by mollusk-rich silts and sand and argillaceous muds, respectively. Pinnacle-type patch reefs of coral occur in the outer ramp.
Characteristic storm beds or tempestites in mid-ramp settings were identified by Aigner (1982) in the European Triassic Muschelkalk beds. Tempestites exhibit characteristics such as sharp bases, sole marks, graded bedding, hummocky cross-stratification, and ripple marks. Because there are no larger outcrops in modern ramps and shelves showing complete sedimentary bodies or large-scale sedimentary structures, our knowledge of storm beds is largely based on the fossil record.

**Temperate- and cool-water shelves and platforms**

Apart from the abundant typical warm water and low latitude deposits, about one third of the modern carbonate shelves and platforms are characterized by temperate and cool-water carbonate sedimentation (Lees and Buller, 1972; James and Clarke, 1997). Prominent and well-studied modern examples are the Great Australian Bight or the Mediterranean realm. Temperatures in the cool-water realm are below 20°C and heterotrophic carbonate producers predominate. They include mollusks, echinoderms, bryozoa, foraminifera, coralline algae, barnacles, and serpulids ("heterozoan association"). Constituent carbonate particles of the same taxa are also present in warm-water carbonates; however, there, they are swamped by carbonate grains from rapidly growing producers that have photosymbionts such as corals and codiacean algae as well as by mud and non-skeletal carbonate formation as in ooids, peloids, and cements ("photozoan association"). There are also cases where heterozoan carbonates occur in warm-water settings, e.g., in western Florida or to some degree on Campeche Bank, Mexico, as well as in several fossil examples. Elevated nutrient contents and primary production are usually responsible, and hamper abundant photozoan carbonate production (Figure 21).

Temperate and cool-water carbonates usually form unrimmed shelves or platforms, and they are strongly influenced by hydrodynamic energy. Submarine cementation is rather weak. In modern examples, kelp (brown algae) forests occur in the constantly wave-dominated inner ramp. Siliciclastic sediment may derive from local sources from the mainland. Sand waves and dunes are found on the mid-ramp, which is prone to storm-reworking. Sediments on inner and mid-ramps are mostly coarse-grained and frequently redeposited. The outer ramp environment may contain fine-grained sediment with bryozoan, sponge, or non-zooxanthellate coral mounds.

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**Carbonate Environments, Figure 12** Scheme of carbonate platform types and morphologies. (Modified from Tucker and Wright, 1990.)

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**Carbonate Environments, Figure 13** Underwater photograph of *Callianassa* mounds, Florida Bay.
Reefs
Reefs are classical objects of geobiological research because they are rock formations built by the biological activity of organisms. Reefs normally form topographic highs above the surrounding sea floor. Reef organisms comprise large numbers of taxa, which either construct a framework or baffle and bind sediment, which is rapidly consolidated. In addition, there are reef dwellers, which do not necessarily contribute to reef growth, and, there are reef-destroying organisms, which bore into, dissolve, or

**Carbonate Environments, Figure 14** Sediment distribution in Florida Bay and the reef tract. (Modified from Ginsburg, 1956.)

**Carbonate Environments, Figure 15** SEM photo of carbonate mud from Belize with abundant aragonite needles and nanograins, which originate from codiacean algae.
erode reef limestone. This ecological guild concept of constructors, bafflers, binders, dwellers, and destroyers was developed for reef organisms by Fagerstrom (1987), who compiled a geological history of reef building. Like Wilson (1975) before, he stressed that reef building is a geologically old phenomenon that reaches back into the Archaean, however, reef-building organisms have repeatedly changed with time. Recently, Wood (1999), Stanley (2001), and Kiessling et al. (2002) have provided additional monographs and editions on the long history of reef building. The most abundant groups of reef builders during earth history have been microbes, corals, sponges, bryozoa, and rudist bivalves. Reef destroyers include boring sponges, bivalves, worms, and microborers as well as grazing gastropods, echinoderms, and fish. Reef building is a complex interplay of reef construction, destruction, sedimentation, and cementation, processes which occur at the same time and eventually produce what geoscientists would term reef limestone (Scoffin, 1992) (Figure 22).

Tropical reefs
Modern tropical reefs cover an area of about 600,000 km² in tropical-subtropical latitudes around the globe. Reefs thrive in warm waters between 18 and 30°C, normal marine salinity, and clear and oligotrophic waters. They represent the most diverse marine ecosystem with several tens of thousand of species including taxa from all known animal phyla. Scleractinian corals and red coralline algae are the dominant builders (Figure 23). Other calcareous

**Carbonate Environments, Figure 16** Underwater photographs of calcareous codiacean algae (a) Penicillus and (b) Halimeda, Belize.
Holocene marine facies

Sample Location

January July

Sand
Sandy Marl
Thin-shelled Bivalve Marl Wackestone/Mudstone
Halimeda-Thin-shelled Bivalve Wackestone/Mudstone

>10 % Miliolid Line

Honduras

Guatemala

Mexico

Belize

Landscape Terrain

Quaternary
Topographic Contours in feet

Carbonate

Non-Carbonate

Organic-rich Halimeda & Mollusc Wackestone
Peloidal-Skeletal Wackestone/Packstone
Micritized Grain Wackestone/Packstone
Peneroplid Packstone/Wackestone
Mollusc-Foram Wackestone
Halimeda Packstone/Wackestone
Coralgal Framestone/Grainstone/Packstone

Terrigenous

Transitional

Carbonate Environments, Figure 17  Carbonate sediment distribution on the Belize shelf and platforms. (With permission of Purdy and Gischler, 2003.)
algae such as codiaceans, mollusks, benthic foraminifera, and echinoderms are important sediment producers on tropical reefs. Reef-building Scleractinian corals live in symbiosis with dinoflagellates ("zooxanthellae"), which live in their tissues and photosynthesize. The symbionts are both important for the coral metabolism by providing food and also enhance skeletal growth of the aragonite coral skeleton because they consume carbon dioxide.

\[
\text{CO}_2 + \text{H}_2\text{O} \rightarrow [\text{CH}_2\text{O}]_n + \text{O}_2
\]  

(2)

Because of the symbiosis, the tops of tropical reefs are at or close to sea level. Fossil coral reefs are therefore used as gauges for past sea levels. The annual banding of coral skeletons and the longevity of colonial reef corals makes them ideal archives of historical climate change ("sclerochronology").

Since Darwin (1842), tropical reefs are morphologically subdivided into fringing reefs, barrier reefs, and atolls. Fringing reefs occur close to the coast with a shallow moat or lagoon in between reef and coastline. These reefs are abundant in the Caribbean Sea and the Indonesian archipelago. Barrier reefs are larger structures that are separated from the coast by a deep channel, like in northeastern Australia, New Caledonia, or Belize. The Great Barrier Reef is the longest barrier reef with more than 2,000 km in length. Barrier reefs in the sense of Darwin surround volcanic islands like, e.g., Bora Bora in the Tuamotu archipelago. Atolls are circular reefs, which enclose a deep lagoon. There are about 400 atolls worldwide. They are most common in the Pacific and Indian Oceans, and have a volcanic basement. According to Darwin’s (1842) elegant subsidence theory, fringing reef, barrier reef, and atoll are genetically connected by the simple process of subsidence of a volcanic island. Tayama (1952) carried the theory even further and explained the formation of small circular reefs with shallow or filled lagoons, so-called table reefs, as the final stage in reef development before drowning and seamount (guyot) formation. Even so, the subsidence theory does not explain large barrier reefs that are attached to large continental land masses, and, it neglects the significance of eustatic sea-level change, which was first considered in Daly’s (1910) glacial control theory. Indeed, drilling on Enewetak Atoll in the 1950s has demonstrated about 1.3 km of shallow-water reef limestone on top of an Eocene volcano, but numerous hiatuses were found, which are the result of repeated subaerial exposure. The antecedent karst model of Purdy (1974) takes into account the importance of limestone dissolution by meteoric waters during eustatic lowstands of sea level, which are even able to produce fringing, barrier, and atoll reef morphologies as well as spur-and-groove patterns in subaerially exposed limestone and limestone-volcanic islands. Recent studies have shown that modern barrier reefs are comparably young features that only came into existence about 400–600 ky ago when high amplitude, eccentricity-driven eustatic sea-level changes became more and more dominant (International Consortium of Great Barrier Reef Drilling, 2001; Multer et al., 2002).

Tropical reefs are characterized by ecological zonation, with zones running more or less parallel to bathymetry (see Figure 18). Modern reefs also exhibit provincialism between Atlantic and Indo-Pacific realms. For high sea-level greenhouse climates in earth history, like in the Devonian or Jurassic and Cretaceous, however, no evidence of provincialism was found. Common reef zones include from outboard to inboard the fore reef, reef crest, back reef, sand apron, and lagoon. Surface sediments are coarse sands of coral and calcareous algae in reefal environments and more fine-grained, mollusk- and foraminifer-rich sediments in lagoonal areas. Several shallow lagoons of Caribbean reefs are rich in non-skeletal grains. Milliman (1974) explained this difference to Indo-Pacific reefs, which he termed the “ooid problem,” by the shallower and smaller lagoons, in which water exchange is much faster. Carbonate-rich water circulates more rapidly and thereby preferentially produces non-skeletal grains such as ooids, peloids, and aggregates by submarine cementation (Figure 24). However, the discovery of abundant modern non-skeletal grains such as ooids and cemented fecal pellets in the shallow lagoons of Aitutaki Atoll (Cook Islands) and Bora Bora (Tuamotus) in the south Pacific opposes this widely held concept (Rankey and Reeder 2009; Gischler 2010).

Drilling in the barrier reef around the island of Tahiti (Montaggioni and Camoin, 1993) and investigations in cavities of the Great Barrier Reef (Reitner, 1993) have shown the importance of abundant microbial activity in
tropical coral reef cavities. Especially in Tahiti,stromatolite-type formations are quite abundant, even though microbial activity was apparently most widespread during the early Holocene. In the Great Barrier Reef example, sponges and sponge tissue decay are additional agents besides microbes that trigger carbonate formation.

**Deep- and cold-water reefs and mounds**

After the first discovery of abundant coral reefs in deep and cold water off the Scandinavian coast (Teichert, 1958), it took several decades before these reef structures were investigated in great detail (Freiwald and Roberts, 2005; Roberts et al., 2006). These reefs are largely built by non-zooxanthellate branched corals such as *Lophelia*, *Madrepora*, or *Cladocora* in water depths below 200 m and temperatures between 4 and 2°C. Reef thickness may reach more than 30 m and individual reef structures have lengths of more than 10 km. Species richness in the north Atlantic examples is around 1,300 taxa. Deep-water reef foundations are usually submarine topographic highs such as, e.g., iceberg plough marks. Meanwhile, it has become clear that deep- and cold-water reefs in the north-eastern Atlantic are not restricted to the Scandinavian area but are also abundant off the Atlantic shelf margin as far south as the Mediterranean and in the Caribbean.

Somewhat similar deep- and cold-water reefs, termed lithoherms, were discovered in strong currents at the bottom of the Straits of Florida in 600–700 m of water (Neumann et al., 1977). Lithoherms are several 100 m long and up to 50 m high. Their shape is ellipsoidal with the long axis parallel to the current. The faunal elements mainly include non-zooxanthellate corals, crinoids, sponges, and bryozoans. Interstitial muddy to sandy detrital carbonate sediment is abundant and largely a product of baffling. The mounds are cemented at the surface in that concentric, hard carbonate crusts occur. Stromatactis-type fenestral
cavities were discovered and attributed largely to burrowing and subsequent sediment infill.

It is tempting to interpret the Florida lithoherms as modern analogues of fossil deep-water mud mounds. Mud mounds are common in the fossil record, especially in the Devonian and Carboniferous (Monty et al., 1995). The most prominent examples are probably the Carboniferous ("Waulsortian") mud mounds. Waulsortian mounds or banks also formed in deep water and contain biota with crinoids, sponges, bryozoans, brachiopods, and mollusks. Corals are rare. Stromatactis, large cement and sediment-filled fenestral cavities with flat bases and irregular tops, is very common in Paleozoic mud mounds (Krause et al., 2004). Even so, major differences between
the lithoherms and the Waulsortian mud mounds appear to be the abundance of carbonate mud in the fossil examples. Lithoherms have higher quantities of coarse sediment. Also, lithoherms are largely biodetrital mounds whereas the Waulsortian ones are microbial mounds in which in situ mud production prevailed (Monty et al., 1995).

Also, mud mounds and deep-water carbonate mounds have been found to be associated with cold seeps or hot vents (Beauchamp and von Bitter, 1992; Mounji et al., 1998). Around locations of seepage of methane (CH₄) and hydrogen sulfide (H₂S) on the seafloor, authigenic carbonate precipitation may be triggered by bacteria via chemosynthetic processes that produce bicarbonate:

\[
\begin{align*}
\text{CH}_4 + \text{SO}_4^{2-} & \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O} \quad (3) \\
\text{SO}_4^{2-} + 2(\text{CH}_2\text{O})_n & \rightarrow 2\text{HCO}_3^- + \text{H}_2\text{S} \quad (4)
\end{align*}
\]

Usually, metazoans such as certain bivalve mollusks (Calyptogena, Bathymodiolus, lucinids), vestimentiferan worms (Riftia), and thyasirid shrimps, which have

**Carbonate Environments, Figure 22** (a) Scheme of reef limestone formation with synchronous processes. (From Scoffin, 1992; with permission of Springer: Berlin, Heidelberg.) (b) Quaternary reef limestone from Belize Barrier Reef. C coral; M microbialite, D detritus.
chemosynthetic bacteria in their gills and tissue thrive at these seeps. Interestingly, fossil seeps often have abundant brachiopods (dimerelloids, e.g., *Peregrinella*), which do not have modern representatives that harbor chemosynthetic bacteria (Campbell and Bottjer, 1995). Meanwhile, fossil seep carbonates have been reported from rocks as old as Silurian (Little et al., 1997). Apart from their characteristic fauna and texture, and their patchy distribution, methane seep carbonates may be identified rather easily in the fossil record by geochemical means. Carbon isotopes of these carbonates are typically very strongly depleted (Beauchamp and von Bitter, 1992).

**Slope**

Carbonate slopes may have lower angles as in depositional margins or higher angles in bypass margins. In general, carbonate and siliciclastic slopes have similar heights, however, carbonate slopes are usually steeper (Schlager, 2005). James and Ginsburg (1979) have compiled slope morphologies of several western Atlantic carbonate margins, which usually exhibit a threefold division (Figure 25). There is a low-angle (20–30°) fore reef slope, a steep slope to vertical wall between ca. 50–150 m and a 50° slope below the wall, which is dominated by carbonate debris and blocks transported down from shallower depths. Foliaceous corals, *Halimeda*, crinoids, and sponges may be found colonizing the walls. In general, the slope is characterized by sediment redeposition as seen, e.g., in the occurrence of talus blocks, debris-flows, and slump deposits, as well as breccias (James and Ginsburg, 1979; Schlager, 2005). Limestone turbidites (Meischner, 1964) originating from platform tops occur on lower slopes and in the adjacent
Carbonate Environments, Figure 25  (a) Schemes of fore reef slopes. (Modified from James and Ginsburg, 1979.)  (b) Sedimentary processes on slopes. (From Enos and Moore, 1983; with permission of American Association of Petroleum Geologists.)
toe-of-slope among pelitic deposits. Limestone turbidites have similar characteristics as siliciclastic turbidites, with sharp bases, graded beds of debris, and lenticular geometries of individual turbidite bodies. Highstand shedding describes the fact that limestone turbidites in the Quaternary are more common during sea-level highstands when platforms are flooded and abundant carbonate produced as opposed to lowstands of sea level when platforms are subaerially exposed (Droxler and Schlager, 1985). Recent investigations in fossil slope deposits have revealed the formation of so-called auto-micrites as a result of microbial activity on carbonate slopes (Keim and Schlager, 2001). Automicrite facies is apparently a significant agent for platform margin and slope stabilization in these Triassic examples.

**Basin**

Basinal carbonate environments are in depths between about 200 and 5,000 m (Hsü and Jenkyns, 1974; Crevello and Harris, 1985). The basins of the Atlantic, Indian Ocean, and large parts of southern Pacific Ocean are covered by very fine-grained carbonate sediments, which are termed oozes. Consolidated oozes are called chalk. Carbonate deposition and accumulation is controlled by carbonate supply from the pelagic realm and largely includes tests of planktonic foraminifera, pteropod gastropods, and coccoliths (Figure 26). These particles are transported to the sea floor as “marine snow,” which is largely composed of fecal pellet aggregates. Fecal pellets derive from arthropods such as krill and copepods, and sinking velocities of pellets are much faster as compared to those of individual tests. Other minor contributors of calcium carbonate to the deep sea include shells of ostracods, shells of pseudoplanktonic organisms such as arthropods and bivalve mollusks, and cephalopod shells from *Nautilus*, *Sepia*, and *Spirula*. Carbonate accumulation is also dependent upon the position of the aragonite and calcite compensation depths (ACD, CCD), respectively. The ACD lies at about 2,000–2,500 m and the CCD at about 5,000–5,500 m depth. Below the CCD all calcium carbonate is dissolved. Biota in basinal environments include benthic foraminifera, echinoderms, sponges, and crustaceans. Ichnospecies are *Planolites*, *Chondrites*, and *Zoophycos*. Thallassinoides-type bioturbation, often filled by chert, is typical in Cretaceous deep-water limestone. Except for fine lamination, sedimentary structures are usually rare. In Quaternary deposits, carbonate cycles were discovered, which are caused by changing carbonate saturation states during glacial and interglacials. During glacial periods, lower sea-level positions result in higher carbonate saturation in the pelagic realm, because the neritic carbonate factory is largely shut off. During high sea levels, carbonate production is more or less equally distributed between the neritic and pelagic realm, leading to lower carbonate content in basinal deposits. The genesis of deep-water carbonates during the Paleozoic is somewhat enigmatic, because calcareous plankton only evolved in the Mesozoic. Potential Paleozoic contributors include cephalopods, ostracods, tentaculites, calcispheres, and possibly very fine-grained detritus from the neritic realm. Cephalopod limestone is a typical Paleozoic deposit of shallow basins or deeper submarine rises with no modern counterpart (Wendt and Aigner, 1985).

**Summary**

Carbonate depositional environments were described in an idealized cross section from terrestrial to transitional, shallow marine, and to deep marine realms. Environments discussed include springs, lakes, subaerial exposure horizons, beaches and shores, tidal flats, restricted, open-rimmed, isolated shelves and platforms, ramps, tropical and cold deep-water reefs and mounds, slopes, and deep sea basins. The great majority of carbonates forms in the
marine realm, and carbonate formation is largely a product of biological activity. Biological control is either direct, e.g., during growth of shells and skeletons of invertebrates such as corals, mollusks, brachiopods, echinoderms, and sponges, tests of foraminifera or carbonate particles produced by calcareous algae or coccolithophorids. Indirect biological control relates to metabolic processes, e.g., photosynthesis of microbes, which triggers carbonate precipitation. Abiotic formation of carbonates is the exception and includes the formation of some carbonate muds, cements, certain dolomites, and non-skeletal grains such as grapestones, lumps, or ooids. Elevated temperature and salinity, high concentrations of calcium, magnesium, and carbonate ions, low pressure, and CO₂-removal are major environmental parameters that potentially trigger abiotic carbonate formation.

Bibliography


Cross-references
Algae (Eukaryotic)
Alkalinity
Anaerobic Oxidation of Methane with Sulfate
Bioerosion
calcified Cyanobacteria
Calcite Precipitation, Microbially Induced
Calcium Biogeochemistry
Carbonates
Cold Seeps
Cyanobacteria
Dolomite, Microbial
Foraminifera
Hydrothermal Environments, Marine
Karst Ecosystems
Microbial Biominalization
Microbial Degradation
Microbial Ecology of Submarine Caves
Microbialites, Modern
Microbialites, Stromatolites, and Thrombolites
Mud Mounds
Reefs
Saline Lakes
Sediment Diagenesis – Biologically Controlled
Sinter
Soda Ocean Hypothesis
Sponges (Porifera) and Sponge Microbes
Stromatactis
Stromatolites
Symbiosis
Thrombolites
Tidal Flats
Tufa, Freshwater
Waulsortian Mud Mounds

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Synonyms
Carbonate minerals

Definition
Carbonates. The term carbonates comprises solid phases such as minerals and amorphous substances, which basically consist of cations and triangular carbonate groups.

Introduction
The most common carbonate minerals in natural surroundings are calcite (CaCO₃) and dolomite (CaMg(CO₃)₂). These minerals comprise about 90 wt.% of carbonates within the Earth’s crust and 10–15 wt.% of sedimentary rocks. The proportion of dolomite to calcite increases with decreasing geological age, representing the potential for transformation of calcium carbonate to dolomite at elevated temperature and high molar Mg²⁺ to Ca²⁺ ratio during geological history. Additional carbonate minerals are numerous, but occur in rather special natural surroundings.

The occurrence and formation of carbonate minerals is mostly related to the precipitation from aqueous solutions and to a lesser extent to the crystallization from carbonate melts. In aquatic systems carbonate formation may be inorganically or biogenically induced (e.g., Morse and Mackenzie, 1990; Dove et al., 2003; Morse et al., 2007). Inorganic precipitation occurs from supersaturated solutions to form evaporites in marine and terrestrial environments, ooids in shallow water, saline lake deposits, speleothems in caves, sinter from spring water, hydrothermal ores, etc. Biogenic precipitation is mostly related to carbonates as skeletal parts and in tissues of marine and non-marine organisms such as foraminifera, coccoliths, corals, bivalves, and prokaryotes. The main mass proportion of carbonates is built from biogenic activity, whereas less arises from inorganic origin.

Once carbonate particles are formed, sedimentation may yield shelf deposits of carbonate sand or mud, deep sea ooze, or mud in lakes. Compaction, cementation, and diagenetic reactions result in lithification to limestone or calcareous marl and sandstone. Under elevated temperatures and pressures, the primary limestone may be transformed to, for example, dolomite rocks or marble.

Crystal structure and chemical composition
The basic units of carbonate minerals are cations and the triangular carbonate groups (CO₃²⁻). The crystal structure of carbonate minerals is strongly related to the ionic radius of the cation versus the oxygen ion (e.g., Reeder, 1990). As the Ca:O ionic radius ratio in CaCO₃ is close to the upper limit for 6-coordination, CaCO₃ can occur in 6- and 9-coordination of Ca to O yielding calcite and aragonite, respectively. Accordingly, anhydrous carbonate minerals of smaller and larger cations compared to Ca²⁺ crystallize isostructurally to calcite and aragonite, respectively: (1) In the calcite type (trigonal carbonates), the carbonate group lies in planes at right angles to the threefold c-axis and the metal ions in alternate planes with a sixfold coordination of metal ions to oxygen ions (Figure 1). Metal ions of anhydrous carbonates of the calcite type are, e.g., Fe²⁺, Mg²⁺, Mn²⁺, Cd²⁺, and Zn²⁺ (small cations; see minerals in Table 1). (2) In the aragonite type (orthorhombic carbonates), a ninefold coordination of the cation to oxygen ions exists. Respective metal ions of anhydrous carbonates are, e.g., Ba²⁺, Sr²⁺, and Pb²⁺ as well as rare earth elements such as Sm, Eu, and Yb (large cations; Table 1).

Beside calcite and aragonite, the third CaCO₃ polymorph vaterite (hexagonal; see Figure 2) and amorphous calcium carbonate phases (ACC) can occur. Hydrous CaCO₃ minerals comprise amorphous CaCO₃·H₂O, monohydrocalciumcarbonate (CaCO₃·H₂O), and ikaite (CaCO₃·6H₂O). In analogy to calcium, a set of hydrous magnesium carbonates appears: Nesquehonite (MgCO₃·3H₂O), landsfordite (MgCO₃·5H₂O), and hydromagnesite (Mg₅(OH)₂(CO₃)₄·4H₂O).
Ordered double carbonate minerals are dolomite, ankerite (CaFe(CO₃)₂), kutnahorite (CaMn(CO₃)₂), huntite (Mg₃Ca(CO₃)₂), and shortite (Na₂Ca₂(CO₃)₃). In the structure of dolomite, distinct Mg²⁺ and Ca²⁺ layers alternate with CO₃²⁻ layers, whereas in the disordered protodolomite (MgCa(CO₃)₂) Mg²⁺ and Ca²⁺ are randomly distributed in the cation layer, as in Mg calcite. Carbonate minerals may contain hydroxide ions, like the copper carbonates malachite (Cu₂(OH)(CO₃)) and azurite (Cu₃(OH)(CO₃)₂), where the respective formation is sensitive to the CO₂ partial pressure. Typical alkali carbonate minerals are natron (Na₂CO₃·10H₂O), nahcolite (NaHCO₃), trona (Na₃(CO₃)(HCO₃)·2H₂O), and kalicinite (KHCO₃).

**Solubility and stability**

The solubility of carbonate minerals is expressed by the solubility product, \( K_S = (Me^{2+}) \cdot (CO_{3}^{2-}) \) for divalent cations. Parentheses denote the activities of aqueous species at thermodynamic equilibrium. In aqueous environments the solubility of minerals to each other is given by the individual values of the solubility product. At standard condition, aragonite and vaterite are more soluble than calcite (lowest \( K_S \)-value for calcite in Table 1). Accordingly, calcite is the thermodynamically stable CaCO₃ polymorph. At higher pressure, the more dense phase aragonite is the stable polymorph, although aragonite has a larger cation site due to 9-coordination of Ca to O.

Elevated temperatures cause a decrease of carbonate mineral solubility. Most reliable values of solubility products as a function of temperature are obtained by the empirical equation as compiled by Nordstrom et al. (1990). High pressure favors the dissolution of carbonate minerals as observed in seawater below ≈3–5 km depths, the so-called carbonate compensation depth (CCD, e.g., Morse and Mackenzie, 1990). Deep-sea sediments below the CCD are free of calcite and aragonite.

At elevated pH in alkaline solutions, precipitation of carbonate minerals may occur due to high \([CO_3^{2-}] / [CO_2] \) ratios (\([CO_3^{2-}] / [DIC] \) ratios). Components of DIC comprise CO₂(aq), H₂CO₃, HCO₃⁻, and CO₃²⁻, where CO₂(aq) is dominant over H₂CO₃ by a factor of 1,000 (see Usdowski, 1982). In acidic solutions the dissolution of carbonates is favored by a high H₂CO₃ content \((H_2CO_3^+) = [CO_2(aq)] + [H_2CO_3] \) and low \([CO_3^{2-}] / [DIC] \) ratios.

High ionic solute content generally results in high-dissolution capacities due to a decrease of ion activities versus the concentrations of total dissolved ions. Accordingly, calcite is more soluble in seawater than in freshwater. Seawater is slightly supersaturated with respect to calcite. Supersaturation means that the present ion activity product of the calcium and carbonate ion, \( IAP = (Ca^{2+}) \cdot (CO_3^{2-}) \), exceeds the \( K_S \)-value for calcite (saturation degree: \( \Omega = IAP/K_S > 1 \)), and precipitation of calcite may occur. In terrestrial solutions, like drip water in caves and spring water in karst areas, supersaturation with respect to carbonate minerals and sinter formation are mostly related to the degassing of CO₂, which results in an increase of pH.

**Precipitation and dissolution**

Several carbonate minerals such as calcite, wetherite, and strontianite can be easily precipitated from aqueous solutions, whereas others, such as dolomite, are not easily precipitated. In the classical experimental study by Plummer et al. (1978), expressions for the dissolution and precipitation kinetics of calcite are given. Since the Plummer et al. study, various approaches have been used to model the
general relationships. Calcite dissolution kinetics is described by empirical rate expressions depending on the reaction rate constant, the reactive surface area, the volume of the solution, and the supersaturation state. More recently, Arakaki and Mucci (1995) interpreted the dissolution of calcite based on the surface complexation theory.

In natural environments, the observed precipitation and dissolution rates \( R \) (in mol m\(^{-2}\) s\(^{-1}\)) often disagree with the rates estimated from experimental approaches. This may be caused by an incorrect estimation of the reactive mineral surface. Lower rates in natural surroundings may also be due to inhibition by foreign ions or organic matter (e.g., Zaihua et al., 1995).

Gutjahr et al. (1996) showed that in addition to anions like phosphate, the occurrence of divalent ions such as Mg\(^{2+}\), Fe\(^{2+}\), Sr\(^{2+}\), and Ba\(^{2+}\) significantly decreases the precipitation rates of calcite. In seawater, the transformation into the more stable phases is hindered by the high Mg\(^{2+}\) content. Thus, aragonite and high-Mg calcite are often found in marine sediments, although they are metastable against calcite and low-Mg calcite, respectively (Berner, 1966).

A main aspect of inhibition of carbonate precipitation in natural environments (e.g., biomineralization) and applied systems (e.g., scale formation) is the presence of dissolved organic matter, such as organic acids (e.g., Mann, 2001). For instance, polyaspartic acid can systematically and significantly modify the morphology and growth kinetics of calcite.

**Isomorphic substitution**

In natural systems, carbonate minerals are generally not pure substances. Impurities can be caused by isomorphic substitution of a host ion by foreign ions in a given crystal lattice. Isomorphic substitution of divalent cations in carbonate minerals depends on the cation radius, which accounts for the crystal structure and the solubility of carbonate minerals. Isomorphic substitution may result in solid solution formation, as in the irregular but unsymmetrical SrCO\(_3\)-CaCO\(_3\) (aragonite type) solid solution.

Mechanisms of co-precipitation of foreign elements into carbonate minerals are complex and equilibrium thermodynamics may not be appropriate for dealing with many low-temperature reactions. On the other hand, trace element substitution in carbonate minerals can provide information about the environment conditions during formation.

The literature dealing with incorporation of trace elements into carbonate minerals is voluminous, especially with respect to the most abundant calcium and magnesium carbonates. As both the anhydrous magnesium and calcium carbonate crystallize in the calcite type, natural skeletal calcite can include up to about 25 mol\% MgCO\(_3\) to form so-called Mg calcite, which is isostructural with calcite. Low- and high-Mg calcites are defined by a limit of 4 mol\% MgCO\(_3\) with high-Mg calcite being less stable than low-Mg calcite. Only traces of magnesium are incorporated into aragonite as the cation sites are larger due to 9-coordination of Ca to O. Consequently, the larger Sr\(^{2+}\) ion is readily incorporated into aragonite, and less Sr\(^{2+}\) substitution occurs in calcite.

In the case of a solid CaCO\(_3\) phase, a foreign element is incorporated into the crystal according to the overall expression

\[
x\text{Ca}^{2+} + (1 - x)\text{Me}^{2+} + \text{CO}_3^{2-} = \text{Ca}_x\text{Me}_{(1-x)}\text{CO}_3.
\]

The related distribution coefficient is defined by the mole fractions \( X_{\text{MeCO}_3} \) and \( X_{\text{CaCO}_3} \) in the solid CaCO\(_3\) and the molar Me\(^{2+}\) to Ca\(^{2+}\) ratio of the aqueous solution according to

\[
D_{\text{Me}} = (X_{\text{MeCO}_3} / X_{\text{CaCO}_3}) / ([\text{Me}^{2+}] / [\text{Ca}^{2+}]).
\]

The values of the experimental distribution coefficients for trace elements in carbonate minerals show a systematic behavior, but one that is different from that proposed by a thermodynamic equilibrium approach (e.g., Rimstidt et al., 1998; Lea, 2004; Boettcher and Dietzel, 2010).
The distribution coefficient of Mg$^{2+}$ between calcite and aqueous solution is less than unity ($D_{\text{Mg}} \approx 0.02$ at 25°C). The higher the temperature, the more Mg$^{2+}$ is incorporated into the calcite lattice (e.g., Oomori et al., 1987). In contrast, the distribution coefficients of Sr$^{2+}$ in aragonite is larger than unity, and an increase in temperature results in less incorporation of Sr$^{2+}$ into aragonite (Dietzel et al., 2004). Tesoriero and Pankow (1996) clearly showed that the incorporation of divalent cations into inorganically precipitated calcite is a function of precipitation rate. At low rates, a constant value of the distribution coefficient is reached representing thermodynamic equilibrium. Besides temperature and precipitation rates, the apparent $D_{\text{Sr}}$ values depend also on the concentrations of additional foreign ions via competitive substitution, the ionic strength of the solution, and the differential partitioning of the foreign cation on nonequivalent crystallographic faces of the carbonate mineral.

Trace element incorporation into carbonate minerals is widely used to estimate conditions of formation. For instance, Mg$^{2+}$ to Ca$^{2+}$ and Sr$^{2+}$ to Ca$^{2+}$ ratios in calcium carbonate minerals of foraminifera, coccoliths, bivalves, or in speleothems are used to estimate paleotemperature and environmental records as well as diagenetic alterations (e.g., Purton et al., 1999; Huang and Fairchild, 2001). The interpretation of datasets for trace element distribution in carbonate minerals is related to saturation states, precipitation rates, temperatures, and metabolisms of the organisms. Accordingly, recent studies of Elderfield et al. (2006) show a carbonate ion saturation state effect on Mg$^{2+}$ incorporation in benthic foraminifera, and Freitas et al. (2006) show that Sr$^{2+}$ to Ca$^{2+}$ ratios in carbonate shell might be controlled by kinetic effects, the latter driven by seasonal variation in shell growth rate that is in turn influenced in part by seawater temperature.

**Isotope geochemistry**

Stable isotopes of carbon and oxygen of CO$_3^{2-}$ molecules as well as cation isotopes in carbonate minerals are frequently considered in environmental studies to decipher the origin of components, conditions during formation, alteration effects, etc. (e.g., Ripperdan, 2001). For instance, stable oxygen isotopic fractionation between calcite and water has been widely used as a proxy for temperature and environmental changes in paleoenvironment, speleology, and applied mineralogy (e.g., Dietzel, 2000; Spötl and Mattey, 2006). The basic concept is referred to the temperature sensitive oxygen isotope fractionation factor between calcite and water, $\frac{\delta^{18}O_{\text{calcite}}}{\delta^{18}O_{\text{water}}} = (\frac{\delta^{18}O_{\text{calcite}} - 1000}{\delta^{18}O_{\text{water}} + 1000})$. For example, Kim and O’Neil (1997) and more recently Coplen (2007) adopted from inorganic precipitation experiments and field studies the $\frac{\delta^{18}O_{\text{calcite}}}{\delta^{18}O_{\text{water}}}$ values for equilibrium fractionation. From laboratory experiments and field studies it is widely accepted that the use of $\delta^{18}O$ as a temperature proxy is complicated by nonequilibrium fractionation (e.g., Kim and O’Neil, 1997; Mickler et al., 2004; Dietzel et al., 2009). In natural biogenic carbonates, the effect of growth rate on $\delta^{18}O$ values of carbonates is noticeable, with a rapid growth rate resulting in a lower $\delta^{18}O$ value (e.g., Adkins et al., 2003). Numerous temperature functions are used to estimate $\frac{\delta^{18}O_{\text{calcite}}}{\delta^{18}O_{\text{water}}}$ values for the respective cation content, crystal structure, and organism (e.g., Lea, 2004).

In the case of nontraditional stable isotopes, Mg and Ca isotopic distributions between aqueous cations and carbonate minerals are promising proxies to reconstruct environmental conditions during carbonate formation and to provide insight into geochemical cycles (e.g., Young and Galv, 2004; Tang et al., 2008, respectively). For example, $^{44}$Ca is discriminated versus $^{40}$Ca during calcium carbonate crystallization. Various models exist for the calcium isotope fractionation during precipitation of calcium carbonate from aqueous solution (e.g., Fantle and DePaolo, 2007). Although, effects of temperature, precipitation rate, crystal structure, and metabolism are identified as main factors for calcium isotope fractionation, future research is required to decipher individual fraction mechanisms.

**Biominalization**

Biominalization refers to the process by which living organisms form minerals. The most abundant carbonate biominalizers with respect to quantities and widespread distribution are calcium carbonates such as calcite and Mg-calcite, and to a lesser extent aragonite, monohydrocalcite, and ACC (e.g., Weiner and Dove, 2003). Biominal phases often have shape, size, crystallinity, isotopic and trace signatures quite unlike their isotopic inorganically formed counterparts as a result of the interaction between organic macromolecules and an amorphous–crystalline phase transformation during early stages of biominalization. Sorting out the physiological effects from environmental signals provides a challenge for further scientific research.

Although, ACC is thermodynamically metastable it can be introduced as an important precursor of biominalization. Biominalization mechanisms and strategies for the biomimetic preparation of functional materials can be followed by transformation of ACC into crystallized calcium carbonates regulated by additives, e.g., functional polymer, proteins, and inorganic ions (Xu et al., 2008).

Han and Aizenberg (2008) showed that the ACC phase can be doped with foreign ions (e.g., Mg$^{2+}$) and organic molecules (e.g., dyes) and that these compounds later function as growth modifiers of calcite crystals. Such foreign components are incorporated into the crystals during the transformation process of ACC to calcite, which may indicate a vital effect on biogenically induced calcite. Lam et al. (2007) synthesized ACC in the presence of Mg$^{2+}$ and polyaspartic acid. While the first Mg rich ACC precipitates showed short-range structures most similar to aragonite, the initial polyaspartic ACC precipitates possessed short-range structures resembling vaterite. The
influence of these additives on the crystallization of calcium carbonate is apparent even in the precipitation of the first amorphous precursor phase.

Summary
Carbonate minerals have a high diversity and are widely distributed throughout the Earth’s crust. The most abundant carbonates contain calcium and magnesium such as calcite, dolomite, Mg calcite, and aragonite. For biogenically formed carbonates, precursor phases such as ACC are of special interest. In aqueous systems the stability of carbonates is related to the solubility product and the kinetics of dissolution and precipitation. Incorporation of foreign cations and isotopes into carbonates is mostly controlled by the composition of the solution, temperature, and precipitation rate. Thus, the chemical and isotopic composition of carbonates can be used as promising proxies to decipher environmental conditions during mineral formation and alteration. Lab experiments will be essential to provide insight about environmental changes throughout the geologic history from field data.

Bibliography
CATHODOLUMINESCEENCE MICROSCOPY

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Definition
Cathodoluminescence (CL) is the emission of visible light (also ultraviolet [UV] and infrared) of a solid material that is irradiated by an electron beam (in vacuum). During irradiation CL often changes. Besides CL, the emission of element-specific X-rays and backscattered, secondary and Auger electrons is caused (e.g., Walker and Burley, 1991). Moreover, the sample is heated by the electron beam, which represents an important cause for the alteration of CL characteristics during electron bombardment. Cathodoluminescence microscopy (CLM) combines CL with optical microscopy (in geosciences, generally polarizing microscopy). CL microscopic studies are mostly carried out on thin sections (uncovered, preferably polished), sometimes on up to one cm-thick samples, the latter preferably with polished surface as well. Depending on the instrument used (see below), samples can be studied uncoated or with a coating to avoid charging by the electron beam.

Two types of CL are known: intrinsic and extrinsic (for details see discussion by Remond et al., 2000). Intrinsic CL is purely due to lattice features, generally very weak and only visible if there are no activators present in adequate amounts. Extrinsic CL, the generally observable case, is caused by activators, which occur as trace elements. The activators may be supported by sensitizers that transmit energy to the activators. Quenchers are elements that reduce or even inhibit CL. The significance of sensitizers and quenchers is still in discussion. An important example is iron, which is generally considered as a quencher in carbonate minerals, and lead, which seems to be a sensitizer (e.g., Machel, 2000). In contrast to the view of iron as a quencher, Cazenave et al. (2003) showed in a recent study of doped calcite that Mn-activated CL in calcite can be uninfluenced by iron in a relatively wide range of iron concentrations. The question whether Pb and Zn can be sensitizers in calcite was discussed by Budd et al. (2000), who could not find any evidence for sensitizing by these trace elements.

In geosciences, CLM is a powerful tool for detailed analyses of rocks and minerals, including biogenic mineral substances (for a summary of CL data and earlier literature, see for instance Marshall, 1988, Remond et al., 1992 and Pagel et al., 2000). Intensity and wavelengths (colors) of emitted light may vary strongly for the same mineral phase or even within the same mineral grain, depending on genetically determined features. This is especially significant when a mineral substance is growing or recrystallizing under changing ambient conditions, often causing changes of CL intensity or color, sometimes revealing patterns of complex zoning. In most cases, such effects are not visible by optical microscopy. Therefore, for studies of biogenic mineral substances, CLM can...
provide important information. In geosciences, CLM has been used for more than 40 years, resulting in a huge number of publications (for an outline of history, see for instance Pagel et al., 2000). In biology, probably the first CL study was performed by means of an electron microscope by Cavallier (1975).

**Instrumentation**

CL studies can be carried out by (1) CLM with cold cathode equipment, (2) CLM with hot cathode equipment, and (3) scanning electron microscopy (SEM) and electron probe micro-analysis (EPMA), if the latter are equipped with CL detector or with an instrumentation for light optical observation/recording. For CLM, CL images can be recorded by conventional camera technique using film, video technique, or digital camera. However, today, digital camera and video techniques have generally replaced film techniques. For detailed and reproducible recording of CL colors, which is important for the determination of activators, optical spectrometers are applied (e.g., Gillhaus et al., 2001). Moreover, CLM can be combined with energy-dispersive X-ray spectroscopy (EDS), providing rapid elemental analysis (see below).

An overview for the application of SEM and EPMA for CL studies is given by Remond et al. (2000). These instruments allow the combination of CL with electron images and elemental analysis by means of EDS or wavelength-dispersive X-ray spectroscopy. Due to the influence of the chemistry of analyzed materials on backscattered electrons (BSE), corresponding electron images are especially interesting in combination with CL. Moreover, SEM offers the advantage of high spatial resolution, including spectrometric recording of CL colors. Monochromatic CL mapping by SEM, with the same resolution as for element mapping by means of EDS, was recently described by Edwards et al. (2007).

For cold cathode CLM, a vacuum chamber with fixed electron gun and $x$–$y$ stage for sample movement is attached to the stage of a microscope. As the electron beam is directed from above, i.e., from the same side as the objective of the microscope, besides thin sections, up to one cm-thick samples can be studied as well. Care has to be taken with cold cathode CLM systems, to avoid too high energy flux, which can damage thin sections and increase the changes of CL intensity and colors during electron bombardment. Due to low vacuum, residual gas is ionized, preventing electron charging of the sample surface. Therefore, coating is not necessary. A disadvantage of cold cathode CLM systems, often mentioned in the literature (e.g., Walker and Burley, 1991), is that objects with very low CL intensity are not observable, or at least require too long exposure times for conventional camera film techniques. Witkowski et al. (2000), for instance, published excellent CL images of objects with low CL intensity, showing that former problems can be overcome by using a high-sensitivity digital video camera linked to a computer.

Besides optical spectroscopy, EDS is also possible with cold cathode CLM systems (e.g., Vortisch et al., 2003). Cold cathode CLM systems equipped with EDS can also be used for the analysis of opaque and nonluminescent materials as well as finely ground pressed powders (Vortisch, 2004). Cold cathode CLM systems are less expensive and easy to operate. Instrumental working conditions, vacuum, high tension and beam current are now automatically stabilized. The CL images used in this chapter were obtained by means of a cold cathode CLM system.

Hot cathode CLM systems operate at high vacuum, comparable to SEM. To prevent charging during electron bombardment, coating is recommended. Microscope, vacuum chamber with hot cathode, and $x$–$y$ stage for sample movement are integrated. In contrast to cold cathode CLM systems, the sample may be less heated. Hot cathode CLM systems are generally regarded as being more suitable for objects with low CL intensity. Therefore, many publications including optical spectroscopy for the analysis of CL-activating trace elements are based on hot cathode CLM (e.g., Gillhaus et al., 2001; for interesting examples concerning geobiology, see e.g., Barbin, 2000; see below).

The irradiation of the sample is normally from below, opposite to the objective of the microscope, therefore only transparent objects (i.e., thin sections) can be studied.

**CL in geobiology**

CLM proved to be of special interest in the following areas of geobiology:

- Biogenic minerals in shells, bones, and other mineralized parts of organisms; structures and conditions of growth
- Processes of fossilization and transformation of biogenic mineral matter during diagenesis; mineralization of organic matter
- Microbial mineralization processes

Structures and growth conditions of biogenic minerals often reveal more details when CLM is applied. Barbin et al. (1991a), for instance, were able to distinguish winter growth rings from other parts of the shell of *Pecten maximus* by means of CL, observing an inverse relation between growth velocity and CL intensity. In a recent study of experimental biology, Barbin et al. (2008) were testing the uptake of manganese of the oyster species *Crassostrea gigas* (Mn$^{2+}$ was added as MnCl$_2$ to a tank with seawater where the oysters were living). By means of CLM and microchemical analysis, the authors could prove the daily incorporation of Mn to the growing shell. Mn was incorporated simultaneously to calcitic and aragonitic parts of the shell. CL colors were yellowish for calcite and greenish for aragonite. A comparable differentiation in CL color between calcite and aragonite was observed in the shell of *Haliotis* by Hawkes et al. (1996) during
Cathodoluminescence Microscopy, Figure 1 Thin section of Lower Ordovician limestone from Mőckleby (southern Öland, Sweden), revealing various stages of diagenetic alteration of carbonate fossils (interpretation Maurits Lindström); optical ((a), plane polarized light) and cathodoluminescence (CL) images (b); height of images: 1.6 mm. 1. Trilobite fragment, completely recrystallized but still clearly separable from surrounding sediment by CL. 2. Recrystallized echinoderm fragment with uniform extinction under crossed polarizers, showing changing chemical conditions during recrystallization. The innermost part with dull CL indicates an (almost) unchanged chemical composition. 3. Brachiopod fragment, consisting of fibrous calcite with sediment matrix entering between the fibers and (later) diagenetically recrystallizing. A larger inner part of this fragment shows very low CL intensity, indicating recrystallization without considerable alteration of the original chemical composition. 4. Series of cement generations filling a small cavity (probably after diagenetically dissolved, siliceous sponge needle), demonstrating the chemically complex early diagenetic history of this limestone layer that was already lithified near the interface sediment/seawater.

Cathodoluminescence Microscopy, Figure 2 Thin section of Upper Cretaceous glauconitic sandstone, showing intergrowth of glauconite (no CL) and calcium phosphate (CaP, pink CL); optical ((a), plane polarized light) and CL images (b); height of images: 2 mm. The intergrowth with CaP (determined by scanning electron microscopy–energy-dispersive X-ray spectroscopy (SEM–EDS)) indicates glauconitization of organic substances, especially fecal pellets (Schinagl and Vortisch, in prep.). 1. Glauconite with an outer rim of CaP. 2, 3. Compared with grain 1, less intensive glauconitization of organic material, indicated by considerable proportions of CaP inside the grains. 4, 5. Grains consisting predominantly of CaP, with minor proportions of glauconite. 6, 7. Glauconite grains with small inclusions of carbonate minerals. Bright yellow CL: carbonate (probably dolomite); bright blue CL: potassium feldspar; brownish to violet CL: quartz.
a similar experimental study. CL colors observed by these authors were orange-red for calcite and yellow-green for aragonite.

Biogenic minerals are generally transformed during diagenesis by processes such as recrystallization or replacement. By means of CLM, it is often possible to observe primary structural details even after diagenetic alteration of the biogenic material. Baumgartner-Mora and Baumgartner (1994) have shown good examples for Miocene foraminifera from Costa Rica. When biogenic minerals are investigated with regard to primary stable isotope geochemistry, it is important to know whether the geochemistry of the analyzed material has been changed or not. By means of CLM, Wierzbowski and Joachimski (2007) selected carbonate shells (calcitic and aragonitic), which were nonluminescent or showed only intrinsic bluish CL, for oxygen and carbon isotope analysis, with the argument that diagenetic alteration of carbonate minerals generally results in the enhancement of CL intensity. Barbin et al. (1991b) already recommended the usage of only nonluminescent biogenic carbonate for the investigation of past sea water composition. These authors emphasize that even recent carbonate shells may show CL of considerable intensity. Figure 1 shows examples for different steps of diagenetic recrystallization of fossils in a Lower Ordovician limestone from Sweden.

The postdepositional mineralization of organic materials may also represent interesting objects for CLM. Götte and Rößler (2000) studied silicified Permian wood. On the base of CL, they concluded that a polyphase process of silicification included an initial hydrothermal phase. Among other analytical procedures, CLM was also applied by Scott and Collinson (2003) for the analysis of Tertiary and Jurassic permineralized woods that contain calcite and Ca phosphate as important mineral phases. Coprolites of Upper Triassic carnivore vertebrates were investigated by Hollocher et al. (2005), who suggest an early onset of mineralization, starting with the formation of apatite. Further minerals are calcite and glauconite. An example for the association of glauconite and apatite, probably due to the mineralization of fecal pellets, is shown in Figure 2.

Reitner et al. (2005, 2006) applied CLM, among other methods, for the study of microbial mineralization processes in methane-derived carbonate buildups in the Black Sea sediments. An example of this work is given in

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\text{Cathodoluminescence Microscopy, Figure 3 Thin section of methane-derived carbonates from the Black Sea (Reitner et al., 2005, 2006). Comparison of light, ultraviolet (UV), and CL microscopy with element distribution of Mg, Sr, and Mn. Length of individual image 40 mm. (Light optical image and CL image compiled of about 200 images, UV image of about 600 single images.) The carbonates were precipitated within thick microbial mats, mainly constructed of anaerobic methane-oxidizing microbial communities. The microbial mats are differentiated in an outer black zone, characterized by the so-called ANME 2 (\textit{A}naerobic \textit{M}ethanotrophs) microbial community, and an inner orange/pink zone characterized by the so-called ANME 1 microbial community. ANME 2 community precipitates high Mg–calcite (HMGC), which is rich in Mn. The incorporation of Mn within the calcite crystal lattice causes a strong CL of the HMGC. ANME 1 community precipitates a Sr-rich nonluminescent aragonite.}
\]
Figure 3. Microbial mats with their methane–sulfate redox systems are the place where strongly luminescent high Mg–calcite is formed as a first carbonate phase. This first carbonate phase interfingers with nonluminescent aragonite, with the latter finally creating the thrombolithic fabric of the towers (Reitner et al., 2005). Another example for the significance of microbial activity at the methane–sulfate interface for early mineralization of marine sediments, also studied by means of CLM, was described by Meister et al. (2007). This work reports on early, near-surface dolomite cementation in Quaternary sediments from the Peru continental margin.

Conclusions

CLM is successfully applied in all areas of geobiology where mineral substances are involved. Important examples are biogenic minerals in shells, bones, and other mineralized parts of organisms; their structures and conditions of growth and processes of fossilization; mineralization of organic matter; microbially influenced processes of mineralization and formation of sedimentary structures. Minerals often studied by CLM are carbonates, phosphates, and silica minerals.

Acknowledgments

I am grateful to late Prof. Dr. Maurits Lindström for the interpretation of the Lower Ordovician sample (Figure 1), to Prof. Dr. Joachim Reitner for providing Figure 3 (including figure caption), to Jay Morgan for important discussions and language corrections in the manuscript, and to Dr. Reinhard Gratzer for his help with graphical work.

Bibliography


Chemolithotrophy, Table 1 Some common reactions performed by chemolithotrophs

<table>
<thead>
<tr>
<th>Electron donor</th>
<th>Reaction</th>
<th>Organism</th>
<th>ΔG° (kJ/reaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>H₂ + 1/2 O₂ → H₂O</td>
<td>Hydrogen bacteria</td>
<td>−237.2</td>
</tr>
<tr>
<td>Sulfide</td>
<td>HS⁻ + H⁺ + 1/2 O₂ → S⁰ + H₂O</td>
<td>Sulfur bacteria</td>
<td>−209.4</td>
</tr>
<tr>
<td>Sulfur</td>
<td>S⁰ + 1/3 O₂ + H₂O → SO₄²⁻ + 2H⁺</td>
<td>Sulfur bacteria</td>
<td>−587.1</td>
</tr>
<tr>
<td>Ammonium</td>
<td>NH₄⁺ + 1/2 O₂ → NO₂⁻ + 2H⁺ + H₂O</td>
<td>Nitrifying bacteria</td>
<td>−274.7</td>
</tr>
<tr>
<td>Nitrite</td>
<td>NO₂⁻ + 1/2 O₂ → NO₃⁻</td>
<td>Nitrifying bacteria</td>
<td>−74.1</td>
</tr>
<tr>
<td>Iron</td>
<td>Fe³⁺ + H⁺ + 1/2 O₂ → Fe²⁺ + 1/2 H₂O</td>
<td>Iron oxidizing bacteria</td>
<td>−32.9</td>
</tr>
</tbody>
</table>

Source: Adapted from Madigan and Martinko (2006)
CO₂ as their carbon source (most are) they are referred to as chemolithoautotrophs. Well-known examples of chemolithotrophs relevant in geobiology are sulfur-oxidizing bacteria (e.g., Beggiatoa; Thiomargarita) and iron-oxidizing bacteria (see entries “Fe(II)-Oxidizing Prokaryotes,” “Gallionella”) (Figure 1).

Bibliography

Cross-references
Acetogens
Anaerobic Oxidation of Methane with Sulfate
Archaea
Bacteria
Beggiatoa
Cold Seeps
Fe(II)-Oxidizing Prokaryotes
Gallionella
Hydrothermal Environments, Marine
Sulfate-Reducing Bacteria
Sulfur Cycle
Thiomargarita

CHERTS
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Chert is a sedimentary rock mainly consisting of micro- or cryptocrystalline (=submicroscopic) quartz (SiO₂) varieties. Cherts often have an “indirect” biogenic origin as they result from re-precipitation of dissolved opal-A shells derived from microorganisms such as diatoms (diatomite, diatomaceous cherts, see also entry “Diatoms”) and radiolarians (radiolarite, see also entry “Protozoa (Heterotroph, Eukaryotic)” ). Postsedimentary dissolution of these shells leads to the accumulation of silica-rich fluids within pore waters, thus, inducing precipitation of metastable cristobalite, also known as opal-CT (Konhauser, 2007). Opal-CT may, under increasing temperatures and pressures, be converted into stable quartz. Chert may also originate from microbial silification, and it may form, with or without microbial mediation, in hydrothermal environments (Konhauser et al., 2004; Jones et al., 2004, see also entries “Sinter” and “Hot Springs and Geysers”). Depending on the content of clay and other minerals, chert may grade into siliceous shales or -mudstones. The color of cherts may vary greatly with the content of accessorist elements. Iron (hematite), for instance, gives rise to red varieties called “jasper.” Banded cherts, with often colorful, rhythmic layers, are called “agate.”

Chert may occur as extensive layers (bedded cherts) within sedimentary formations as well as in concretionary
nODULES. For example, vast deposits of bedded cherts, alternating with iron minerals, are found within the Precambrian “Banded Iron Formations” (see entry). “Flintstones” on the other hand, are dark-colored chert nodules that precipitated in chalk such as in the Upper Cretaceous Maastrichtian Formation in Middle Europe. Here, the SiO₂ mainly derived from diagenetically dissolved, and re-precipitated, sponge spicules. Deposits with a high content of sponge-derived SiO₂ dispersed in sedimentary rocks are called “spiculites.”

Cherts may provide an excellent matrix for the long-term preservation of macro- and microfossils as well as chemical and isotopic signatures, due to their density and resistance. Examples are the Rhynie Chert that uniquely preserved an early Devonian community of plants, animals, bacteria, and fungi (Taylor et al., 2009), and numerous Precambrian cherts that provide an exciting view on the early dawn of life on Earth (Derenne et al., 2006, see entry “Biosignatures in Rocks”).

Bibliography

Cross-references
Algae (Eukaryotic)
Banded Iron Formations
Biogenic silicification (or Passive)
Diatoms
Hot Springs and Geysers
Hydrothermal Environments, Terrestrial
Microbial Silicification – Bacteria (or Passive)
Pore Waters
Protozoa (Heterotroph, Eukaryotic)
Sinter
Silica Biomineralization, Sponges
Sponges (Porifera) and Sponge Microbes

CHROOCOCCIDIOPSIS

The unicellular cyanobacterial genus Chroococcidiopsis was first described by Geitler (1933) from Sumatra, where it was found in warm springs.

Definition
The cyanobacterial genus Chroococcidiopsis is defined as having more or less spherical cells surrounded by a thin, firm, colorless, sometimes layered extra-cellular polysaccharide sheath (EPS). The cell has an S-layer of a special ribbon-like type, not found in other cyanobacteria so far (Büdel and Rhiel, 1985). It often occurs in large agglomerations of spherical or irregular shape. Fully grown cells (usually 2–6 μm in diameter) divide in two modes: (1) after one or two binary divisions with planes rectangular to each other, resulting daughter cells continue to divide in different planes without intermediate growth (successive multiple divisions); (2) by successive or almost spontaneous irregular cell division (multiple fission) without intermediate growth. In both types, the final daughter cells are very small (1–3 μm in diameter) and are released by rupture of the sheath envelope.

Presently, the genus Chroococcidiopsis comprises 14 different species (Komárek and Anagnostidis, 1998). Chroococcidiopsis is closely related to the heterocyst-containing, filamentous cyanobacteria (Nostocales, Stigonematales, see entry “Cyanobacteria”) and forms their natural sister group (Fewer et al., 2002). The current species concept needs revision.

In terms of ecology, Chroococcidiopsis is most interesting because of the wide variety of habitats it colonizes. Among these are different extreme biotopes such as thermal or mineral springs (see entry “Hydrothermal Environments, Terrestrial”), and extremely hot and cold deserts (Antarctica), where it is epilithic or endolithic on rocks (Figure 1a–h; see entry “Endoliths”). The genus is a common photobiont of the cyanolichens of the

CHORDRITES

Chordates are the most common class of stony meteorites. Chondrites largely consist of iron- and magnesium silicate minerals and remain unaffected from melting and differentiation. Therefore, chondrites are considered to reflect the elemental composition of the original solar nebula. Carbonaceous chondrites are a rare variety of chondrites containing numerous organic compounds, including amino acids. See “Meteoritics” for further reading.

CHOND RITES

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Lichinomycetes group (see entry “Fungi and Lichens”), mostly growing on rock surfaces of hot deserts. As an endolith, it contributes to biogenic weathering (see entry “Bioerosion”) of silicate rocks by photosynthetic alkalization (Büdel et al., 2004).

**Bibliography**


**Cross-references**

Biodeterioration (of Stone)
Bioerosion
Biological Volcanic Rock Weathering
Cyanobacteria
Desert Varnish
Endoliths
Extreme Environments
Fungi and Lichens
Geomycology

CLAY AUTHIGENESIS, BACTERIAL

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**Definition**

The formation of clay minerals in situ through the direct or indirect action of bacteria.

**Overview**

Clays are one of the largest and most diverse assemblage of minerals known. The vast majority of clay minerals are aluminum silicate in composition, with various other cations substituted into the crystal lattice. The replacement of either Si or Al can lead to an excess of negative charge, which is either neutralized by the adsorption of additional cations onto the outer mineral surfaces or within the interlayer spaces.

Within the past 2 decades, field studies have led to the general recognition that bacteria can mediate the formation of clay minerals. Some clays form as replacement products from the alteration of primary minerals. In this regard, the bacterial community growing on the rock surface potentially function initially as the weathering agents (through the production of organic acids or ligands that facilitate mineral dissolution) and later as reactive surfaces for the nucleation of secondary clay phases. For instance, Konhauser et al. (2002) documented that highly altered rocks found within active steam vents at Kilauea Volcano, Hawaii, contained bacteria with small (<500 nm in diameter) grains of smectite \([\text{Al}_{1.6}\text{Fe}_{0.4})(\text{Si}_{3.8}\text{Al}_{0.2}) \text{O}_{10} (\text{OH})_2 \text{K}_{0.2})\] attached directly to the outer cell walls.
Many of the elements now incorporated into the clays originated when the volcanic glass dissolved and released them into the pore waters. Leveille et al. (2002) similarly documented the formation of kerolite \([\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2\cdot n\text{H}_2\text{O}]\) in association with microbial mats colonizing the surfaces of walls and ceilings within basaltic sea caves on the north coast of Kauai, Hawaii. That microorganisms were in some way involved in mineral formation was suggested by the lack of kerolite within basalt vesicles near the rock surface or in the carbonate-rich evaporitic coatings found on other exposed surfaces without the presence of microbial mats.

Other clays form in environments far removed from the actual sites of weathering. The best examples come from biofilms retrieved from the sediment–water interface of lakes and rivers. Ferris et al. (1987) initially described complex (Fe,Al)-silicates on bacterial cells growing in metal-contaminated lake sediment in northern Ontario. These precipitates ranged from poorly ordered and uncharacterized phases to crystalline forms of the Fe-rich chlorite, chamosite \([\text{Fe}_3(\text{Si}_3\text{Al})\text{O}_{10}(\text{OH})_2]\). Since then, similar clayey precipitates have been reported from various rivers around the world (e.g., Konhauser et al., 1998). What is particularly remarkable about bacterially mediated clays is that they share a number of properties, irrespective of the chemical composition of the waters from which they were sampled: (1) They are generally amorphous to poorly ordered structures. (2) All have grains sized <1 \(\mu\)m, although the majority are <100 nm. (3) The grains have a composition dominated by iron, silicon, and aluminium in varying amounts.

Based on the observations from natural studies, a general sequence of events leading to clay biomineralization can be adduced (Konhauser and Urrutia, 1999). In the initial stages, a bacterium adsorbs any number of different free aqueous iron species, i.e., \(\text{Fe}^{2+}\), \(\text{Fe}^{3+}\), \(\text{Fe(OH)}^2^{+}\), and \(\text{Fe(OH)}_2^{+}\), depending on solution chemistry and redox potential. Adsorption can occur on either the cell wall, which is frequently lined with anionic carboxyl and phosphate functional groups, or to the more neutrally charged carbohydrate-rich extracellular layers that surround the cell, including sheaths and capsules – the latter are the organic components that make up the bulk of biofilms. If the soluble iron concentration around the cell surface exceeds the solubility product of ferric hydroxide, then the reaction between the bound iron with dissolved iron will lead to the development of small (~100 nm in diameter), dense, mineral aggregates on the outer cell surface (Figure 1a). Indeed, it has been shown experimentally that a continuum exists between cationic iron sorption and precipitation reactions at bacterial surfaces; with the three stages of (1) sorption, (2) surface site saturation and (3) precipitation, all evident in Langmuir-type isotherms relating the solid-phase concentration of Fe(III) to the equilibrium proton and soluble Fe(III) concentrations (Warren and Ferris, 1998). Alternatively, bacteria can attract preformed nanometer-sized ferric hydroxide particles from suspension (Glasauer et al., 2001), thereby negating the need for the nucleation step. In most solutions, iron is only found in trace amounts compared to other ions, particularly silica. Under those conditions, the adsorbed/particulate iron may instead serve as kinetically favorable site for the development of more complex precipitates of variable clay composition, morphology, and structure (Figure 1b).

Why these clays form is likely as follows. In the pH range of most natural water bodies, negatively charged counter-ions, or those molecules that are neutrally charged but exhibit residual surface electronegativity (e.g., oligomeric and colloidal silica species), accumulate near the solution-adsorbed iron/solid interface to neutralize the net positive charge of iron. Two surface species of iron oxide exist in this pH range; \(\text{FeOH}_2^{+}\) and \(\text{Fe(OH)}^3\), but the majority of the surface charge is positive at circumneutral pH. The initial (Fe,Al)-silicate phases then form via hydrogen bonding between the hydroxyl groups

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**Clay Authigenesis, Bacterial, Figure 1** Transmission electron micrographs (TEM) of bacteria (stained) from the Rio Solimões in Brazil. (a) Fe-mineralized capsule with formation of dense Fe-rich aggregates on the outer edges (arrow). (b) Partially encrusted cyanobacterium with various amorphous clay precipitates (arrow) tangential to the cell wall and surrounding sheath. Scale bars = 500 nm.
associated with the cell-bound iron and the hydroxyl groups in the dissolved silica, aluminum, or aluminosilicate complexes (e.g., Taylor et al., 1997; Davis et al., 2002). Exactly how these reactions occur in nature has not been ascertained, but we do know that dimeric silica (the species that accounts for more than 99% of the oligomeric silica in natural waters) is highly reactive toward iron hydroxide surfaces (Reaction 1), and that oligomeric silica exhibits a strong affinity for dissolved aluminum, forming soluble hydroxy-aluminosilicate ions (e.g., Reaction 2) that subsequently react with cell-bound iron.

\[
\begin{align*}
2\text{Si(OH)}_3^- + \text{FeOH}^- &\rightarrow \text{FeSi}_2\text{O}_3(\text{OH})_4^- \\
+ 2\text{H}_2\text{O} + \text{H}^+ &\quad (1) \\
2\text{Si(OH)}_4^- + \text{AlOH}^{2+} &\rightarrow \text{Si(OH)}_4\text{SiO}_4\text{Al}^- \\
+ \text{H}_2\text{O} + 3\text{H}^+ &\quad (2)
\end{align*}
\]

This arrangement of ions forms an electric double layer with iron attaching to the bacterial surface as an inner sphere complex, while the silica-aluminosilicate species attach as more diffuse outer layers. The surface charge of these composites is inevitably dependent upon the solution pH, the ionic strength of the solution, and the time of reaction, such that as the particles age more silica (and aluminium) sorb. Interestingly, this mechanism of binding Fe to the bacterial cell surface and subsequent reaction with Si and Al from solution has been shown in experimental systems with Bacillus subtilis (e.g., Urrutia and Beveridge, 1994). Accordingly, if the microbial mats are subject to sufficiently concentrated solutions, then continued reaction of the dissolved components to the Fe-bearing solid interface eventually results in the formation of amorphous to poorly ordered clay phases. Often, these reactions lead to the partial and complete encrustation of some bacterial cells as abiological surface reactions accelerate the rate of mineral precipitation: in some biofilms, the density of clayey material surrounding individual cells can extend hundreds of nanometers away from the cell surface (Figure 2). Over time, these hydrous compounds dehydrate, some converting to more stable crystalline products. This latter material presumably uses some fraction of the precursor surface as a template for its own growth, in effect circumventing the need for direct nucleation (Steefel and van Cappellen, 1990). Once it begins to grow, the more stable clay phase increases its own surface area until it can control the composition of the proximal solution. When this happens, the saturation state of the solution moves below the solubility of the precursor, inhibiting further growth, or even dissolving the precursor.

The reactions above are naturally an oversimplification because a number of inorganic processes are also at play. It is well known that soluble Al–Si complexes precipitate as poorly ordered Al–silicates when a state of supersaturation is achieved (Wada and Wada, 1980). Moreover, Fe ions can readily be incorporated into those structures leading to clay-like products (Farmer et al., 1991). All these reactions can conceivably take place in the proximity of bacterial surfaces or within the extensive EPS, particularly since diffusion through the polysaccharide matrix is inherently slow, and a microenvironment can be established that is conducive to mineralization. Additionally, colloidal species of (Fe, Al)-silicate composition that either form initially in the water column or are products of weathering and soil formation, may react directly with the outermost cellular layers. It follows that anything which will neutralize or diminish the charge of the colloids (e.g., bacterial surface if colloids are positively charged or adsorbed iron if the colloids are negatively charged) will cause the particles to flocculate out of solution.

One of the intriguing questions that arises from the observation of bacterial cells completely encrusted in clays is why do they allow for mineralization to occur? It has been established that bacteria have the ability to partially control their surface charge (Doyle, 1989). During metabolism, a membrane-induced proton motive force continuously pumps protons into the wall fabric that effectively compete with metal ions for anionic wall sites (Urrutia et al., 1992). Subsequently, living bacteria may be able to reduce authigenic mineral formation (and detrital mineral adhesion) on their cellular surfaces, unless environmental conditions exceed their capacity to compensate. In most aqueous environments with low dissolved solutes, this condition should not arise. Therefore, the presence of epicellular minerals on bacteria suggests that (1) the cells observed under the electron microscope had lysed prior to mineralization, or (2) that the mineralized matrix is in some way advantageous to the microorganism. Several possibilities for the latter
exist. First, clays provide a good source of exchangeable inorganic nutrients readily available to microbes (Tazaki et al., 1994). Second, clays such as montmorillonite stimulate the respiration of bacteria by maintaining the pH and water content of the environment at a level suitable for growth (Stotzky and Rem, 1966). Third, the formation of an external mineralized matrix may protect the living cells from the detrimental effects of toxin-producing microorganisms (Habte and Barrion, 1984) and predation by grazing protozoans (e.g., Heynen et al., 1988).

Summary
Microorganisms are commonly associated with fine-grained (Fe, Al)-silicates of variable composition. The inorganic phases develop in a predictable manner, beginning with the adsorption of cationic iron to anionic cellular surfaces, supersaturation of the proximal fluid with Fe (III), nucleation and precipitation of a precursor ferric hydroxide phase on the cell surface, followed by reaction with dissolved silica and aluminium, and eventually the growth of an amorphous clay-like phase.

Bibliography

Cross-references
Black Shales
Extracellular Polymeric Substances (EPS)
Microbial Biominalerization
Microbial Silification – Bacteria (or Passive)
Microbial Surface Reactivity
Soils

COCCOLITHOPHORES

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Coccolithophores (coccochloriophorids) are a group of unicellular, marine, planktonic algae belonging to the haptophytes (Prymnesiophyta). The coccolithophores are capable of controlling the intracellular precipitation of calcite onto organic plates and the assembly of the mature carbonate scales at the cell surface. These scales, the coccoliths, are spherical or oval, often intricately patterned and generally less than 20 μm in diameter. Given the abundance of coccoliths in sediments, coccolithophorids have been playing a major role in the global carbon budget. They were very abundant in the Mesoicic era, particularly in the Cretaceous period, in which they became a major component of the “Chalk” lithology (e.g., White Cliffs of Dover, England). Today, coccolithophorids are accounting for about a third of the total marine CaCO₃ production, and coccoliths are a major component of the
modern deep-sea calcareous oozes. The most widespread modern species is *Emiliania huxleyi*. Recent studies showed that calcification and net primary production in *E. huxleyi* have increased by ~40% over the last 220 years due to ocean acidification in response to rising atmospheric CO₂ partial pressures (Iglesias-Rodriguez et al., 2008). Contributions of sedimentary organic matter from coccolithophorids, and other haptophyte algae, can be tracked by particular lipid biomarkers, the long-chain alkenones. These compounds are often used as a “paleothermometer” for the estimation of past sea surface temperatures (see entry “Biomarkers (Organic, Compound-Specific Isotopes)”). For detailed reading and further references please see entry “Algae (Eukaryotic).”

Bibliography

COLD SEEPS

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Synonyms
Diffusive seep; Ground water seep; Hydrocarbon seep; Methane seep; Oil seep. The terms “seep” and “seepage” can be used as alternates

Definition
Fluid flow containing reduced compounds, e.g., methane or hydrogen sulfide (see entry Sulfur Cycle), with almost no temperature anomalies compared with ambient seawater gushing out from or reaching very close proximity to the seafloor.

Introduction
Active water circulation systems can be found at seafloor level and beneath the seafloor along the plate boundaries such as mid-ocean ridges and trenches. It is well known that at ocean ridges, hydrothermal fluid containing reduced compounds and minerals vent out from the seafloor. In contrast to the hydrothermal vents, focused flow containing reduced compounds, e.g., methane and hydrogen sulfide, gushing out from the seafloor can also be found along active and passive continental margins and around diapirs of serpentine and salt rock (halite) with no or less temperature anomalies compared to the ambient seawater. This fluid flow is called a cold seep.

The first cold seep was found at the Florida Escarpment off Oregon in 1984 by the submersible Alvin (Paull et al., 1984). The Alvin found a large number of alleged tube worms and white clams which were thought to be highly endemic to hydrothermal vents. It turned out that there are fluid flows which contain reduced compounds and which support chemosynthesis-based life, similar to hydrothermal vents (Kulm et al., 1986).

Examples of the reduced compounds contained in cold seeps are methane and hydrogen sulfide. These compounds lead to extensive biogeochemical reaction when the fluid encounters oxic interstitial water which has penetrated into sediments from seawater. The reaction is mainly mediated by microbes as a way of obtaining chemosynthetic energy to synthesize their organic compounds. Primary production based on the chemosynthesis found in cold seeps results in the formation of characteristic life forms similar to those which can also be found in hydrothermal vents.

The important role played by methane in global warming is due to the fact that the greenhouse effect that results from it is more than 20 times that of carbon dioxide (Kvenvolden, 1988). Hence if we think of cold seeps as a pipe connecting the deep subsurface and the oceans, we can understand their importance in terms of global climate change and biogeochemical cycles at the sediment–water interface.

Distribution
Cold seeps are widely distributed along the active and passive continental margins of the world’s oceans (Figure 1; Sibuet and Olu, 1998; Levin, 2005). The depth range of a cold seep varies from shallow water less than 15 m (Montagna and Spies, 1985) to deep water more than 7,400 m in the Japan Trench (Fujikura et al., 1999).

The total number of cold seeps reported to date represent a minimum, because finding a cold seep is significantly more difficult than finding a hydrothermal vent. The difficulties of identification result from the natural characteristics of cold seeps which are difficult to observe by the use of a submersible or remote operated vehicle (ROV).

Natural characteristics of cold seeps
Cold seeps vary in accordance with their natural characteristics. Most cold seeps are very calm, i.e., the low seepage intensity and low fluid flow rate has no effect on the sea floor. The almost total absence of any temperature anomaly in relation to the ambient seawater also makes it difficult to distinguish the flow by visual observation. The occurrence of chemical anomalies is found over too narrow an area and the anomalies usually occur only in the sediment. Thus, this kind of calm cold seep, called a diffusive seep, is rather difficult to find on the basis of geological and geochemical signatures which exist only beneath the sea floor. One good indicator pointing to the existence of a cold seep is the occurrence of chemosynthesis-based life obtaining energy from the reduced compounds contained in the seep fluids (Sibuet and Olu, 1998). Such biota often comprises large bivalves and/or
worm tubes which are clearly visible (Figure 2). However, seep-associated infaunal animals (i.e., animals living only in the sediments) and microorganisms are potentially difficult to detect.

Direct measurements of cold seep fluid chemistry started around the end of the last century (e.g., Tryon and Brown, 2001, 2004). The results show that the fluid pattern and rate of flow are more complex than was previously thought to be the case. Although most cold seeps are calm, there are some effusive and explosive cold seeps. When the saturation level of dissolved methane in sea water is exceeded, the excess methane forms gas bubbles (Figure 3) or gas hydrate (Figure 4) depending on the physical conditions. The bubbly gaseous methane can be found by visual observation (Figure 2) and also by hydroacoustic sounders, like a fisheries sonar (Figure 5; Paull et al., 2007; Aoyama and Matsumoto, 2009). The acoustic sounder makes it possible to see the plume due to the relatively low density of the methane bubbles compared to the ambient sea water. In waters off Niigata, Sea of Japan, methane bubble streams venting out from the sea floor at about 900 m water depth produce a plume reaching to about 300 m below the sea surface (Figure 5; Aoyama and Matsumoto, 2009).

Cold seeps associated with a mud volcano or serpentine diapir show that the flow gushes out together with muddy sediment or brecciated serpentine rocks. It may also happen that methane erupts from the seafloor due to a pressure increase in the sediments caused by an accumulation of methane gas. Such an eruption forms chaotic structures of strata, like landslides, and crater-like topographically depressed areas, so-called pock marks. The size of a pock mark may vary from a few meters to a few hundred meters in diameter and from 1 m to 80 m in depth (Gay et al., 2006).

Pathway and migration of fluid flow

Generally, reduced compounds increase with depth in marine sediments. These reduced compounds can be oxidized by oxidants such as oxygen, nitrate, and sulfate and usually occur at a shallow depth under the sea floor. Although these reactions do also occur elsewhere below the sea floor, substantial reaction occurs in cold seeps.
because of the high amounts of reduced compounds brought up to the sea floor from the deep subsurface. Accumulation and migration of reduced compounds are required to form a cold seep.

The pore water containing reduced compounds favors migration through physically weak paths, e.g., faults, and permeable sediments. Tectonic stresses, e.g., plate subduction or diapirs, induce the formation of such pathways. Thus, occurrences of cold seeps are largely dependent on the tectonic background of the area concerned.

Cold seep pathways take the form of a fault or faulted anticline, salt or serpentine diapirs, or a cut bank of strata. These structures can be revealed by seismic profiles, direct observations of the outcrops in the ocean, and drill core samples with chemical profiles of the pore water (Figure 6; Moore et al., 1990, 2001).

Iodine radioisotopes (see entry Isotopes, Radiogenic) have recently been identified as a useful tool to determine the age of the strata from which the methane originated, as the iodine will be released at the same time as methane is generated from organic matter, and the diffusion rates of methane and iodine are very similar (Fehn et al., 2003).
For example, pore water from a cold seep in the Japan Sea passed through and probably originated from strata which sedimented during 30–20 Ma (Tomaru et al., 2007). Faults associated with cold seeps occur particularly on the landward wedge of subduction zones, e.g., the Barbados accretionary wedge, the Cascadia margin and the Japan Trench. Faults created by tectonic stress due to subduction of plates play the role of physically weak pathways for the fluid flow. It may also happen that faults cut through a methane reservoir formed under impermeable strata, and the methane then migrates upward through the fault. Indeed, the direction formed by Calyptogena (bivalve) colonies, which mostly live in cold seeps and/or hydrothermal vents, is coincident with a theoretical stress fracture pattern caused by subduction tectonics (Ogawa et al., 1996).

The main driving forces of the migration of pore water containing reduced compounds are density differences and the compression of pore water by increasing pressure within the sediments. The buoyancy of gaseous and dissolved methane and light hydrocarbons in pore water is the main driving force for upward migration. Gaseous methane, the main hydrocarbon gas in the fluid flow, has a strong buoyancy, for example, the density of gaseous methane beneath 3–4 km of water is 200–300 kg m$^{-3}$ compared with 1,024 kg m$^{-3}$ for seawater (Clennell et al., 2000). Water in which methane is dissolved is also lighter than methane-free water. Thus, the pore water containing gaseous and dissolved methane potentially tends to migrate upward within permeable sediments. Entrapment of the fluid usually occurs where impermeable sediments are found. When strata composed of impermeable sediment are tilted, the fluid migrates within the permeable sediment in a lateral-upward direction directly beneath the impermeable strata. Thus, the hinge zone of an anticline is suitable for the accumulation of methane contained in pore water. Methane-rich fluids may further migrate upwards through faults that cut through the impermeable strata. The fluid also gushes out from outcrops where there is a break in the strata caused by erosion.
Increasing pressure arising from compaction due to continuous loading of sediments or tectonic stress squeezes out pore water containing reduced compounds into overlying strata. The increasing pressure causes not only pore water migration but also mud diapirs. This is why mud diapirs (or mud volcanoes) are often associated with methane or other reduced compounds.

Heating of the pore water is the other factor causing upward fluid migration. Heating occurs as a result of geothermal activities in the deep subsurface. After heating by geothermal activity in the deep subsurface, the temperature of the fluid drops during upward migration. Thus, the fluid temperature at sea floor level shows no or fewer anomalies in contrast to hydrothermal vents.

Migration of reduced compounds is also caused by diffusion. Although diffusion can be considered as a factor much less important than buoyancy and pressure increase, it is important for the penetration of oxidants from the sea water into sediments.

Chemistry of fluid flow
Major reduced compounds in the fluid flow in cold seeps are methane and other light hydrocarbons, hydrogen sulfide, ammonia, reduced iron, barium, and hydrogen (e.g., Tunnicliffe et al., 2003; Tryon and Brown, 2004). Methane is the most important by virtue of the fact that it is the most common hydrocarbon gas in marine sediments. Methane has a pronounced greenhouse effect and plays an important role both as an energy source and as a carbon source for microbes; furthermore, the anaerobic oxidation of methane (AOM) coupled with sulfate reduction produces hydrogen sulfide which acts as a major energy source for chemosynthesis-based ecosystems.

Elements contained in the fluid flow change with the depth of sediments, because the generation and consumption occur at different depths. Concentrations of reduced compounds generally increase in line with the depth of the sediments. Drastic changes in the chemical profile occur at shallower depths where the reduced compounds encounter oxidants. These reactions take place mainly within sediments, but it sometimes happens that the reduced compounds discharge directly into the sea water column. In such a case, the oxidation reactions take place within the water column.

The drastic chemical changes in cold seeps are generally responsible for substantial biological activities. Methane, the major reduced compound in a cold seep, is generated at a relatively deep subsurface level under anaerobic conditions. When a fluid flow containing methane migrates toward the sea floor and encounters oxidants such as oxygen and sulfate (see entry Sulfur Cycle) which penetrated into the sediment from oxic sea water, intensive biogeochemical reactions occur. The most important and extensive of these is AOM which occurs where the methane and sulfate coexist in anaerobic conditions. Following the AOM, oxidation of hydrogen sulfide, formation of pyrite (see entry Iron Sulfide Formation), and precipitation of carbonate minerals occur. When the fluid flow gushes out into well-oxygenated conditions, aerobic oxidation of methane (Methane Oxidation (Aerobic)) occurs.

The origin of methane
There are several pathways for the generation of methane of which biogenic and thermogenic processes are the most important. In the case of the former, the methane is produced directly by microbes, and in the case of the latter, the methane is produced through the thermal degradation of organic compounds in the deep subsurface. Methane that is produced neither from organic material nor through organic processes can be found mainly along the edge of hydrothermal vent fields and above serpentine diapirs.

Biogenic methane
Biogenic methane is produced by microbes. The methanogenic microbes (methanogens) belong to the phylum Euryarchaeota within the domain Archaea. Methanogens are obligatory anaerobes, meaning that they live in an anoxic environment which usually occurred below the zone of sulfate reduction. The main substrates linked to the generation of methane are simple carbon-containing molecules such as carbon dioxide, formate, acetate, methanol, and methylamine (Whiticar, 1999). The two simple pathways of methanogenesis, i.e., carbonate reduction and acetate fermentation, are expressed as

\[
\text{CO}_2 + 8\text{H}^+ \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad \text{and} \quad \text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2,
\]

respectively. Archaeal methanogenesis promotes large fractionations of carbon isotopes. The stable carbon isotopic composition of methane produced by carbonate reduction generally ranges from $-60$ to $-80\%$ (vs. PDB), while the methane produced by the fermentation process ranges from $-50$ to $-60\%$ (vs. PDB; Whiticar, 1999). The methane is mostly a unitary hydrocarbon product of the biological process, whereas the thermogenic process explained below produces methane as well as other hydrocarbons such as ethane and propane. However, the recent suggestion that ethane and propane are also produced biologically (Hinrichs et al., 2006) upset the long-held concept of the origin of light hydrocarbons larger than methane, which were thought to be generated by the thermal degradation of organic matter only. The biogenic process is carried out at a relatively shallow depth compared to where thermogenic methane is produced. This is due to the presence of appropriate thermodynamic conditions, i.e., not too high a temperature and pressure, to permit microbial activities.

Thermogenic methane
The methane generated through the degradation of sedimentary organic matter as a result of thermodynamic conditions is called thermogenic methane (Kotelnikova, 2002). The rising temperature and pressure of sediments that accompany the increasing depth accelerates the
degradation of organic compounds. Thus, the production of thermogenic methane generally increases in proportion to the depth of the sediments, usually more than a few hundred meters below the seafloor. Contrarily, a high temperature inhibits microbial activities at a deep subsurface level, thus it follows that the dominant methane in the deep subsurface is thermogenically generated. This process produces not only methane but also ethane, propane, and other light hydrocarbons. There are fewer isotopic effects on carbon and hydrogen from methane produced by thermal maturation compared to biogenic methane. Thus, the carbon isotopic composition of thermogenic methane ranges from −20 to −50‰ (vs. PDB; Whiticar, 1999).

Abiogenic methane
Methane produced from inorganic matter through an inorganic process is called abiogenic methane. Such methane is thought to be generated through the processes of cooling and degassing of mafic igneous rocks and magma and the process of serpentinization of ultramafic rocks. Although the exact mechanism of abiogenic methane formation is still not fully understood, some abiogenic methane formation processes have been revealed (e.g., Horita and Berndt, 1999; Proskurowski et al., 2008). We can now say that abiogenic methane is probably the dominant methane source in hydrothermal vent systems especially along mid-ocean ridges and above serpentine diapirs.

Identification of the origin of methane
Looking specifically at the methane in cold seeps, it has been thought that this originated from the processes of biogenesis and/or thermogenesis. Most abiogenic methane will be found at hydrothermal vents and not at cold seeps except in the case of cold seeps associated with serpentine diapirs. Means of allowing us to estimate the origin of methane are a combination of analyses of the carbon isotopic compositions of the methane and the relative abundance of ethane and propane compared to the amount of methane included in fluid seepage (Schoell, 1988). Specifically, extremely negative carbon isotope values of methane, generally less than −50‰ (vs. PDB), with a high methane/(ethane + propane) ratio indicate that the methane was generated by a biogenic process. In contrast, less negative carbon isotopic composition, generally −20‰ to −50‰ (vs. PDB), with a relatively low methane/(ethane + propane) ratio indicates that the methane was generated by a thermogenic process.

Anaerobic oxidation of methane (AOM)
AOM is a key reaction at cold seeps. Although several probable reactions have been proposed (Valentine, 2002), the details of the AOM reaction are not yet clearly understood. What we can say is that the net reaction of the AOM is expressed as \( \text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O} \). Thus, the relative amounts of both sulfate and methane regulate the activity of AOM. This reaction occurs widely in oceans because large amounts of sulfate are contained in sea water whereas the reaction will not occur in freshwater environments such as ponds, lakes, rivers, and rice fields because freshwater lacks sulfate (Whiticar, 1999). It has recently been recognized that AOM by sulfate reduction is carried out by consortia of anaerobic methane-oxidizing archaea and sulfate-reducing bacteria (Elvert et al., 1999; Boetius et al., 2000; Orphan et al., 2001, 2004). The anaerobic methane-oxidizing archaea (ANME) are found in three different phylogenetic clusters belonging to the Euryarchaeota, i.e., ANME-1, -2, and -3. It has also been revealed by genome analysis that methane is oxidized under anoxic conditions by means of a “reverse methanogenesis” process (Hallam et al., 2004). The ecology of each of these archaeal lineages (ANME-1, -2, and -3) differs from that of the others. Thus, the differences reflect their distribution pattern in cold seeps (Elvert et al., 2005; Knittel et al., 2005).

An important function of the AOM reaction is that it prevents the escape of methane, which has a pronounced greenhouse effect, from sediments into the ocean and the atmosphere. The carbon derived from methane is transformed into bicarbonate or carbonate ions. An increase in the ions leads to an increase in alkalinity and thus to the subsequent precipitation of carbonate minerals given the existence of calcium or other appropriate cations (explained below).

Oxidation of sulfides
The hydrogen sulfide (see entry Sulfide Mineral Oxidation), a byproduct of the AOM, is used for subsequent reactions. One is the oxidation of sulfides mediated by sulfur-oxidizing bacteria under (sub)oxic conditions, and the other is the formation of pyrite (see entry Iron Sulfide Formation) in anoxic conditions. Sulfur-oxidizing (thiotrophic) bacteria, such as Beggiatoa (qv), Thiotrix, and Thioploca, use the hydrogen sulfide as an electron donor and oxidize it by oxygen. The final product of the reaction is sulfate. Most sulfur-oxidizing bacteria are chemolithoautotrophs, but some can use organic compounds for their cell synthesis. The thiotrophic bacteria sometimes form white mats that cover the sea floor and some may live as symbiotic bacteria in animal tissues (e.g., in gills).

Aerobic oxidation of methane (qv)
The aerobic oxidation of methane (see entry Methane Oxidation (Aerobic)) is performed by methane-oxidizing (methanotrophic) bacteria, whereas archaea are responsible for AOM. When the methane is released directly into an oxic water column, it will be aerobically oxidized by these methanotrophs. However, in normal marine environments, methane is mostly oxidized anaerobically by AOM within the sediments. Aerobic oxidation of methane becomes important when methane vents out vigorously into oxic sea water.
Authigenic mineral precipitation in cold seeps (qv “microbial mineralization” and “microbially induced sedimentary structures”)

One result of the substantial biological reactions referred to above is the precipitation of several minerals. The minerals most commonly found in cold seeps are carbonate such as aragonite, high magnesian calcite, and low magnesian calcite. The precipitation of these carbonates occurred because of increasing alkalinity resulting from AOM. When AOM occurs at sea-floor level or above the sea floor in eukynic waters, reef-like carbonate build-ups may be formed on the sea floor, as in the Black Sea (Michaelis et al., 2002). Under an oxic water column, the reaction generally occurred within the sediment, thus the carbonate formed beneath the sea floor. At a shallower depth below the sea floor, aragonite and high magnesian calcite can be formed, whereas low magnesian calcite and dolomite are frequently found relatively deeper within the sediments (e.g., Takeuchi et al., 2007). Methane-derived carbonate bodies are sometimes exposed on the sea floor because of surface erosion. Then the carbonate may function as a hard substrate for sessile organisms or carbonate borers.

Pyrite is another common authigenic mineral found in cold seeps. Hydrogen sulfide produced as a result of AOM may combine with reduced iron to form monosulfide, i.e., a precursor of pyrite. Subsequent reaction of the precursor with elemental sulfur or sulfides promotes the formation of pyrite. It is commonly the case that the pyrite crystal aggregation forms a frambooidal shape, a so-called frambooidal pyrite (for details see Chapter Iron Sulfide Formation). The sulfur isotopic composition of pyrite inherits the composition of the hydrogen sulfide source and indicates whether or not the hydrogen sulfide derived from bacterial sulfate reduction (Peckmann and Thiel, 2004).

Relationship to methane gas hydrate

Cold seeps are frequently associated with gas hydrate (Figure 4) where the environment has appropriate physical conditions for gas hydrate formation. The main component of the gas hydrate is methane but carbon dioxide, ethane, and other gaseous molecules are also present. Gas hydrates form in low temperature – high pressure environments where the appropriate concentrations of methane exist (Zhang, 2003). Such environments are usually distributed in the deep sea around continental margins and in permafrost areas on land. The hydrate is one of the largest methane reservoirs in the world. It has been estimated that more than 2,100 Gt of methane are stored in gas hydrates.

Formation of a mass of methane gas hydrate usually occurs within sediments whereas methane rapidly escapes and does not readily accumulate in water columns. If a substantial amount of methane, e.g., bubbly methane gas, is released into a water column, gas hydrate may form as a thin shell on the surface of a gas bubble (Figure 3). The depth limit of gas hydrate formation and occurrence in the sediments is usually determined by the local geothermal gradient. The gas hydrate itself may act as an impermeable barrier within the sediment. In this case, the methane gas, which has migrated from the deep subsurface, is trapped and accumulates beneath the gas hydrate layer.

The importance of the gas hydrate for cold seeps is that (1) pore water surrounding the methane gas hydrate has an oversaturated level of methane, and (2) the rapid dissociation of methane gas hydrate may result in an episodic increase of cold seeps and sometimes an eruption of methane. When methane-undersaturated water penetrates the sediment and comes into proximity with the gas hydrate, the gas hydrate dissociates so as to create a physical equilibrium between the pore water and the gas hydrate. When the pore water, after taking up a substantial amount of methane, migrates further upward, a continuous methane supply is established between the methane gas hydrate reservoir and the sea floor.

Episodic emission of methane because of the dissociation of methane gas hydrate occurs, for example, in cases where the sea level decreases at the beginning of a glacial period or increasing sea water temperature because of global warming (e.g., Judd et al., 2002). The dissociation of gas hydrates may cause instability of the sea floor inducing landslides (Buffett, 2000). Such an underwater landslide results in additional lowering of pressure and may produce further dissociation of gas hydrate. It should be noted that the meaning of the existence of methane gas hydrate is that the area has large potential for the generation and/or accumulation of methane.

Because the density of gas hydrate is lower than that of sea water, the gas hydrate itself tends to rise upwards. Thus, an insufficient mixture of sediment in the gas hydrate and/or insufficient loading by overlying sediments causes the release of a mass of gas hydrate into a water column.

Ecosystem established at cold seeps – chemosynthesis-based ecosystems

Cold seeps can be considered as chemosynthesis-based ecosystems that support a large amount of life in deep seas (Figures 2 and 7; Sibuet and Olu, 1998). The main primary producers of the ecosystem found in cold seeps are chemolithoautotrophic microbes, such as sulfur-oxidizing bacteria. For the sulfur-oxidizing bacteria, hydrogen sulfide has the role of an electron acceptor, oxygen that of an electron donor, and carbon dioxide that of a carbon source. Such microbes that metabolize via the chemical reaction of inorganic compounds with an inorganic carbon source are called chemolithoautotrophs. An ecosystem based on chemolithoautotrophy is called a chemosynthesis-based ecosystem. This kind of ecosystem is a counterpart to the photosynthetic ecosystem found on the surface of the Earth. Chemosynthetic ecosystems establish themselves especially in areas where light does not penetrate such as the
deep sea and the subsurface below the sea floor. Within these settings, chemosynthesis-based ecosystems particularly flourish at cold seeps and hydrothermal vents because these are environments where large amounts of reduced compounds encounter oxic waters.

With chemosynthetic microbes as a basis, heterotrophic microbes and animals flourish in cold seeps as members of the chemosynthetic ecosystem. In order to flourish in a oxygen-poor cold seep environment which is instead rich in toxic substances such as hydrogen sulfide, the animals found there often have a detoxification system (Felbeck et al., 1985). Most animals living in cold seeps house chemosynthetic bacteria, such as sulfur-oxidizing bacteria and methane-oxidizing bacteria, and obtain organic matter from those symbionts. These symbiotic chemosynthetic bacteria are called as chemosymbiotic bacteria. The animals living in cold seeps are generally endemic at a higher taxonomic level and diverge from the animals belonging to photosynthetic ecosystems. Major animals living in a cold seep environment are vesicomyid, mytilid, thyasirid, lucinid, and solemyid bivalves, provannid, acmaeid and neomphalid gastropods, and vestimentiferan tube worms. Crabs, sea urchins, sponges, and barnacles are occasionally found as minor faunal elements. Nectobenthic animals, i.e., animals that can live near the sea floor, such as hydrothermal vent shrimps, are relatively fewer in number at cold seeps than in hydrothermal vent fields. This is because the reduced compounds, their energy source, are often consumed already within the sediments, and only occasionally appear above the sea floor. In contrast, infaunal animals, i.e., animals that live in the sediment, are more abundant in cold seeps than in hydrothermal vents, because the cold seeps are usually covered by rich soft sediments whereas fresh (recently formed) hard igneous rocks are exposed at hydrothermal vent fields.

The biotic composition of cold seeps is controlled by physicochemical factors including the quantity of reduced compounds such as methane and hydrogen sulfides. One good example of the biotic elements controlled by methane accumulations is seen at Hydrate Ridge (Cascadia Margin, off Oregon). Beggiatoa (sulfur-oxidizing bacteria) mats, Calyptogena (vesicomyid bivalve), and Acharax (solemyid bivalve) all flourish in this cold seep area in a zoned distribution. A large concentration of hydrogen sulfide was detected in the Beggiatoa zone, decreasing toward the Acharax zone through the Calyptogena zone (Sahling et al., 2002). A similar regulation of distribution caused by the concentration of hydrogen sulfide occurred at the species level. Two species of Calyptogena (bivalve), C. kilmeri and C. pacifica, coexisted in a cold seep but they were roughly segregated. The C. kilmeri lived where there was a much higher concentration of hydrogen sulfide and the other lived where the concentration of hydrogen sulfide was much lower (Barry et al., 1997).

The biotic component that lives in cold seep environments looks similar to those found in other reduced environments such as hydrothermal vents, sunken vertebrate carcasses, and driftwood (see Chapter Whale and Wood Falls). Actually, the components are mostly the same at a higher taxonomic level (i.e., genus or family level), although they are restricted to living in a single type of habitat at the species level (Peek et al., 1997), except for vertebrate carcasses (Smith and Baco, 2003). Twenty species found in a cold seep environment also live on/around vertebrate carcasses. Thus, vertebrate carcasses may play a role as a dispersal stepping stone prior to living in a cold seep at least for some species. It is generally thought that the macro organisms in hydrothermal vent environments have evolved from cold seep species (Hecker, 1985; McLean 1985; Craddock et al., 1995). In addition, molecular data indicate that mytilid bivalves living in a cold seep environment adapt to such a reduced environment via whale carcasses as an evolutionary stepping stone (Distel et al., 2000; Smith and Baco, 2003).

**Ancient cold seeps**

**Recognition of ancient cold seeps**

Numerous ancient cold seeps dating from various ages have been found in a variety of tectonic settings (Figures 1 and 8; see review in Campbell, 2006). The identification of ancient cold seeps depends on revealing features which indicate the presence of methane and other hydrocarbons which escaped from the sea floor. In order to make these features evident, a comprehensive approach based on geological, geochemical, and paleontological techniques is a primary requirement.

It is well known that carbonate minerals are forming in modern cold seep environments as a result of increased alkalinity due to AOM (Ritger et al., 1987; Aharon,
Methane-derived carbonates are potentially imprinted with environmental information concerning the location where they were formed. Shapes, textures, and imprinted chemical signatures of modern cold seep deposits can be seen as a good analog facilitating the recognition of ancient cold seeps. A conduit pipe such as a carbonate chimney (Takeuchi et al., 2007), although they are generally formed under the sea floor in contrast to chimneys at hydrothermal vents, is a good example indicating the route of fluid. Cold seep carbonates often have chaotic fractured structures. The fracture is usually ductile and/or accompanied by brittle sediment deformations. The deformations are probably due to active fluid venting. Microbiologically induced carbonate cements, e.g., clotted fabrics and isopachous fibrous cements, are frequently found in the carbonate. The influence of methane and microbial activity can be detected by further biogeochemical analysis (Peckmann and Thiel, 2004).

The carbon isotopic compositions of the carbonate reflect the values of the carbon source. Methane, the main gaseous component of cold seeps, generally shows negative carbon isotopic compositions in the case of thermogenic and biogenic methane (see above). The carbon isotopic compositions of thermogenic and biogenic methane commonly range from $\delta^{13}C$ to $\delta^{12}C$ and $\delta^{12}C$ to $\delta^{13}C$ (vs. PDB), respectively, and are thus distinguished from other carbon sources in the marine environment. These values will be imprinted at roughly the same level in the carbon isotopic compositions of the methane-derived carbonate. Considering that the $\delta^{13}C$-values of carbonate are often somewhat higher than the values of the source methane because of the admixture of other carbon sources (Peckmann and Thiel, 2004), values ca. lower than $\delta^{12}C$ (vs. PDB) suggest that a major portion of the carbonate carbon is derived from methane.

Lipid biomarkers provide information on ancient microbial activities. It has been revealed that AOM is mediated by microbial consortia composed of archaea and sulfate-reducing bacteria (Orphan et al., 2002). Cell membrane lipids produced by those microbes have a strong propensity to be preserved in sediments (Peckmann and Thiel, 2004). Analyzing the carbon isotopic composition of each of these biomarkers (see entry Biomarkers (Organic, Compound-Specific Isotopes)) tells us whether or not the microbes used methane as the carbon source for their biosynthesis. The carbon isotopic compositions of lipids derived from methanotrophic microbes sometimes reach as low as $\delta^{12}C$ or even lower. On the basis of this information, the presence of isotopically depleted lipid biomarkers derived from archaea will be a good indicator for the occurrence of AOM (Elvert et al., 2005).

Cold Seeps, Figure 8 Late Cretaceous cold seep deposit (above) and dense accumulation of lucinid bivalves associated with the carbonate (below) from South Dakota, USA. The carbonate rock was induced by anaerobic oxidation of methane at a late Cretaceous active cold seep in the Western Interior Seaway, located in the eastern part of what is today the Rocky Mountains in northwestern America.

2000). Methane-derived carbonates are potentially imprinted with environmental information concerning the location where they were formed. Shapes, textures, and imprinted chemical signatures of modern cold seep deposits can be seen as a good analog facilitating the recognition of ancient cold seeps. A conduit pipe such as a carbonate chimney (Takeuchi et al., 2007), although they are generally formed under the sea floor in contrast to chimneys at hydrothermal vents, is a good example indicating the route of fluid. Cold seep carbonates often have chaotic fractured structures. The fracture is usually ductile and/or accompanied by brittle sediment deformations. The deformations are probably due to active fluid venting. Microbiologically induced carbonate cements, e.g., clotted fabrics and isopachous fibrous cements, are frequently found in the carbonate. The influence of methane and microbial activity can be detected by further biogeochemical analysis (Peckmann and Thiel, 2004).

The carbon isotopic compositions of the carbonate reflect the values of the carbon source. Methane, the main gaseous component of cold seeps, generally shows negative carbon isotopic compositions in the case of thermogenic and biogenic methane (see above). The carbon isotopic compositions of thermogenic and biogenic methane commonly range from $-30$ to $-50\%$ and $-50$ to $-80\%$ (vs. PDB), respectively, and are thus distinguished from other carbon sources in the marine environment. These values will be imprinted at roughly the same level in the carbon isotopic compositions of the methane-derived carbonate. Considering that the $\delta^{13}C$-values of carbonate are often somewhat higher than the values of the source methane because of the admixture of other carbon sources (Peckmann and Thiel, 2004), values ca. lower than $-30\%$ (vs. PDB) suggest that a major portion of the carbonate carbon is derived from methane.

Lipid biomarkers provide information on ancient microbial activities. It has been revealed that AOM is mediated by microbial consortia composed of archaea and sulfate-reducing bacteria (Orphan et al., 2002). Cell membrane lipids produced by those microbes have a strong propensity to be preserved in sediments (Peckmann and Thiel, 2004). Analyzing the carbon isotopic composition of each of these biomarkers (see entry Biomarkers (Organic, Compound-Specific Isotopes)) tells us whether or not the microbes used methane as the carbon source for their biosynthesis. The carbon isotopic compositions of lipids derived from methanotrophic microbes sometimes reach as low as $-120\%$ or even lower. On the basis of this information, the presence of isotopically depleted lipid biomarkers derived from archaea will be a good indicator for the occurrence of AOM (Elvert et al., 2005).

Oldest cold seep

Ancient cold seeps with chemosymbiotic macrofossils have been traced back to at least Silurian (Barbieri et al., 2004). As it is difficult to find and recognize ancient cold seeps without macrofossils, there is practically no record of older cold seeps. However, a possible cold seep occurring after the severe glacial period around 600 Ma, i.e., the period known as “Snowball Earth,” has recently been
Changes in cold seeps through time

The number of cold seeps varies through the Phanerozoic. In the Paleozoic, there are only five ancient cold seep localities. In spite of the fact that a stratigraphic change in carbon isotope ratios shows a global negative shift at the Permian/Triassic boundary, probably caused by the release of a large amount of methane into the ocean and the atmosphere, probably from methane gas hydrate, there are no records of ancient cold seeps from the Permian and Triassic. However, the number of records of ancient cold seeps in post-Triassic marine sediments has increased. More than 15 localities have been found in the Mesozoic and in an abundance of localities, more than a dozen, from the Cenozoic (Campbell, 2006). The cause of the drastic increase in cold seeps since the late Mesozoic might be due to an increase of organic flux into sediments resulting from increasing planktonic productivity and diversity during the Mesozoic Era (Tappan and Loeblin, 1973; COSOD II, 1987) leading to increasing generation of methane in marine sediments.

The biotic components of cold seeps changed through the Phanerozoic (Campbell and Bottjer, 1995; Little and Vrijenboek, 2003). Mollusks are the most dominant organism in modern cold seeps, despite the dominance of brachiopods in the Paleozoic and mid-Mesozoic. The brachiopods were replaced by mollusks, mainly by bivalves, in the late Mesozoic (Campbell and Bottjer, 1995; Kiel and Peckmann, 2008). The last abundant occurrence of brachiopods in cold seeps is found from the Campanian, late Cretaceous (ca. 80 Ma), from the Omagari, Hokkaido, Japan (Hikida et al., 2003; Kaim et al., 2010). Vesicomiyd and bathymodiolian bivalves, which flourish in modern cold seeps, entered the cold seep environment during the Eocene (ca. 40 Ma; Kiel, 2006; Amano and Kiel, 2007; Amano et al., 2008). It should be noted that the fossil record of vesicomiyd bivalves has recently been reexamined and its origin changed from Cretaceous to Eocene (Amano and Kiel, 2007; Amano et al., 2008; Kiel et al., 2008). Most living molluscan genera which lived in ancient cold seeps originated in the late Mesozoic to Paleogene (see basic data in Kiel and Little, 2006 and updated data in Amano et al., 2007; Jenkins et al., 2007; Campbell et al., 2008; Kaim et al., 2008; Kiel et al., 2008; Kaim et al., 2009).

Impact on climate change

Judd et al. (2002), indicate that the input of greenhouse gases from the sea floor into the world’s oceanic and atmospheric systems may be an important component of the atmospheric carbon budget, because the greenhouse potential of methane is more than 20 times that of carbon dioxide. A catastrophic release of methane may therefore produce global climate changes. Such an event due to the rapid escape of methane into the atmosphere happened at the Paleocene–Eocene boundary (ca. 55.5 Ma). At that time, the rapid dissociation of methane hydrate and subsequent release of methane into the atmosphere resulted in global warming and an increase of 6°C in the sea surface temperature (Dickens et al., 1995). A similar methane release outburst is thought to have occurred at the end-Permian forming one of the biggest known biodiversity crises (see entry Critical Intervals in Earth History) (Ryskin, 2003; Retallack and Krull, 2006). Kennet et al. (2002) proposed that the periodic glacial-interglacial cycle during Quaternary was controlled by the decomposition of methane gas hydrate and the subsequent release of methane gas into the atmosphere. Evidence of these methane releases comes mostly from carbon isotopic anomalies which were recorded in carbonate and organic material. The release of isotopically depleted biogenic methane induces negative carbon isotope excursions because of the transfer of 13C-depleted carbon from methane to carbon dioxide.

Summary

Cold seeps have been the object of considerable attention because of their role as a connecting pipe between the lithosphere, hydrosphere, and biosphere on the Earth. Reduced compounds contained in cold seep fluid result in substantial biogeochemical reactions. The establishment of a cold seep requires specific geological and biological background conditions, such as tectonic stress to make a route for the fluid, sedimentary organic matter supply to provide a source of methane and other hydrocarbons, microbial and/or geothermal activities to form the hydrocarbons, and microbial activities accounting for the oxidation of reduced compounds. Revealing the processes and mechanisms has largely progressed during the last 2 decades. For example, it was revealed at the time of the transition from the twentieth to the twenty-first century that anaerobic methane-oxidizing archaea and sulfate-reducing bacteria are responsible for the anaerobic oxidation of methane. However, there are still many questions about cold seeps that remain unresolved, e.g., the long-term behavior of cold seeps, response to climate change and details of various biological processes and mechanisms. Gaining an understanding of cold seeps is a primary requirement because a cold seep provides a condensed version of
The knowledge that we can obtain from the research on cold seep may help us to understand a much wider range of geobiological phenomena taking place on Earth. Furthermore, methane, the main component contained in cold seep fluids, has a potentially large effect on global warming. It is evident from several geological records through the Phanerozoic that dissociation of methane gas hydrate has drastic effects in terms of global climate change. Methane is also attractive to human beings because of its large potential as a future energy source. In fact, the methane amount stored in gas hydrate beneath the sea floor and permafrost is more than 2,100 Gt. However, as noted above, methane has a tremendous potential to bring about global climate change on the Earth. Thus, any form of intervention in the natural gases in marine sediments should be carefully carried out on the basis of sufficient knowledge of what will happen when the methane is released from the sea floor. Adopting this stance implies a very strong requirement to reveal the entire range of processes and mechanisms within a cold seep.

### Bibliography


COPPER

Definition

Copper (Cu) is a transition metal that serves as an essential nutrient to eukaryotes and many prokaryotes due to its many functions in copper enzymes. At elevated concentrations, Cu is toxic and the many industrial uses of copper...
and its release to the environment has resulted in Cu pollution in soils and surface waters.

Properties: Copper has two stable isotopes $^{63}\text{Cu}$ and $^{65}\text{Cu}$ with a natural abundance of 69.17 and 30.83, respectively, and a standard atomic weight of 63.546 (Coplen et al., 2002; Wieser, 2007). The copper concentration in the bulk continental crust, in the oceanic crust, and in soils is estimated to be 27 ppm (Rudnick and Gao, 2003), 78 ppm (Wedepohl and Hartmann, 1994), and 39 ppm (Han et al., 2002), respectively. The concentrations of copper in polluted seawater is typically around 0.3 ppb and in river water, 1.5 ppb (Gaillardet et al., 2003). The world average concentration of copper in rivers has been estimated as 23.6 nM Cu dissolved load and 100 ppm Cu suspended particulate load (Martin et al., 1993; Martin and Meybeck, 1979; Martin and Thomas, 1994). In natural systems, it occurs in the Cu(I) (cuprous) or Cu(II) (cupric) redox states and rarely as elemental copper. The standard redox potential of the aqueous Cu(I)/Cu(II) couple is at 0.34 V, but the actual redox potential depends strongly on its coordinative environment. This is exploited by organisms using Cu as reactive center of reductases with high redox potentials between +0.2 and +0.8 V.

Copper toxicity: Due to its toxicity to humans, the US-EPA maximum contaminant level goals and EU standards for copper in drinking water has been set at 1.3 (USEPA, 1991) and 2 mg/l (EU, 1998), respectively. Copper is also toxic to marine and terrestrial prokaryotic and eukaryotic organisms (Flemming and Trevors, 1989; Gledhill et al., 1997). Its toxicity derives from its redox reactions producing radical ions similar to Fenton chemistry causing oxidative stress. Phytochelatins (plants) and metallothioneins (animals, fungi) are intracellular metal binding proteins that play an important role in detoxification and homeostasis of Cu and other metal ions.

Copper as a nutrient: Copper-containing oxidoreductase enzymes are widespread among prokaryotic and eukaryotic organisms and mediate important biogeochemical processes, including nitrification, denitrification, and methane oxidation. Examples are particulate methane monooxygenase, ammonia monooxygenase, nitrite reductase, cytochrome c oxidase, and superoxide dismutase. It has been suggested that Cu-bearing reductases evolved only after the oxygenation of the Earth atmosphere, considering the low solubility of Cu in sulfidic environments and the high redox potential of organically bound Cu(I)/Cu(II) up to the limit of the stability field of water (Fraústo da Silva and Williams, 2001). Copper is part of a metalloenzyme involved in the uptake of iron by diatoms so that a Cu/Fe co-limitation of primary productivity in the ocean is conceivable (Maldonado et al., 2006; Peers et al., 2005). Recently, it was discovered that some methanotrophic bacteria possess a high-affinity Cu uptake system involving methanobactin, a structurally distinct chromopeptide metallophore analogous to iron-specific siderophores (DiSpirito et al., 1998). This highlights the potential role of copper availability and copper uptake in regulating biogeochemical cycles.

Bibliography


Cross-references

Metals, Acquisition by Marine Bacteria
Siderophores
Cosmic Molecular Clouds

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Synonyms
Complex molecular clouds; Interstellar clouds; Interstellar dust and molecules; Molecular clouds

Definition
A cosmic molecular cloud is an accumulation of cosmic matter that is composed of organic and inorganic molecules. The dust grains in a cosmic molecular cloud are mainly composed of crystalline silicates (Watson et al., 2009) covered with ice of H₂O, NH₃, and CO₂. The main molecule constituent is H₂-He, but other molecules also occur, e.g., NH₃, CO, and complex organic molecules.

Cosmic molecular clouds are denser than the interstellar medium, with gas densities ranging from about 10 molecules per cm³ on large scales to over 10⁷ molecules per cm³ in cloud cores. These cores are very cold, with temperatures ranging from 8 to 20 K. New stars and protoplanetary discs are formed, when it comes to a gravitational collapse of cosmic molecular clouds. Via nucleosynthesis, these stars produce elements, which may constitute organic molecules (e.g., H, C, N, O, S, P). When stars burn out, they collapse either via catastrophic explosions or by forming expanding shells, thereby recycling modified material back into the interstellar space and, thus, forming new cosmic molecular clouds (Boss, 2004). Some of the interstellar clouds are enriched in prebiotic organic molecules. These molecules are found in cosmic matter, chondritic asteroids/meteorites, and cosmic dust particles.

Based on spectroscopic wavelength analyses, more than 120 different complex organic molecules could be detected within cosmic molecular clouds, including common PAHs (polycyclic aromatic hydrocarbons), amino acids, sugars ("polyols"), hydrogen cyanide (HCN), and formic acid (Figure 1) (Ehrenfreund and Menten, 2001). The largest organic molecules so far reported from cosmic molecular clouds are cyanodecapentayne (HC₃₀N) and diethyl ether (C₅H₁₂O). Not all of these molecules are part of the gaseous phase, but the more complex ones are related with cosmic dust particles. A well studied cosmic molecular cloud is the Sagittarius B cloud, where the most complex organic molecules like ethyl cyanide, acetone, glycine, acetic acid, and glycolaldehyde were detected (Hollis et al., 2000; Snyder, 1997). Glycolaldehyde may react with ribose to form a molecule, which is a major constituent of RNA. It was hypothesized that organic molecules within a cosmic molecular cloud are formed on 1–2 µm-sized dust grains, e.g., via photochemical reactions. These dust grains have a distinctive shell-like structure. An inner core of silicates is covered by an inner rim...
containing complex organic compounds like amino acids, “polyls,” and carbonaceous matter such as hydrogenated amorphous carbon or kerogen-like material. The inner rim is covered by an outer rim consisting of “dirty” ice. In that icy outer rim, due to the heavy ultraviolet radiation, radicals are formed and H₂ is dissociated in monomolecular H that may react with other radicals to form organic molecules (Sorrell, 2001; Pearson et al., 2002). An important finding obtained with the Hubble Space Telescope was the observation of protoplanetary discs in the Orion cloud (Megeath et al., 2005). The size of these discs is more or less similar to our solar system. During solar system formation, an interstellar cloud becomes the building block, from which planets, asteroids, and comets form. Comets and carbonaceous chondrites are chemically very similar to star-forming interstellar clouds and are relics of them (Busemann et al., 2006; Wickramasinghe et al., 2009).

They carry pristine primitive organic matter and represent a plausible source of prebiotic organic matter on the late Hadean early Earth. The stable isotope compositions of organic matter, e.g., from the Murchison carbonaceous chondrite (CM2), provide clear evidence that the organic matter was formed under conditions in stellar clouds and not on Earth (Sephton et al., 2003, see entry “Meteoritics” for further reading). External delivery of water and organic compounds is widely accepted as the origin of pristine earth-related organic matter (Wickramasinghe, 2010) and may have pushed the start of early life with the first microbial organism – LUCA – Last Universal Common Ancestor (Hoenigsberg, 2003).

Cross-references
Asteroid and Comet Impacts
Astrobiology
Chondrites
Meteoritics
Origin of Life

Bibliography
Great Oxygenation Event or the Great Oxidation Event, respectively (GOE, ca. 2.4 Ga), the Cambrian Explosion around 524 Ma (Lower Cambrian), and the evolution of land plants from the Middle Ordovician on (ca. 463 Ma; Figure 1).

Critical intervals of subordinate significance are mass extinction events. It is clear that any of these phases of mass extinction reflect severe perturbation in the global ecosystem, and there is no doubt that the biotic recovery from mass extinctions is also a critical period in the regeneration of the biosphere. However, when carefully considering the most significant phases of mass extinction in the Phanerzoic (the “Big Five”; Figure 1), we see that its aftermaths never resulted in the evolution of any new clades. Although critically for the fauna extinct, new taxa always developed in a saturated evolutionary landscape, where no space was for innovations at a clade’s scale. These events, thus, represent “merely” a convulsion of the taxonomic inventory and are regarded here as subordinate critical intervals.

The study of critical intervals in the history of life aims at the mapping of the paleobiological and geological changes during these intervals, the interaction between the both, and the attempt to extract possible complex triggering mechanisms for the phenological expression in the (biogeochemical) fossil record within its geological framework. The study of critical intervals is, thus, an ideal example of how geobiology is embedded in interdisciplinary research of classic paleontology, genetics, bio- and isotope geochemistry as well as sedimentary geology, to name some of the disciplines inevitably applied.

Great Oxygenation Event, ca. 2.4 Ga (Great Oxidation Event)

Definition

Sedimentological and geochemical data indicate that the atmosphere became suddenly oxygenated up to a \( p_{O_2} \) of ca. 1% of present atmospheric level (PAL) around \( \sim 2.4 \) Ga (Kump, 2008; Figure 2). This period is called the “Great Oxygenation Event” or the “Great Oxidation Event” (GOE). Thus, it marks the boundary between an oxygen-free and an oxygenized world. The GOE approximates stratigraphically the boundary between the Archean and the Proterozoic (2.5 Ga).

Evidences

The evidence for atmospheric oxygenation results from various independent sources, which also provide controversial data (see compilations in Holland, 2006; Falkowski and Godfrey, 2009; Figure 2).

Sedimentological aspects: Prior to the GOE, detrital pyrite, uraninite, and siderite are known from siliciclastic fluvial sediments – minerals that would have been oxidized under the presence of \( O_2 \) either in the atmosphere or water – and this clearly indicates the lack of any free oxygen. There are no records of these minerals younger than \( 2.5-2.3 \) Ga. Around 2.4 Ga, the first red beds developed in the continental areas, resulting from dispersedly distributed ferric oxide in the sediments, which only could have developed by oxygenation of ferrous iron under the presence of free atmospheric \( O_2 \). A while later, the mineral glauconite (containing ferric iron) occurred for the first time.

The occurrence of banded iron formations (BIFs) from the early Archean on (ca. 3.8 Ga) with its peak occurrence
at ca. 2.6 Ga, bearing large amounts of ferric iron, were previously thought to correlate positively with the occurrence of oxygen (see overview in Klein, 2005). However, as shown by Kappler et al. (2005), the precipitation of ferric oxides by anoxygenic phototrophic Fe(II)-oxidizing bacteria could also explain the development of BIFs, which then is not indicative of any O2 availability.

**Geochemical evidence:** Geochemically, the period around 2.4 Ga is characterized by the establishment of mass dependent sulfur isotope fractionation (Bekker et al., 2004; Farquhar and Wing, 2003) and a first enrichment of molybdenum in black shales (Scott et al., 2008), both indicative of a progressively oxidized atmosphere. In addition, chromium isotope fractionation points at slightly elevated O2 levels even earlier than the GOE already at ca. 2.8–2.6 Ga (Frei et al., 2009).

**Possible causes for the GOE**

There are a variety of models that try to explain the GOE. **Onset of oxygenic photosynthesis/anaerobic oxidation of methane (AOM) (2.7–2.6 Ga).** Anaerobic methanogenesis by archaea (*via* methyl-CoM reductase) was responsible for an estimated atmospheric methane concentration of 1,000 ppm and extreme greenhouse conditions in the early atmosphere. The rise of oxygenic photosynthesis by cyanophyta (at ~2.45 Ga) as suggested by biomarker evidence (Buick, 2008), and the onset of AOM by sulfate reduction (at ~2.7 Ga; Hinrichs and Boetius, 2002) resulted in a significant withdrawal of CO2 and CH4 from, and an enrichment of O2 in the atmosphere. Anbar et al. (2007) proposed “a whiff of oxygen” some ca. 90 Ma before the GOE, and Kaufman et al. (2007), based on sulfur isotopes, suggested slightly oxic conditions in the ocean prior to oxygenation of the atmosphere, but the sudden oxygenation event at 2.4 Ga has also been considered as a direct result of increased microbial activity reaching a ‘critical point’ by some scientists.

**Time lag:** An alternative approach derives from the assumption that oxygenic photosynthesis had already been established around 3.2 Ga (deduced from kerogen-rich black shales) or even 3.8 Ga (deduced from U–Th–Pb isotopy; see Buick, 2008 for discussion). The time lag...
between the onset of oxygenic photosynthesis and the GOE has been explained by two models. Konhauser et al. (2009) observed a decrease in nickel concentration of ocean waters around 2.4 Ga. As nickel is a crucial element in several enzymes of methanogens, a decline would have severely dropped its activity (nickel famine). The stagnation of biogenic methane supply would then have favored a rapid increase of environmental oxygenation. Goldblatt et al. (2006) proposed, based on modeling, an alternative explanation. Assuming the onset of oxygenic photosynthesis at 2.7 Ga, the 300 Ma time-lag is explained by the bistability of the atmospheric oxygen system. A low oxygen steady state system existed at oxygen levels around and below \(10^{-5}\) PAL prior to the GOE. With oxygen levels higher than \(10^{-5}\) PAL, UV shielding becomes more efficient, which resulted in a nonlinear increase of atmospheric oxygen’s lifetime and thus, oxygen content. The high-oxygen steady-state system stabilized at values larger than \(5 \times 10^{-3}\) PAL.

Concluding remarks
Even though the timing of the onset of oxygenic photosynthesis is controversially debated, sedimentological and geochemical data clearly point at a time around 2.4 Ga, where the atmosphere became slightly oxygenized with values of \(10^{-3}\) PAL. The presumably microbially induced severe drawdown of the greenhouse gases \(\text{CH}_4\) and \(\text{CO}_2\) and the increase of \(\text{O}_2\) resulted in a temperature drop of estimated 4–9°C (Goldblatt et al., 2006), leading directly to the climate disaster of the Paleoproterozoic Huronian snowball earth glaciations at ca. 2.3–2.1 Ga (Kopp et al., 2005).

The GOE is also regarded as an oxygen catastrophe or oxygen crisis. In fact, from the moment on, when oxygenized environments in the oceans and in/on the ocean floors developed, these oxic environments (which progressively increased) were no longer habitable for anaerobic microorganisms. Thus, the GOE also marks a first fundamental and irreversible turnover of shelf microbial community structure and, thus, a first truly critical interval in Earth’s history.

The Cambrian Explosion
Definition
The Cambrian “Explosion” marks a sudden outburst of morphological disparity (distinctness) and diversity among metazoans during the early Cambrian. Starting around ca. 524 Ma, the number of known genera increased within roughly 10 Ma from something about 200 genera in the Tommotian (Lower Cambrian) to ca. 1,200 genera in the Botomian (highest Lower Cambrian, Figure 3). During this interval, all modern phyla occurred in the sedimentary record, including the vertebrates, thus, being the birth of the “tree of animals” (Shu, 2006). An overview on the ecology of the Cambrian Explosion is given by Zhuravlev and Riding (2001).

The way to the Cambrian Explosion
The explosion of life is impressively documented by the rich and excellently preserved faunas in a number of important Cambrian “fossilagerstätten,” which opened a preservation window to this early period of metazoan evolution and diversification. Maybe, the most famous ones are the Chengjiang and Sirius Passet biota (Lower Cambrian; Figures 4 and 5) and the Burgess Shale biota (Middle Cambrian; Figure 3). There have been repeated discussions in the past, whether we see a true pulse of evolution or whether we face merely exceptional preservational hot-spots, and based on calibration of the molecular clock some authors suggest increasing disparity already around 1 Ga (e.g., Blair and Hedges, 2004). Other molecular clock dating suggests a pulse of evolution of the Metazoa in the Cryogenian, but a first radiation pulse occurred at ca. 635–542 My (Peterson et al., 2008). This approximates roughly the first occurrence of animal macrofossils at 575 Ma. However, at the moment, the literature data suggest a common understanding that we see a true expression of metazoan radiation in the fossil record. To appreciate this event in its significance fully, it is desirable to highlight it in its evolutionary context from the first occurrence of larger animal body fossils and trace fossils from ca. 575 Ma on (Figure 3).

The post-GOE times from 2.4 Ga on were a period of evolutionary stasis – the “boring billion” of Kerr (2005) – in which stromatolites had their abundance and diversity peak from ca. 2.0 to 1.0 Ga. Either complex Cyanophyta colonies or possibly the first eukaryotic algae (Grypania) occurred by 2.0 Ga, and the first red algae are known since 1.2 Ga (Butterfield et al., 1990). The rise of complex animal life (Metazoa: Parazoa) is dated by some authors around 1 Ga, based on carbonate rock texture (Neuweiler et al., 2009) or by molecular clock dating (e.g., Hedges et al., 2004). After the last Snowball Earth glaciation around 580 Ma (see Hoffman and Schrag, 2002 on the Snowball Earth theory), enigmatic body fossil of organisms with unknown taxonomic affinities, the Vendobionta (reaching already sizes up to 2 m!), occurred worldwide at ca. 575 Ma. The Vendobionta show a typical “quilt” bauplan (Figure 6), and they are intimately associated with microbial mats (“elephant skin”), specifically large sulfur bacteria (Steiner and Reitner, 2001). At ca. 560 Ma, unequivocal trace fossils prove the existence of bilaterians (see discussion of problematical trace fossil records from 1 Ga in Jensen, 2003). Dated as 580–570 Ma, the Wengan Phosphorites (Doushantuo Group, China) yielded structures that resemble metazoan embryos, but its metazoan nature has recently been doubted and reinterpreted as phosphatized, large sulfur bacteria with affinities to the Thiomargarita group (Bailey et al., 2007). However, possible body fossil of sponge larvae occur also, and by 550 Ma, fossil sponges (Hexactinellida) are known from the Dengying Formation (China). Of the same age is Kimberella (White Sea region), which is interpreted as a nonmineralized mollusk, which then marks also the first body fossils of a bilaterian metazoan. The same period
also marks the onset of enzymatic biocalcification. The first mineralized skeletons occur in the form of \textit{Namacalathus} and \textit{Namapoikia} in Namibia around 550 Ma, which could be coralline sponges (Grotzinger et al., 2000; Wood et al., 2002). Calcified tubes of \textit{Cloudina} (Figures 7 and 8) show large resemblance with the deep sea vent and cold seep related \textit{Escarpa}, a pogonophorid worm. All three taxa occur together within thrombolithic and finely laminated microbial assemblages in the first reef-like structures in the form of small mud mounds (Figure 9).

In the Lower Cambrian (Nemakit-Daldynian), calcareous Archaeocyatha (possible Demospongia) formed the first Cyanophyta/sponge mud mounds. Concomitantly, phosphatic and calcitic microfossils or mineralized sklerites of parts of unknown organisms, the Small Shelly Fauna, SSF, appeared – partly in large abundances – worldwide. Only little later, in the Lower Tommotian, first shells of brachiopods, mollusks, and arthropods occurred (for an overview of the biotic development prior to the Cambrian Explosion see Valentine, 2002; Xiao and Kaufman, 2007).

Ecologically, most of the Ediacaran period was considered as a peaceful “Garden of Ediacara” by McMenamin (1986), as no evidence of predation is known from this time. Instead, it appears that the biomat-related Vendobionta represented an ecologically and evolutionary very stable steady-state system without any significant innovations for over 30 Ma. Thus, the slowly increasing disparity, which we recognize close to the base of the Cambrian, resulted exclusively from speciation among the Metazoa. Even more, paleobiogeographical and facies affinities (e.g., different species of \textit{Cloudina} occupied different habitats) were now recognizable. And, trouble in paradise became visible by small holes in \textit{Cloudina} – the first evidence of predator–prey interaction (Hua et al., 2003).
The increasing complexity among the Metazoa finds its expression also in progressively more complex burrow systems. While in the Middle Ediacaran sub-biomat miners created simple horizontal furrows on the sediment’s surface, terminal Ediacaran burrows became three-dimensional, such as, for example, *Trichophycus pedum*, the biostratigraphical index marker for the base of the Cambrian system (ca. 542 Ma). It is specifically this trend, which was responsible for the first severe extinction among multicellular animals: approximately at the base of the Cambrian, the Vendobionta decreased significantly in abundance to become completely extinct in the Lower Cambrian. Seilacher and Pflüger (1994) suggested that the progressive complexity among infaunal burrowers and burrow intensities resulted in an irreversible destruction of the microbial mats – the essential for the Vendobionta (agronomic revolution).

Possible reasons for the Cambrian Explosion
For the slow but continuous increase of disparity and diversity over ca. 60 Ma in the terminal Ediacaran and its explosive increase around 524 Ma, a number of possible triggering mechanisms are suggested, proposing changes in the abiotic, biotic, and genetic evolutionary landscapes (see, a.o., Marshall, 2006 and Levinton, 2008 for a compilation).
Changes in the abiotic evolutionary landscape (environmental changes)

(a) Oxygen level: An explanation for the increasing disparity in the Ediacaran might be a second oxygenation event to a \( pO_2 \) near modern values as deduced from chromium isotopes (\( \delta^{33} \text{Cr} \)) around 800–542 Ma (Frei et al., 2009) or by 580 Ma (Canfield et al., 2009). Before this time, geographically widespread occurrences of black shales indicate anaerobic deep oceans: due to the aerobic continental weathering of detrital pyrite and, resulting, riverine sulfate input into the oceans, its bacterial reduction caused deep ocean saturation of \( H_2S \) (“Canfield Ocean,” see Canfield, 1999; Figure 2). This caused a reduction of iron, copper, and molybdenum, all less soluble under the presence of hydrogen sulfide. Because these play important roles in the enzymatic metabolism of the Cyanophyta, the depletion of these elements caused malnourishment, which might have blocked evolutionary advance for more than a billion years. Only after the last episode of the Snowball Earth period (Gaskiers Glaciation), molybdenum isotopes indicate a second oxygenation event and progressively oxygenized deeper part of the oceans. This could have increased the capacity for metabolism of larger Metazoa and the evolution of the Vendobionta and first metazoans can be seen in the light of this atmospheric/oceanic change. However, the long delay between the terminal Proterozoic oxygenation event and the Cambrian Explosion of estimated 50–60 Ma makes a direct link unlikely. On the other hand, the increased \( O_2 \) amount doubtlessly acted as an indispensable prerequisite for size increase and evolution of the Metazoa.

(b) Global carbon cycle: In the Vendian/Cambrian boundary interval, numerous published \( \delta^{13} \text{C} \) curves show vivid fluctuations and a negative \( \delta^{13} \text{C} \) peak with up to \(-12\) per mil in the Upper Ediacaran. The observed \( \delta^{13} \text{C} \) fluctuations are surely expression of heavy perturbations in the global carbon cycle and, thus, indicate environmental disturbances. There is a wealth on literature on this problematic, and variety of complex and differing explanation models exists, as illustrated by two examples: For the terminal Ediacaran negative \( \delta^{13} \text{C} \) peak, Kirschvink and Raub

Critical Intervals in Earth History, Figure 8 First calcareous biomineralization: Thin sections of Cloudina from the Dridoornvlakte area, Namibia. Latitudinal section; Same magnification like Fig. 7.

Critical Intervals in Earth History, Figure 9 Latest Proterozoic mud mounds, exhumed by erosion, with Namacalathus (sponge?), Cloudina, and thrombolithic microbial limestones. Swartkloof area, Namibia.
(2003) postulated methane release, resulting in a rise of global temperatures as a triggering pulse for the Cambrian Explosion, while Bristow and Kennedy (2008) suggested that the negative excursion resulted from a non-steady-state oxidation of oceanic dissolved organic carbon (DOC), resulting from a rise of atmospheric oxygen (see above).

(c) Ocean water chemistry: The ocean water geochemistry changed progressively from a Proterozoic soda ocean towards a late Proterozoic halite ocean during the Ediacaran (e.g., Hardie, 2003). As seawater carbonate alkalinity triggers the possibility of Ca\(^{2+}\) extraction, the decreasing pH values progressively facilitated enzymatic CaCO\(_3\) mineralization. Geochemical data from terminal Proterozoic (~544 Ma) and early Cambrian (~515 Ma) marine halite indicate that seawater Ca\(^{2+}\) concentrations increased approximately threefold during the early Cambrian (Brennan et al., 2004). A high Ca\(^{2+}\) and low [SO\(_4\)]\(^{2-}\) composition of the early Cambrian sea water is recorded by Petrychenko et al. (2005), which then could be responsible for the sudden occurrence of organisms with strongly mineralized shells and sklerites consisting of CaCO\(_3\).

(d) Global sea-level and paleoceanography: The terminal Ediacaran is a period of global sea-level rise, and the break-up of Rodinia from ca. 850 Ma on created a huge amount of environmentally differentiated shelf areas, which became flooded during this interval. These provided an immense potential for specialization and diversification of shelf organisms. However, from the Ediacaran through the Cambrian, there is a continuous flooding of shelf areas (Miller et al., 2005; Haq and Schutter, 2008), and there seems be no or only little relation to the Cambrian Explosion.

Changes in genetic and developmental capacities
With the first occurrence of the Bilateria, the bilaterian development system had developed. When we accept the Ediacaran Kimberella as a molluskan Bilateria, then the bilaterian development system must predate the Cambrian Explosion significantly. As a small increase of genomic complexity can result in high morphological diversity, the Cambrian Explosion cannot exclusively be explained by developmental innovations (see compilation and discussion in Marshall (2006).

Changes in the biotic environment
Interactions among organisms is difficult to recognize in the fossil record. However, predator–prey relations could be reconstructed in some cases as we see traces of predation, manifested by damages in mineralized skeletons. The first record of predation is known from small holes in Cloudina, bioerosive evidence of predation. With the arthropod Anomalocaris from the Lower Cambrian (Chengjiang Biota), a first top predator occurred. This shows that predation pressure and the need to predate had well established, which opened a wide window for any imaginable evolutionary interactions and its morphological manifestation in the form of a biological arms race.

Biomineralization
After all, the Cambrian Explosion is phenologically also an expression of increased biomineralization, why it is also a biominalization event. We have no knowledge about diversity of any nektonic and benthic nonmineralized bilaterians before the Cambrian Explosion, because they are simply not preserved. To argue that the increase of trace fossil diversity matches that of the body fossil diversity is in a strict sense not feasible because trace fossils are expression of behavior, and it is impossible to deduce body fossil diversity from trace fossil diversity (the same type of trace fossil can be generated by several organisms of different groups).

Concluding remarks
The rise of the Metazoa and increasing bioturbation of the sea floor resulted in the extinction of the Vendobionta – the first period of multicellular animals extinction in Earth’s history (if one accepts that the Vendobionta are multicellular eukaryota). The succeeding Cambrian Explosion is still difficult to interpret. Although there are a number of possible explanations for environmental and biological changes during the Lower Cambrian, none of the mechanisms alone provides sufficient argument to exclusively explain the explosive radiation of invertebrates within this short period of only ca. 10 Ma so far.

The rise of land plants
Generalities
The first multicellular marine plants are known since ca. 1.2 Ga (Butterfield et al., 1990; Figure 2), and although molecular data suggest some kind of very primitive land plants around 700 Ma (Heckman et al., 2001), it took further 230 Ma until first multicellular complex land plants (Embryophyta) occurred in the Lower(?)/Middle Ordovician (Darriwillian, ca. 465 Ma). These records are phytodebris and cryptospores with uncertain affinities (Steemans and Wellman, 2004; Figure 1). In the Upper Ordovician to Lower Silurian, cryptospore diversity and abundance decreased significantly, while trilete/hilare spores became frequent, radiated, and finally, the first vascular plant remains occurred in the Silurian (Wenlock) ca. 40 Ma later (e.g., Wellman et al., 2003; Gensel, 2008). A first spectacular fossilgerstätte of advanced Embryophyta is the Lower Devonian Rhynie Chert with excellently preserved cellular structures (Figure 10), and it is possibly the best investigated case study of an early terrestrial ecosystem (see Wellman, 2004; Taylor et al., 2005; with additional references therein). Still in the Devonian, first seed plants, trees and multi-storied forests occurred. Overviews over the (early) land plant evolution can be found, a.o., in Graham et al. (2000), Gensel and Edwards (2001) or Gensel (2008).
Associated environmental changes
There can be no doubt that the occupation of land with its succeeding evolutionary evolutions over ca. 60–80 Ma represents possibly the most significant step in plant evolution since the development of the first eukaryotic plant cell. However, the progressively increasing occupation of the continents by land plants resulted also in significant changes in Paleozoic ecosystem structures and had severe impact on a number of geological processes such as weathering, the development of the atmosphere, and global carbon and hydrological cycles.

Development of land animals
The spread of land plants represented a first significant occurrence and increase of terrestrial primary productivity and, thus, the prerequisite for animal life on the continents. However, DiMichele and Hook (1992) suggested that there existed no direct positive correlation between land plant and early land animal evolution in the sense of herbivory. Instead, the biomass affected the animal food web via a detritus chain. The increasing complexity of terrestrial animal ecosystems can be deduced by the progressive diversification of arthropods as well as trace fossil abundance in paleosols and continental deposits from the Ordovician on (see Retallack and Feakes, 1987; Buatois et al., 1998), and the occurrence of simple food webs are suggested by the finds of predatory arthropods already for the Silurian (Jeram et al., 1990).

Effect on continental weathering, erosion and nutrients
The evolution of plants and the occurrence of deep roots triggered the development of paleosols, which are known since the Ordovician (see overview in Retallack, 2005). The roots favored physical weathering, and the evolution of root symbionts such as mycorrhizae acted as a chemical weathering agents. A further pedogenic factor is the increasing amount of bioturbation, which, by its large surface and permeability, favored chemical weathering. The development of soils advanced likewise erosion and an increased continental run-off of nutrients in to the oceans (see Algeo and Scheckler, 1998).

Effect on the atmospheric composition
With the occurrence of land plants, a feedback system between atmospheric CO2 evolution and land plants established (see Beerling and Berner, 2005). The withdrawal of CO2 by photosynthesis and by increased weathering of Ca–Mg silicates resulted in progressive drop of atmospheric pCO2 from the Silurian on (Beerling and Berner, 2005).

Concluding remarks
Although the development of continental ecosystems is much less appreciated in the literature than marine environments, the significance of the land plant evolution is enormous. Apart from the above-mentioned geological aspects, the development of continental primary productivity was the base for animal life on land and the progressive establishment of complex ecosystems as we see them today. Plants did the first step, animals followed. The shore leave of the vertebrates in the Devonian would not have been possible without the occurrence of land plants.

Extinction events
Generalities
An extinction event is the – in a geological sense – abrupt decrease of speciation, diversity, and abundance within a geologically very short time. It affects all groups of organisms at various degrees. As mentioned above, we do not consider extinction events as classical critical intervals, as we never see the extinction, or in the aftermath of extinction, the entry, of new phyla. Instead, we see something like a phylogenetic steady-state, where even after heavy extinction no evolutionary playground for complete innovations at high levels was created.

The “Big Five”
Classically, five main extinction events are distinguished in the Phanerozoic, in the literature referred to as “the Big Five.” These are (Figure 1)

1. The Upper Ordovician event with an estimated extinction of ca. 85% of the marine species and ca. 50% of marine genera (see overview in Krug and Patzkowsky, 2004 and Sheehan, 2001).
2. The Upper Devonian Kellwasser and Hangenberg events (Frasnian/Famennian boundary) with an extinction rate of ca. 20% of all animal families and 70–80% of all animal species (see overview in McGhee, 1996; Over et al., 2005; Becker and Kirchgasser, 2007).
3. The Permian–Triassic boundary (P/Tr boundary event) with an extinction of ca. 95% of the shelf biota and
65–70% of the vertebrates. Nicknamed “The Big Dying,” it was the most dramatic mass extinction in the Phanerozoic (see overview in White, 2002; Over et al., 2005; Şengör and Atayman, 2009).

4. At the Triassic–Jurassic boundary (Tr/J boundary event) with a loss of ca. 77% of all species and ca. 41% of all mesobenthic and macrobenthic genera (see overview in Tanner et al., 2004; Hesselbo et al., 2007).

5. At the Cretaceous/Tertiary boundary (K/T boundary event). This event caused extinction of ca. 65% among the species. Among the victims were so popular groups such as ammonoids and dinosaurs (see overview in Ryder et al., 1996; MacLeod et al., 1997).

Causes

There exists a flood of ideas and literature about the possible causes of mass extinctions with complex interactions and feedback mechanisms. Here, some of the popular concepts will be briefly introduced, which either focus at the trigger or the kill mechanisms.

**Impact events.** The trend-setting discovery was the recognition of an iridium anomaly at the K/T boundary Event by Alvarez et al. (1980), who suggested that a bolides’ impact was responsible for the end-Cretaceous extinction of dinosaurs and a great number of typical Cretaceous marine invertebrates such as ammonites, belemnites, inoceramids, and rudist bivalves. Later, tektites (impact glasses) were discovered in the boundary interval in numerous sections worldwide. Today, the Chixulub structure on the Yucatan peninsula of Mexico (ca. 170 km diameter) is seen as the associated impact structure, although there is still some controversy about the stratigraphical fit of the extinction and the dating of the impact (Keller et al., 2007). The inferred relation between mass extinction and large impacts resulted in a search of impact evidences such as craters, spherule layers, iridium anomalies, or certain noble gases in fullerenes that correlate stratigraphically with other large extinction events. The Siljan impact structure in Sweden (diameter: ca. 55 km, age: ca. 368 Ma) was shown to correlate with the Upper Devonian Kellwasser-Events, from which also microtektites are known (Claeys et al., 1996). In the case of the Permain/Triassic boundary interval (ca. 250 Ma), the dating of the Araguainha structure of Brazil (diameter ca. 40 km, age: ca. 445.5 +/- 3.5 Ma: Hammerschmidt and Engelhardt, 1995) shows good temporal accordance. For the Triassic/Jurassic boundary extinction (ca. 199.6 Ma, Gradstein et al., 2004), the Manicouagan impact structure, Quebec, Canada (diameter: ca. 100 km) was made responsible (Olsen et al., 1987). However, refined dating shows that its age is 215.5 (Ramezani et al., 2005), postdating the extinction level significantly (see Kelley, 2007 for a discussion of the problematical stratigraphical accuracy of impact – mass extinction correlation). Possible killer are believed to be direct physical effects such as shock waves, heat and fire, impact winter, succeeding warming, collapse of the food chain, or environmental acidification. However, it appears that of the Big Five only for the K/T boundary extinction, an impact as a possible cause is still seriously discussed.

**Flood basalt volcanism.** Catastrophic outflow of gigantic flood basalts (continental flood basalt provinces, large igneous provinces) co-occur with the Permian/Triassic boundary event (Siberian flood basalts), the K/T boundary event (Deccan Trapps, India) and the Triassic/Jurassic boundary event (Central Atlantic Magmatic Province, CAMP). They are believed to have triggered mass extinction by the production of large amounts of CO_2 (e.g., Fraiser and Bottjer, 2007) and SO_2 either via climate changes resulting in the either warming or cooling or via feedback loops causing oceanic anoxia or gas hydrate release (see Wignall, 2001 for a summary and Kelley, 2007).

**Anoxic events.** Oceanic anoxic events, indicated by the occurrence of laminated marine black shales, are common phenomena in Earth’s history and were made responsible for some extinction events such as the Kellwasser events (late Devonian), the P/Tr and Tr/J boundary events and some subordinate extinctions such as the Cenomanian/Turonian boundary event. For the P/Tr boundary event, even photic zone anoxia was suggested, caused by release of deep-sea originated hydrogen sulfide to the surface (Kump et al., 2005; Meyer et al., 2008; the superanoxic event of Grice et al., 2004). However, oceanic anoxia alone fails to explain the extinction of land plants and animals. H_2S release into the atmosphere. In the cause of massive oceanic anoxia, hydrogen sulfide is believed to have been released into the atmosphere. H_2S toxicity and hypercapnia are suggested as possible killing mechanisms (Meyer et al., 2008).

**Explosive methane release.** The release of methane is believed to be an associated process of catastrophic global warming in the context of catastrophic volcanisms and greenhouse gas emission (s.o). However, Ryskin (2003) suggested in the case of the P/Tr boundary event that the explosive release of methane by the collapse and instability of gas hydrates brought anoxic deep waters with hydrogen sulfide to the surface, causing mass extinction of the shelf biota.

**Glaciations, warming, cooling.** The succession of a severe cooling, followed by warming was made responsible for the end-Ordovician extinction. The model proposes an equator-shift of boreal taxa and extinction of warm-water fauna. *Vice versa*, in the next step, warming caused a poleward-shift of warm waters and extinction among cold-water faunas. In addition, the associated glacioeustatic sea-level low diminished shelf areas and caused stress on the shelf biota (see discussion in Hallam and Wignall, 1997 and an ecological approach in Twitchett, 2006). The reasons for Phanerozoic glaciations are not always clear to elaborate; in the case of the late Ordovician glaciation, withdrawal of atmospheric CO_2 as a result of increased continental weathering due to volcanism-induced atmospheric SO_2 emission is suggested (Young et al., 2009).
Sea-level changes. Sea-level changes are observed to co-occur with mass extinctions and are suggested to be a possible triggering mechanism. However, while for the Ordovician a glacio-eustatic sea-level low is suggested to be responsible by some authors (see above), strong transgression with the establishment of anoxia is suggested by others (e.g., Kellwasser Event: Bond and Wignall, 2008). There is no universal model for how sea-level changes effects extinction. A more general discussion on that topic is given by Peters (2008).

Gammaray burst. For the end-Ordovician extinction, a gamma ray burst was suggested by Melott et al. (2004). It resulted in a depletion of the ozone layer and increased UV radiation, which is believed to be lethal to numerous groups of surface-dwelling organisms and the phytoplankton. Furthermore, a gamma ray burst can produce atmospheric opaque nitrogen dioxide, resulting in an increased back-scatter of sunlight, which provides a feasible mechanism for a global cooling.

Urease dead zones. Wooldridge (2008) suggested that the pH dependency of the enzyme urease might be the explanation for the end-Devonian, the P/Tr, the Tr/J and the K/T boundary events. Urease catalyzes the hydrolysis of urea into ammonia and CO2. It enables organisms to access urea as a source for metabolism, and it is a very important enzyme for biomineralization of carbonate and nitrogen accessibility during early stages in ontogenetic development. It is equally important in numerous plants, bacteria, fungi, and invertebrates, but not the vertebrates. At pH 7.9, the enzyme becomes inactivate, forming a dead zone between some pH optima. Thus, any sudden or long-term changes in the CO2 concentration (e.g., flood basalt volcanisms), and, resulting, fluctuations of pH values crossing the magic 7.9 boundary, might potentially be phases of extinction.

Halogenated gas emission. Based on recent investigations in evaporitic environments, Weissflog et al. (2008) suggested that during evaporation in the central European Zechstein basin (South Permian Basin; Upper Permian) bacterially-derived, toxic volatile halogenated hydrocarbons (HCC; e.g., chloroform, trichloroethylene, tetrachlorethylene) emitted into the atmosphere. They damaged the ozone layer and – by increased UV radiation – the Permian biosphere. Likewise, the phytotoxicologic effect of the HCC provided a direct kill mechanism for Permian biota.

Concluding remarks

When reviewing the literature it becomes clear that for the “Big Five” no single cause mechanism is accepted (Elewa, 2008). A number of possible “killer” are discussed for each period of mass extinction, and in the case of the Permian/Triassic event, 6 potential suspects (cooling, warming, anoxia, volcanism, impact, methane release) were summarized by White (2002). Even the K/T boundary event is still split into two groups, one favoring an impact, the other flood basalt volcanism. Overviews on the possible triggering mechanisms, with explanations of the complex feedback interrelations based on case studies can be found, for example, in Ryder et al. (1996), Wignall (2001), Taylor (2004), Hallam (2005) and Twitchett (2006), and some data on the biotic response in the context of mass extinctions can be found in Hart (1996) or Hallam and Wignall (1997). The still large number of newly published papers and debates show that extinction is still a hot topic, and while some theories will turn out to be persistent, other will be volatile.

Bibliography


### Cross-references
- Algae (Eukaryotic)
- Anaerobic Oxidation of Methane with Sulfate
- Animal Biocalcification, Evolution
- Animal Skeletons, Advent
- Archaea
- Asteroids and Comet Impacts
- Bacteria
- Banded Iron Formations
- Early Precambrian Eukaryotes
- Ediacaran Biota
- Isotope Fractionation (Metal)
- Mass Extinctions, Phanerozoic
- Mat-Related Sedimentary Structures
- Methane, Origin
- Methane Oxidation (Aerobic)
- Microbialites, Stromatolites, and Thrombolites
- Nickel, Biology
- Origin of Life
- Photosynthesis
- Salinity History of the Earth’s Ocean
- Snowball Earth
- Soda Ocean Hypothesis
- Stromatolites
- Sulfate-Reducing Bacteria
- Sulfur Cycle
- Trace Fossils: Neoproterozoic

### CRYOBIOSPHERE

The term “cryobiosphere” refers to organisms living in extremely cold environments. For detailed reading, see entries “Permafrost Microbiology” and “Extreme Environments.”

### CYANOBACTERIA

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**Synonyms**

Blue-green algae; Chloroxybacteria; Cyanophyta; Cyanoprokaryota

**Definition**

Cyanobacteria are a large and morphologically rather diverse group of photoautotrophic prokaryotes and are classified as a monophyletic phylum within Domain Bacteria. Cyanobacteria share oxygenic photosynthesis with the eukaryotic algae. Cyanobacteria belong to the oldest organisms on earth. Their broad environmental tolerance range makes cyanobacteria well suited to live in habitats of extreme and fluctuating conditions where they can outcompete eukaryotic algae.

Many cyanobacteria have large cells like the eukaryotic algae with which they share the same life-style: Consequently, they are often treated as prokaryotic algae or, formerly, blue-green algae. Some cyanobacteria even form macroscopic colonies from the size of a few millimeters to up to 30 cm (Dodds and Castenholz, 1988).

Cyanobacteria occur in a broad variety of forms, from solitary unicells over colony-forming and undifferentiated filamentous types to more complex forms that even display the hallmarks of multicellularity. Photosynthetic cyanobacteria produced earth’s first oxygen atmosphere, which fostered the rise of eukaryotes. In addition, ancient cyanobacteria produced massive undersea carbonate deposits, thereby reducing atmospheric carbon dioxide levels and influencing global climate. The first plastids arose from cyanobacteria through a process called primary endosymbiosis (McFadden, 2001; Keeling, 2004; Palmer, 2003; Reyes-Prieto et al., 2007). Modern cyanobacteria are recognized for their ability to occupy diverse aquatic and terrestrial habitats, cyanobacteria produce organic compounds used by other organisms, and they stabilize sediments and soils. Nitrogen-fixing cyanobacteria increase soil and water fertility and foster the growth of certain plants and fungi in symbiotic associations. There is strong evidence that cyanobacteria are the oldest microorganisms performing oxygenic photosynthesis, dating back to about 2.45 billion years (Rasmussen and Wilmotte, 2008) and with an evolutionary history stretching over at least 1.5 and possibly 2.7 billion years (Blank, 2004). Cyanobacteria are able to live in some of the most extreme habitats on earth (e.g., Seckbach, 2007 for an overview). The broad environmental tolerance range of cyanobacteria in extreme habitats is in contrast to most other prokaryotic and eukaryotic organisms from extreme environments that occur only within a small window of almost constant conditions. From an evolutionary point of view, it appears that cyanobacteria which are
among the oldest organisms on earth may have been forced by the “modern” eukaryotes to withdraw themselves into habitats of extreme and fluctuating conditions (Whitton, 1992; Bhattacharya et al., 1999).

**Cell structure**

Unlike most groups of prokaryotes, there is substantial morphological and life history variation within the cyanobacteria (Hayes et al., 2007). Although some cyanobacteria occur as solitary unicells, in most species the cells adhere at each other to form at least pairs of cells (representing the products of a recent cell division, *Synechococcus* Figure 1a) or group of cells are held together to form colonies (e.g., in *Myxosarcina*, Figure 1b). Very common growth forms are filaments (trichomes) where the cells are joined end to end through regular divisions into one plane (Figure 1c–g). Such filaments can be even joined into bundles or can be branched (true-branched, Figure 1b or false-branched, Figure 1d). Many filamentous cyanobacteria produce specialized cells known as heterocytes (formerly heterocysts; Figure 1d and g) that function in nitrogen fixation. Such cyanobacteria are usually capable to produce specialized cells for dormancy, known as akinetes, under adverse environmental conditions. Some filamentous cyanobacteria are capable of gliding, a slow uniform motion on a solid substrate. The gliding occurs by an interaction of slime secretion and certain proteinaceous fibers (Hoiczyk, 2000). Cyanobacteria lack sexual reproduction, but gene exchange may be realized through viral transduction by specialized cyanophages (Coleman et al., 2006). The only mode of reproduction is by cell division (binary fission, resulting in two equal-sized cells) or simply by fragmentation of a colony. In unicellular cyanobacteria, baeocytes (endospores) are formed by the division of the cytoplasm several times and in different planes whereby the mother does not increase in size. Filamentous cyanobacteria form hormogonia (Figure 1e), which are short filaments resulting from a breakup of a longer filament, promoted by the controlled death or collapse of certain cells. Hormogonia exhibit gliding motility for several hours, are positively phototactic and lack heterocytes. Light is absorbed by thylakoids, photosynthetic membranes, which are arranged concentrically at the cell periphery, not stacked and not connected to the plasma membrane. They contain the chlorophylls *a* and *d*, in some cyanobacteria also chlorophyll *b*, as well as carotenoids and phycobilins as accessory pigments to capture light energy and convey it to the photosynthetic reaction centers located inside the thylakoid membranes. Phycobilins are blue or red water-soluble pigments and are present in large amounts. They are bound to proteins that form up hemispherical phycobilisomes which occur on the outer thylakoid surfaces. Several particles in the cytoplasm of cyanobacteria may represent storage products, that is, cyanophycin (a polymer of the nitrogen-rich amino acids asparagine and arginine), cyanophycean starch (a polyglucan), and polyphosphate. In addition, lipid droplets, carboxysomes (polygonal aggregations of Rubisco, the key enzyme in CO₂ fixation), and gas vesicles may be present in the cytoplasm. Gas vesicles (assemblies of hollow, pointed cylinders, but not delimited by membranes) occur frequently in aquatic cyanobacteria and enable them for buoyancy and to confer vertical mobility in the water column. The cell wall of cyanobacteria is basically the same as for Gram-negative bacteria, that is, a thin peptidoglycan layer (a polymer composed of sugar derivatives and amino acids) is outside of the cell membrane (cytoplasm membrane). Outside of the peptidoglycan layer is a space surrounded by another membrane, termed outer membrane. A very characteristic feature of cyanobacteria is that they usually are surrounded by a mucilaginous sheath (extracellular polymeric substances [EPS], see entry “Extracellular Polymeric Substances (EPS)”), mostly composed of polysaccharides, which protects the cells from drying and enables them to attach to substrates.

**Ecology**

Cyanobacteria are widely distributed in aquatic and terrestrial environments, including such extreme habitats as hot springs, deserts, and polar regions (Whitton and Potts, 2000). Many cyanobacteria are diazotrophic, that is, they can fix atmospheric molecular nitrogen gas (N₂) into ammonia which is then further assimilated into amino acids and proteins. Nitrogen fixation is an inducible process and is exceptionally energy demanding. The environmental levels of ammonium or nitrate regulate it and it can only be performed if sufficient ATP (produced by photosynthesis) is available. The key enzyme of nitrogen fixation is nitrogenase, which is reversibly inhibited by oxygen and, therefore, nitrogen can only be fixed under anaerobic conditions. Cyanobacteria that produce heterocytes are capable to fix nitrogen even in aerobic habitats, because several processes within the heterocytes (e.g., intracellular respiration) consume oxygen and the heterocytes’ thick cell walls reduce diffusion of oxygen into the cells. Cyanobacteria without heterocytes may fix nitrogen in the dark. Bloom-forming filamentous cyanobacteria devoid of heterocytes are responsible for fixing one-quarter of the total nitrogen in the oceans (Bergman and Carpenter, 1991). These cyanobacteria fix nitrogen in randomly distributed vegetative cells adjacent to each other within the filaments. Here and inside the colonies, the filaments may be depleted from oxygen. In addition, these species occur in tropical seas that are relatively low in dissolved oxygen. The ability to fix molecular nitrogen makes cyanobacteria attractive as symbionts for many eukaryotes because eukaryotes are not able to fix nitrogen. Very common occurrences of nitrogen-fixing cyanobacteria as extracellular symbionts are lichens, that is, symbioses with certain fungi (Friedl and Büdel, 2008, see entries “Symbiosis” and “Fungi and Lichens”). In the marine environment, cyanobacterial symbioses are known
Cyanobacteria, Figure 1 Different cyanobacterial morphotypes from pure culture. Figure a, d–g show strains recovered from tufa stromatolite biofilms of hardwater creeks: (a) coccoid unicellular *Synechococcus* sp. The cells adhere at each other to form at pairs representing the products of a recent cell division (Photograph taken by A. Hodačová); (b) *Myxosarcina* sp. (strain SAG 30.84) with adhering groups of cells forming irregular colonies (Photograph taken by A. Hodačová); (c) filamentous, true-branched *Stigonema* sp. (strain SAG 49.90) (Photograph taken by A. Hodačová); (d) filamentous, false-branched *Tolypothrix* sp.; (e) thick unbranched filaments of *Lyngbya* sp. forming hormogonia; (f) thin unbranched filaments of *Pseudanabaena* sp. which are fragmenting (Photograph taken by A. Hodačová); (g) filamentous *Nostoc* sp. forming gelatinous colonies. Insert, filament with a heterocyte located within the filament (intercalary) (Photographs taken by A. Hodačová); (h) Fluorescence in situ hybridization using an oligonucleotide probe specific for bacteria at a cryosection of the top of a tufa-forming biofilm. The red filaments represent cyanobacteria (*Phormidium* sp.). Cyanobacterial autofluorescence is shown in red and calcite in white (Photograph taken by G. Arp, printed with permission of the publisher). Scale bar, 20 μm.
with sponges, ascidians (sea squirts), and echiuroid worms in the benthos and diatoms, as well as dinoflagellates in the plankton. These symbioses can be significant in terms of the biogeochemistry of coastal and open-ocean areas (Carpenter, 2002). Planktonic diatom–cyanobacteria symbioses play a significant role in the ecology and biogeochemistry of the surface ocean (see entry *Algae, Eukaryotic*). Symbiotic associations with cyanobacteria are also known from plants. In the water fern *Azolla* and cycads where filamentous cyanobacteria with heterocytes occur within certain cavities inside the leaf and intercellular spaces inside the root, respectively (Lee, 2008; Graham et al., 2009). N₂-fixing cyanobacteria are common in waterlogged rice fields and the importance of these naturally occurring prokaryotes in the nitrogen economy of rice cultivation has long been known (Whitton, 2000). To support the demand for nitrogen in rice cultivation, input of chemical N fertilizers is a prerequisite, but the inoculation with free-living N₂-fixing cyanobacteria as biofertilizers has been shown to increase growth and crop yield of rice. An example for intracellular associations involving cyanobacteria can be found in certain diatoms (see entry *Diatoms*).

The cyanobacteria metabolic activities make quantitatively important contributions to the carbon, nitrogen, sulfur, and other biogeochemical cycles (Hayes et al., 2007). There is an enormous variety of habitats where cyanobacteria play important roles, but to discuss this comprehensively is beyond the scope of this article. Therefore, we confine the discussion of cyanobacteria ecology just to a few selected examples. Other examples may be equally intriguing and important. In the littoral zone of marine environments, cyanobacteria form thin adhering films on porous or soft granular rocks within the spray zone. Cyanobacteria of the littoral zone make a significant contribution to the productivity of rocky shores (Lee, 2008). In the open-ocean minute unicellular cyanobacteria (less than 2 µm in diameter) may occur in high concentrations due to their adaptation to low light and effective nutrient uptake kinetics. Here, cyanobacteria represent the major organisms of the picoiphytoplankton (Ferris and Palenik, 1998) that dominates biomass and production of these oligotrophic habitats. Massive development of nitrogen-fixing filamentous cyanobacteria occur in certain tropical waters and the large surface area produced by the cyanobacterial filaments forms miniature ecosystems (Carpenter et al., 1992; Lee, 2008). Also in freshwaters blooms of cyanobacteria are common. Bloom-forming cyanobacteria often release significant toxic substances, termed cyanotoxins, with physiologically two types, neurotoxins and hepatotoxins (e.g., Codd et al., 1999). Cyanotoxins may function as anti-herbivore chemicals and can inhibit the growth of other algae. Cyanobacteria are also well known from hot acidic springs throughout the world (e.g., Ward and Castenholz, 2000). Many physiological properties that make the cyanobacteria capable to adapt to a large variety of extreme and environmental conditions may also indicate their long evolutionary history. Many cyanobacteria are able to tolerate low oxygen conditions and even free sulfide at levels much higher than those tolerated by most eukaryotic algae. Many terrestrial cyanobacteria can tolerate high UVB and -C radiations, which may also be reminiscent of an adaption to early earth’s conditions. Some cyanobacteria can even confer anoxygenic photosynthesis where H₂S is utilized as an electron donor. Cyanobacteria are also very successful primary colonizers in terrestrial habitats due to their tolerance of desiccation and to water stress. In biological soil crusts of semideserts, for example, cyanobacteria play a key role in maintaining the stability of the surface (e.g., Büdel et al., 2009). As they bind sand and soil particles, cyanobacteria prevent erosion. They help to maintain moisture in the soil and contribute nitrogen to it through nitrogen fixation. That may even assist higher plant growth by supplying growth substances (Lee, 2008). The cyanobacterial genus *Arthrosira* (formerly *Spirulina*) is used as human food supplement; the *Spirulina* industry is of increasing economic importance. Large filamentous colonies of *Nostoc* are used in China as “hair vegetable” (Facai).

Many species of cyanobacteria have calcium carbonate in the enveloping mucilage of the cells; crystals of calcite may also be trapped in the mucilage excreted by the cells (see entry *Calcite Precipitation, Microbially Induced*). In the marine environment, trapping and binding of the sediments as well as carbonate precipitation by cyanobacteria led to the formation of stromatolites (see entries *Microbialites, Modern*, *Microbialites, Stromatolites, and Thrombolites*). The production of laminae in the stromatolites depends on fluctuations ultimately derived from movements of the earth, sun, and moon, and requires rhythmicity such as daily photosynthetic cycle of the organisms in the stromatolites. Up to 2,000 million years ago, there were no grazers and boring organisms and, therefore, stromatolites grew uncontested. The occurrence and size of stromatolites declined dramatically after the evolution of grazing and boring organisms. Today stromatolites grow only in warm hypersaline waters inhospitable to grazers and borers (e.g., Shark Bay, Australia). Extant cyanobacteria do not calcify in open-ocean sea water because this water is normally not supersaturated sufficiently with calcium carbonate to allow calcification (Riding, 2000; see entry *Calcified Cyanobacteria*). However, even in extant freshwaters, calcification processes somehow similar to the stromatolite formation in marine environments occur, for example, in tufa-forming biofilms of karstwater streams (Arp et al., 2010). Epilithic cyanobacterial species may enhance calcium carbonate deposition, either by trapping particles or by contributing to communities where calcite and aragonite (in freshwaters) or other carbonates are precipitated. Endolithic species mainly occur in soft and porous limestone (Figure 1h) where they grow actively into the limestone at sites where removal of surface material occurs (Pentecost and Whitton,
of cyanobacterial sheath, their treatment under the bacteriological code of nomenclature seems more appropriate (e.g., Anagnostidis and Komárek, 1985). However, significant influence of cyanobacterial photosynthesis in the regulation of tufa-forming processes has been found recently (Bissett, 2008a, b; Shiraiishi et al., 2008).

Fossil history and classification

Fossil evidence indicates that major lineages of cyanobacteria had originated 2.3–2.45 billion years ago (Eigenbrode and Freeman, 2006; Tomitani et al., 2006; Graham et al., 2009), well before the establishment of eukaryotes 1.5 billion years ago (Javaux et al., 2001). Even older fossils resembling cyanobacteria have been found in ancient stromatolites (e.g., Schopf, 2006) and geochemical evidence may also indicate an even earlier origin of cyanobacteria, but this is not undisputed. Collectively, the available data suggest that oxygenic photosynthesis arose sometime between 3.4 and 2.3 billion years ago (Allan and Martin, 2007). For classification of the cyanobacteria, more recently a combination of phenotypic and genotypic characters has been used (Castenholz, 2001). The fact that cyanobacteria have an algal way of life – cyanobacteria “usually behave like algae” (Wilmutte, 1994) – led to treat them as plants under the botanical code of nomenclature until the late 1970s (e.g., Anagnostidis and Komárek, 1985). However, as cyanobacteria are essentially prokaryotes, their treatment under the bacteriological code of nomenclature seems more appropriate (e.g., Castenholz, 2001). For a review of the difficulties of cyanobacteria classification, see Hayes et al. (2007). Based on the most recent broad-based description of cyanobacterial taxonomy (Castenholz, 2001), cyanobacteria are classified into five subsections (Subsections I–V) which correspond to the five orders Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales, and Stigonematales which have been erected under the botanical code of nomenclature. Some of the groups described by this current classification scheme are known to be polyphyletic in molecular phylogenies (for reviews see, e.g., Hayes et al., 2007). This shows that taxonomy of cyanobacteria is still in a state of flux. Still more work using multiple gene analyses as well as correction of misidentified strains in DNA sequence databases and culture collections is required to achieve progress toward a more robust taxonomy of the cyanobacteria.

Summary

Cyanobacteria form a large and morphological diverse group of photoautotrophic Gram-negative bacteria. Many cyanobacteria have large cells like the eukaryotic algae with which they share oxygenic photosynthesis. Cyanobacteria occur in a broad variety of forms, from solitary unicells over colony forming and undifferentiated filamentous types to more complex forms that even display the hallmarks of multicellularity. There is strong evidence that cyanobacteria are the oldest microorganisms performing oxygenic photosynthesis, dating back to about 2.45 billion years. Cyanobacteria exhibit a broad environmental tolerance range, which makes them well adapted to live under extreme and fluctuating conditions. From an evolutionary point of view, it appears that cyanobacteria may have been forced by the “modern” eukaryotes to withdraw themselves into such habitats. Modern cyanobacteria are recognized for their ability to occupy diverse aquatic and terrestrial habitats, cyanobacteria produce organic compounds used by other organisms, and they stabilize sediments and soils. Many cyanobacteria can fix atmospheric molecular nitrogen gas (N2) into ammonia which is then further assimilated into amino acids and proteins. Nitrogen-fixing cyanobacteria increase soil and water fertility and are particularly attractive as symbionts, for example, to form lichens with fungi or being endosymbionts in plants, some metazoa, dinoflagellates and diatoms. The cyanobacteria metabolic activities make quantitatively important contributions to the carbon, nitrogen, sulfur, and other biogeochemical cycles.

Bibliography

DEEP BIOSPHERE OF SALT DEPOSITS

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Definition

The biosphere is defined either as “the place on Earth’s surface where life dwells” (Eduard Suess’ original definition, 1875, in The Origin of the Alps), meaning the zone on Earth where life naturally occurs, or in a more narrow sense as just the organisms themselves. Recent advances in microbiology have demonstrated that microorganisms live deep beneath the Earth’s terrestrial surface and that the total mass of microbial life may, in biomass, exceed all animal and plant life on the surface (Whitman et al., 1998). Microorganisms live at such extremes that the “thickness” of the biosphere extends from 5,400 m above sea level to at least 9,000 m below sea level.

Salt deposits are accumulations of solid materials and are usually derived from evaporation of seawater. There are >70 elements dissolved in extant seawater, but only 6 ions make up >99% of all the dissolved salts (wt% in brackets): Na⁺ (30.51), Cl⁻ (55.08), SO₄²⁻ (7.69), Mg²⁺ (3.67), Ca²⁺ (1.17), K⁺ (1.10) (Hay et al., 2006).

Introduction

About 250 million years ago the continents on Earth were close together and formed Pangaea, a supercontinent, which persisted for about 100 million years and then fragmented. The land masses at that time were located predominantly in the southern hemisphere. The climate was arid and dry, and the average temperature is thought to have been several degrees higher than at present. This was one of the time periods in the history of the Earth, when huge salt sediments formed. A total of about 1.3 million cubic kilometers of salt were estimated to have been deposited during the late Permian and early Triassic period alone (250–192 million years ago; Zharkov, 1981); newer research has discovered additional vast salt deposits, which were previously unknown – especially deposits below the Gulf of Mexico, and extensive Miocene salt (about 20 million years old) underlying the Mediterranean Sea, the Red Sea, and the Persian Gulf (Hay et al., 2006).

The thickness of the ancient salt sediments can reach 1,000–2,000 m. When Pangaea broke up, land masses were drifting in latitudinal and northern direction. Mountain ranges such as the Alps, the Carpathians, and the Himalayas were pushed up by the forces of plate tectonics. In the Alpine basin and in the region of the Zechstein Sea, which covered northern Europe, no more salt sedimentation took place after the Triassic period. Dating of the salt deposits by sulfur-isotope analysis (ratio of ⁳²S/⁳⁴S as measured by mass spectrometry), in connection with information from stratigraphy, indicated a Permo-Triassic age for the Alpine and Zechstein deposits (Holser and Kaplan, 1966). This age was independently confirmed by the identification of pollen grains in the sediments (Klaus, 1974). Figure 1 shows pollen grains from extinct coniferous trees, which were found in Alpine Permian salt and also in Zechstein salt (Klaus, 1963).

Microorganisms and signature sequences from salt deposits

As recently as 1981, Larsen (1981) described mined rock salts as free from bacteria, although isolations of halophilic microorganisms from ancient salt sediments had occasionally been reported since the early decades of the twentieth century (see Grant et al., 1998; McGenity et al., 2000). From Alpine Permian rock salt, which was collected from the salt mine in Bad Ischl, Austria, a haloarchaeon (see “Halobacteria – Halophiles”) was
isolated, which was recognized as a novel species and named *Halococcus salifodinae* strain BIp (Denner et al., 1994). This was the first isolate from ancient rock salt, which was formally classified and deposited in international culture collections. Two independently isolated strains, Br3 (from solution-mined brine in Cheshire, England) and BG2/2 (from a bore core from the mine of Berchtesgaden, Germany) resembled *Hc. salifodinae* strain BIp in numerous properties, including the characteristic morphology of coccoid cells arranged in large clusters (see Figure 2, right panel, in “Halobacteria – Halophiles”); in addition, rock salt samples were obtained 8 years later from the same site and several halococci were recovered from these samples, which proved to be identical to strain BIp (Stan-Lotter et al., 1999). The data suggested that viable halophilic archaea, which belong to the same species, occur in geographically separated evaporites of similar geological age. Another halococcal isolate from the Bad Ischl salt formation, which differed from the previously described strains, was identified as a novel species and named *Halococcus dombrowskii* (Stan-Lotter et al., 2002). *Hc. salifodinae* and *Hc. dombrowskii* have so far not been found in any hypersaline surface waters, or any location other than salt mines. Recently, a non-coccoid novel haloarchaeon, *Halobacterium noricense* was obtained from a freshly drilled bore core (Figure 2) at the salt mine in Altaussee, Austria (Gruber et al., 2004). Other isolates from ancient salt deposits include a single rod-shaped *Halobacterium* from 97 000 year old rock salt in the USA (Mormile et al., 2003), which was deemed to resemble the well-characterized *Halobacterium salinarum* NRC-1 (see “Halobacteria – Halophiles”). From the Permian Salado formation in New Mexico, a novel strain, *Halosimplex carlsbadense*, was isolated (Vreeland et al., 2002).

Although the microbial content of ancient rock salt is low – estimates range from 1 to 2 cells/kg of salt from a British mine (Norton et al., 1993) to $1.3 \times 10^5$ colony forming units (CFUs) per kg of Alpine rock salt (Stan-Lotter et al., 2000), and up to $10^8$ CFUs per g of Permian salt of the Salado formation (Vreeland et al., 1998), equivalent to a range of 1 pg to 10 µg of biomass per kg of salt – the reports showed that viable haloarchaeal isolates were obtained reproducibly by several groups around the world. The data support the hypothesis that the halophilic isolates from subterranean salt deposits could be the remnants of populations which inhabited once ancient hypersaline
seas; in addition, they provide strong evidence against the notion that the recovered strains could be the result of laboratory contamination, since the isolates were obtained independently from different locations.

The amplification of diagnostic molecules, such as the 16S rRNA genes of bacteria and archaea, by the polymerase chain reaction is now the standard method for obtaining material for subsequent nucleotide sequencing. For this technique it is not necessary to cultivate the microorganisms; the genes can be amplified by using DNA prepared from the material of interest. Analysis of dissolved Alpine rock salt with molecular methods was performed by extracting DNA and sequencing of 16S rRNA genes. The results provided evidence for the occurrence of numerous haloarchaea, which have not yet been cultured (Radax et al., 2001). Similarities of the 16S rDNA gene sequences were less than 90–95% to known sequences in about 37% of approximately 170 analyzed clones (Radax et al., 2001; Stan-Lotter et al., 2004); the remaining clone sequences were 98–99% similar to isolates from rock salts of various ages (McGenity et al., 2000) and to known haloarchaeal genera. In a similar experimental approach, using halite samples ranging in age from 11 to 425 millions of years, Fish et al. (2002) found haloarchaeal sequences and, in the older samples, also evidence for bacterial 16S rRNA genes which were related to the genera Aquabacterium, Leptothrix, Pseudomonas, and others. These data suggested the presence of a probably very diverse microbial community in ancient rock salt.

Long-term survival of cells
The reports cited above provide evidence that viable extremely halophilic archaea were isolated from salt sediments, which are thought to have been deposited about tens of thousands or even millions of years ago. The fluid inclusions in Permian rock salt were reported to contain cations and anions in a similar composition as today’s seawater (Horita et al., 1991; Hay et al., 2006). While there is no direct proof that haloarchaea or other microorganisms have been entrapped in rock salt since its sedimentation, it would also be difficult to prove the opposite, namely that masses of diverse microorganisms entered the evaporites in recent times (see also McGenity et al., 2000, for further discussion).

If a Permo–Triassic age is postulated for some of the haloarchaeal isolates and DNA signatures, then it becomes necessary to explain the biological mechanisms for such extreme longevity. Grant et al. (1998) discussed several possibilities, such as the formation of resting stages other than spores – since archaea are not known to form spores – or the maintenance of cellular functions with traces of carbon and energy sources within the salt sediments, which would imply an almost infinitely slow metabolism. At this time, there are no methods available to prove directly a great microbial age, whether it be a bacterium or a haloarchaeon. However, it can be shown, when simulating the formation of halite in the laboratory by drying salty solutions, which contained microorganisms that the cells accumulate within small fluid inclusions (Figure 3). The cells can be pre-stained with the fluorescent dyes of the LIVE/DEAD kit (Fendrihan et al., 2006), which provides information on the viability status of a cell (green fluorescence indicating viable cells); the procedure improves also the visualization of cells within crystals. The fluid inclusions were square or rectangular, as is common in the rectangular mineral halite, and the cells were rather densely packed within the fluid-filled spaces. From such experiments it appeared that the cells accumulated always in the fluid inclusions; there were no stained cells within the mineralic halite (Figure 3; Fendrihan et al., 2006). Suggestions have been made that fluid inclusions migrate within evaporites and thus, new nutrients might become accessible for the entrapped cells (McGenity et al., 2000).

Extraterrestrial halite and conclusion
Traces of halite were found in the SCN meteorites (named after the locations where they were found — Shergotty in India, Nakhl in Egypt, and Chassigny in France), which stem from Mars (Treiman et al., 2000). The Monahans meteorite, which fell in Texas in 1998, contained macroscopic crystals of halite, in addition to potassium chloride and water inclusions (Zolensky et al., 1999). Recently,
evidence for salt pools on the Martian surface was obtained (Osterloo et al., 2008). These results are intriguing – they suggest that the formation of halite with liquid inclusions could date back billions of years and occurred probably early in the formation of the solar system (Whitby et al., 2000). Could halophilic life have originated in outer space and perhaps traveled with meteorites, could haloarchaea have persisted in environments as they are found today on Mars?

Viable extremely halophilic archaea, representing novel strains, were isolated repeatedly from Permo-Triassic and other ancient salt sediments, suggesting their capacity for long-term survival under dry conditions. Together with the discovery of extraterrestrial halite it appears thus feasible to include into the search for life on other planets or moons specifically a search for halophilic microorganisms.

Bibliography


Cross-references

Archaea

Astrobiology

Bacteria

Deep Biosphere of the Oceanic Deep Sea

Extreme Environments
Halobacteria – Halophiles
Saline Lakes
Salinity History of the Earth’s Ocean
Terrestrial Deep Biosphere

DEEP BIOSPHERE OF SEDIMENTS


DEEP BIOSPHERE OF THE OCEANIC DEEP SEA

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Definition and overview
Although used in many different ways, the term “biosphere” is principally defined either as a zone in which life occurs, thereby overlapping the atmosphere, the hydrosphere, and the lithosphere, or as the entity of living organisms on Planet Earth. Both perceptions commonly focus on the Earth’s near-surface environment, with all domains sharing solar energy used in the process of photosynthesis. The deep-sea realm takes a special position in this context, as deep-sea pelagic and the majority of benthic organisms live in the ocean’s aphotic zone and inhabit the widespread abyssal plains, respectively. For a long time, their main food source has been considered to be based on particulate organic matter (POM) from the ocean’s surface primary production and its sedimentation to abyssal depths (Gage and Tyler, 1991 and references therein, D’Hondt et al., 2002, 2004). With the discovery of “ocean vents” in the late 1970s (Corliss et al., 1979), this general perspective was broadened by the perception of the enormous potential of chemical energy through the reaction of seawater, rock material, and fluids rising from the Earth’s interior. According to this concept of energy for life, the term ‘surface biosphere’ has been opposed to ‘subsurface biosphere’ (also commonly found in literature as ‘deep biosphere’). Following this definition, the deep-seafloor with its highly diverse topography from heterotrophic to pure chemotrophic habitats has to be treated as a transition zone between both biospheres. Opposed to the “deep hot biosphere” (Gold, 1992), occurring by definition in oceanic as well as terrestrial subsurface environments, stands the “deep cold biosphere” as defined for permafrost sediments (Vorobyova et al., 1997) and ice cores from the depths of Lake Vostoc (Venter, 2001).

Life in the deep sea
Comprising approximately 65% of the Earth’s surface, the deep-sea environment is characterized by hyperbaric, aphotic, and low-temperature conditions and highly diverse seascapes. Canyons, seamounts, ridges, fractures, and trenches, but also biogeochemical oases such as cold seeps, mud volcanoes, carbonate mounds, brine pools, gas hydrates, hot vent systems, and deep-water coral reefs provide ample niches for a highly diverse pelagic and benthic deep-sea community (Tyler, 2003). It was only during the construction of the transoceanic telegraphic communication network that people realized the ocean’s topographic alterations and astonishing depths. In 1861, the repair of an overgrown cable from 1,800 m water depth in the Mediterranean finally aroused the scientific community which by then adhered to Edward Forbes’ theory on a completely “azotic” zone below a water depth of 550 m. Though, 11 years had elapsed before the first global, scientific expedition onboard the “Challenger” (1872–1876) finally convinced people that a flourishing life in fact exists in the deep-sea realm. Numerous, further expeditions and a rushing development of technical facilities allowed deep-sea researches in 1960 to reach even the ocean’s deepest surveyed point, the Challenger Deep at 10,911 meters below sea level (mbsl), located at the southern end of the Mariana Trench within the western Pacific Ocean (Piccard and Dietz, 1961). Since then, several studies on large-scale patterns and the zoogeographical origins of deep-sea organisms evidenced a high macrobenthic diversity (Gage and Tyler, 1991 and references therein). These organisms display a depth-dependent zonation as a result of basin age, deep currents (as barriers or dispersal), topographic boundaries, disturbance processes, and sedimentation in connection with depth-related environmental patterns (for review see Levin et al., 2001; Stuart et al., 2003). Macro- and meiofauna are loosing importance with increasing water and sediment depth, whereas microorganisms like bacteria, archaea, and fungi account for up to 90% of the deep-sea benthic biomass (Pfannkuche, 1992). Sinking particles may carry large numbers of microorganisms from upper zones (10^5–10^10 cells m^{-2} d^{-1}), inoculating deep marine surface sediments with an autotrophic and heterotrophic microbial community, as demonstrated by results from sediment traps (Turley and Mackie, 1995; Danovaro et al., 2000; Vanucci et al., 2001) or the deep-seafloor (Lochte and Turley, 1988).

Particulate organic matter (POM)
Due to the fact that most deep-sea benthic species are deposit feeders (Sanders and Hessler, 1969), the locally qualitatively and quantitatively, variable import of POM from the ocean’s surface waters plays a crucial role for macro-, meio-, and microorganisms living in deep surface sediments (Gooday and Turley, 1990). Mainly consisting of phytoplankton, marine snow, fecal pellets, (dead) zooplankton and molts, this material undergoes different steps of degradation during its passage from the photic, epipelagic (0–200 mbsl), through the mesopelagic (200–1,000 mbsl) to the actual deep-sea zones, in particular the bathypelagic (1,000–4,000 mbsl), the abyssal
(4,000–6,000 mbsf), and the hadal zone (6,000–11,000 mbsf). Depending on the residence time in the water column, the bioavailable part of POM finally reaching the deep-seafloor may be small (De La Rocha and Passow, 2007 and references therein). The refractory remainders such as animal skeletons are continuously accumulating at the seafloor and turn into deeply buried sediment over time, thereby representing the largest global reservoir organic carbon (Parkes et al., 2000 and references therein).

Deep-sea sediment types
Grain size (Gray, 1974) and sediment heterogeneity (Etter and Grasse, 1992) may additionally govern community composition and distribution of macro-, meio-, and micro-organisms in deep-sea benthic environments. In relation to their basic sources, deep-sea sediments may be biogenic (POM from pelagic primary production, benthic in-situ production), lithogenous (terrestrial weathering and transport by wind and rivers), hydrogenous (precipitation from seawater or pore water), volcanic, or cosmic (Seibold and Berger, 1996). According to grain size and settling velocity, lithogenous gravel and sandy fractions usually are deposited along the coast, while silt and clay are transported farther offshore through waves and currents, hence dominating the basically biogenous deep-sea sediments. Regional deviations may be linked to currents, downslope slides, submarine canyon dynamics, or to a release of ice-trapped rock material in polar waters (e.g., Ramseier et al., 2001). Covering almost one-half of the shelves and more than half of the deep ocean bottom, biogenous sediments mainly consist of calcite, aragonite, opal, and calcium phosphate, originating from foraminifera, diatoms, and radiolarians (Hay et al., 1988).

Deep biosphere of deep-sea sediments
Microbiological studies on sediment cores collected during several cruises of the Deep Sea Drilling Project (DSDP), the Ocean Drilling Program (ODP), and the Integrated Ocean Drilling Program (IODP) gave evidence for the presence of complex microbial communities in deeply buried marine sediments down to several hundred meters below seafloor (e.g., Whelan et al., 1986; Parkes et al., 1994; Rousset et al., 2008). Most striking, new insights into subsurface microbiology were gained during the ODP cruise Leg 201 to the equatorial Pacific Ocean and the continental margin of Peru, including sites recognized as most typical for oceanic subsurface environments (D’Hondt et al., 2002). A large fraction of the sub-seafloor bacteria has been proven to be alive and culturable, displaying turnover rates (based on sulfate reduction as dominating mineral process at these sites) comparable to surface sediment communities (D’Hondt et al., 2004; Schippers et al., 2005).

After a logarithmic decline within the uppermost 6 meters below seafloor (mbsf) (Parkes et al., 1994) to about 40 mbsf (Schippers et al., 2005), bacterial cells have proven to be more or less evenly distributed down to several hundred mbsf. Local peaks within these deeply buried sediments seem to mirror sulfate (diffusing from crustal fluids) and methane (from in-situ production) concentration shifts (Engelen et al., 2008). However, published variations of absolute cell numbers (by a factor of up to 3) have to be treated with caution: varying estimations not only depend on the geochemical conditions at the respective sampling sites, but also on the enumeration techniques applied. Calculations based on early results revealed that sub-seafloor sediments comprise – at least – half of all prokaryotic cells and up to one-third of the living biomass on Earth pointing to a slow-growing strategy of high biomass in areas of low-energy flux (Whitman et al., 1998).

The prokaryotic community in deeply buried sediments can not exclusively be traced back to contaminations from biologically active surface layers or reactivation of spores and dormant cells (Parkes et al., 2000 and references therein).

Porewater chemistry data obtained from sites throughout the world’s oceans (ODP, DSDP) showed that sulfate reduction, methanogenesis, and fermentation are the principal degradative metabolic processes in subsurface sediments. These results give evidence for significant lower metabolic rates for the subsurface compared to the surface biosphere and for methanogenesis becoming more important the more sulfate gets depleted with increasing sediment depth (D’Hondt et al., 2002 and references therein).

Windows to the subsurface biosphere
Hydrothermal vents
The discovery of the “ocean vents” near Galapagos Island (Corliss et al., 1979) was the first proof for the active movement of the gigantic oceanic plates of the Earth’s crust creating series of cracks in the ocean floor, teeming with life. At these discharge areas, hydrothermal fluids with temperatures of more than 400°C (Haase et al., 2007) mix up with the cold ocean seawater, resulting in a precipitation of dissolved metals and in the formation of characteristic chimneys over time. Iron and sulfide precipitates turn the smokers black (“black smokers,” Figure 1), while barium, calcium, and silicon minerals result in “white smokers.” Thermal precipitation and/or direct magma degassing of H2, H2S, CH4, CO, and CO2 in combination with oxygen as electron acceptor provide enough energy to support a highly productive and physiologically diverse chemosynthetic microbial community (Reysenbach and Shock, 2002).

As the highly diverse and dense hot vent macrofauna (e.g., vestimentiferan tubeworms, bivalve mollusks, provannid gastropods, alvinellid polychaete, and bresiliid shrimps) cannot feed on the released chemicals themselves, they either feed on chemosynthetic microbes or host them as symbionts. The predominant endosymbionts are mesophilic to moderately thermophilic chemosynthetic (mostly Gammaproteobacteria), whereas most
Episymbionts belong to the Epsilonproteobacteria, which can oxidize H₂ and sulfur compounds while reducing oxygen, nitrate, and sulfur compounds (for review, see Nakagawa and Takai, 2008). The vent habitat proved to harbor methanogens (*Methanococcus*), sulfate-reducers (*Archaeoglobus*), and facultative autotrophs and heterotrophs such as the thermophilic aerobic *Thermus* and *Bacillus* (Harmsen et al., 1997) or, for example, *Thermococcus*, *Phyrococcus*, *Desulfurococcus* (Prieur et al., 1995; Teske et al., 2000; Nercessian et al., 2003; Schrenk et al., 2003). Generally detected archaeal phylotypes were affiliated with hyperthermophilic *Crenarchaeota*, *Euryarchaeota* Group I, II, III (Takai and Horikoshi, 1999) and the “Deep-sea Hydrothermal Vent Euryarchaeotal Group” (Hoek et al., 2003).

**Cold seeps and mud volcanoes**

Just a few years after the discovery of the hydrothermal vent systems, cold seep ecosystems were reported from active and passive continental margins and subduction zones all over the world (Aharon, 1994 and references therein).

High-pressure, low oxygen and low-temperature conditions favour the formation of marine gas hydrates. In the subsurface realm, such gas reservoirs are stored in a crystalline form, whereas they get dissolved in pore waters and finally leave the sediment surface in gaseous form. High fluxes of methane, sulfide, and other reduced elements characterize these ecosystems such as cold seeps, hydrocarbon vents and mud volcanoes, often leaving mineral precipitation in their immediate surroundings. Coupled to sulfate reduction, rich bacterial and archaeal communities perform anaerobic oxidation of hydrocarbons, but predominantly of methane (Boetius et al., 2000; Borowski et al., 2000; Treude et al., 2005). Conversion of methane is mainly mediated by two different groups of anaerobic methanotrophic archaea (ANME-I and ANME-II) (Nauhaus et al., 2005), forming syntrophic consortia with the sulfate-reducing bacteria (SRB) *Desulfosarcina* and *Desulfococcus* (Hinrichs et al., 1999; Boetius et al., 2000; Michaelis et al., 2002; Knittel et al., 2003).

The methane-emitting Haakon Mosby Mud Volcano (HMMV, Barents Sea) has shown to harbor three key communities in methane conversion such as aerobic, methanotrophic bacteria (Methylococcales), anaerobic methanotrophic archaea (ANME-2) thriving below siboglinid tubeworms, and a previously undescribed clade of archaea (ANME-3) associated with bacterial mats (Niemann et al., 2006). Similarly, some cold seeps on the deeper Black Sea shelf are characterized by intense methane bubble discharge, mainly related to microbial methanogenesis (Pape et al., 2008 and references therein). Diffuse gas seeps in more shallow, oxic Black Sea waters often exhibit a netlike coverage of microbial mats similar to *Beggiatoa*-mats observed at HMMV (Figure 2). *Beggiatoa* spp. are discussed as keystone members of seep communities owing to their ability to (directly and indirectly) influence the metabolic activity of δ-Proteobacteria, Planctomycetales, and ANME archaea by providing sulfate and ammonia as reactants (Mills et al., 2004).

The question remains, to which extent such seep systems influence the global methane cycle, as the quantification of bubble dissolution and/or the release of methane-rich pore fluids from the sediment into the hydrosphere is difficult to achieve (Vogt et al., 1999; Reeburgh, 2007). Niemann et al. (2006) estimated that methanotrophy at active marine mud volcanoes consumes less than 40% of the total methane flux, due to limitations...
of the relevant electron acceptors in the upward flowing, sulphate- and oxygen-free fluids.

Deep biosphere of the oceanic crust

The fact that microorganisms are present in the subsurface realm had been reported decades ago in terrestrial subsurface environments (Farrell and Turner, 1931; Lipman, 1931). Early drilling operations performed for commercial purposes such as mining, oil and hot water recovery, and the search for underground waste repositories reported on the existence of a large community of microorganisms obviously involved in geochemical processes in the deep biosphere (Gold, 1992; Pedersen, 1993 and references therein). Hence, it was only in the early 1990s that scientists started to focus on the investigation of prospering life beneath the Earth’s crust, thanks to a chance encounter of a deep ocean, volcanic eruption during a dive onboard the submersible Alvin, releasing white microbial bulk mats (Haymon et al., 1993). The upper layers of the oceanic crust are characterized by high basaltic porosity, hosting a vast hydrothermal reservoir (Johnson and Pruis, 2003) inhabited by a microbial community composed of species that are also found in deep-sea waters, sediments, and the deep oceanic crust (Thorseth et al., 2001; Huber et al., 2006). Among the most prominent anaerobic thermophiles indigenous for the oceanic crust, the Amphitrichon group of bacteria (Nakagawa et al., 2006) or groups within Crenarchaeota, Eurarchaeota, and Korarchaeota (Ehrhardt et al., 2007). Since 3.5 billion years, basalt-associated glass textures and vesicular cavities within the basaltic matrix provide niches for microbial colonization (Furnes et al., 2004; Peckmann et al., 2008). For instance, the fossil record of the oceanic crust even gives evidence for a previous fungal life in deep ocean basaltic rocks (Schumann et al., 2004).

Much effort has been put into the investigation of the deep biosphere of the deep sea during the past 20 years. However, we still are neither aware of the final composition of the living subsurface community, nor of its interrelationship to, for example, crustal fluid-derived compounds, nor of its global impact.

Bibliography


Cross-references

Anaerobic Oxidation of Methane with Sulfate
Archaea
Bacteria
Basalt (Glass, Endoliths)
Beggiatoa
Biotufms
Carbon Cycle
Carbon (Organic, Cycling)
Chemolithotrophy
Cold Seeps
Deep Biosphere of Salt Deposits
Extreme Environments
Hydrothermal Environments, Marine
Methane Oxidation (Acrobic)
Microbial Biomineralization
Microbial Degradation
Microbial Mats
Microbialites, Modern
Microbialites, Stromatolites, and Thrombolites
Mud Mounds

Piezophilic Bacteria
Sulfide Mineral Oxidation
Sulfur Cycle
Terrestrial Deep Biosphere

DEEP FLUIDS


DEGRADATION (OF ORGANIC MATTER)

Transformations of organic matter within the range of temperatures, pressures and environmental conditions found at or near earth surface environments. If biological activity is confirmed to cause the transformations, termed biodegradation. See entries “Carbon (Organic, Cycling)” and “Carbon (Organic, Degradation)” and “Microbial degradation” (of organic matter) for further reading.

DENITRIFICATION

Denitrification is the biological reduction of nitrate (NO₃⁻) to N₂ and, to a minor extent, other gaseous species such as N₂O. See entry “Nitrogen” for further reading.

DESERT VARNISH

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Synonyms

Rock Varnish, Rock Glaze, Silica Glaze, and Schutzrinde

Rock Varnish, Rock Glaze, Silica Glaze, and Schutzrinde were introduced in 1891, from a translation of the French term manteau protecteur: “Schutzrinde” for all dark coatings, including “Wustenlack” a thin, polished coating that may be an eolian polished patina; “Dunkle Rinden” as a dark brown to black coating, possibly more similar to Sonoran and Mojave desert varnish; and “Schweinfurth,” which describes black coatings on rocks in Egypt that have spread on surrounding sands.

Definition

Desert Varnish. In arid environments such as Death Valley (California), rocks are covered with black opalescent desert varnish (Figure 1). Desert varnishes have been found in all continents, in locations such as the Gobi, Sonoran, Mojave, Namibian, Victorian, and Atacama
Deserts. The dark, lustrous coatings have attracted the interest of scientists for centuries. The German naturalist and explorer, Alexander Humboldt, observed desert varnish on a transatlantic expedition and questioned how this enigmatic feature would have formed. His contemporary, Darwin (1887), also engaged in the search for explanations for this unusual rock coating. To date, many other noteworthy scientists have examined desert varnish and have commented on its bulk chemistry, the arid conditions in which it forms and the concentration of manganese that makes it opaque and causes it to be black (cf. Perry et al., 2006, 2007; Perry, 1979; Staley et al., 1992).

Geochemists, planetary geologists, and microbiologists as well as archeologists are interested in desert varnish, as petroglyphs are incised in varnish coatings all over the world.

Yet, despite years of scientific effort, the origin of desert varnish is still shrouded in controversy. Most investigators have looked into biological causes where microbes create varnish and preferentially concentrate manganese relative to the local soils and rocks. However, more modern theories propose a nonbiological origin with sequential episodes of inorganic chemical reactions dominating formation processes.

“Desert varnish” is used here to define coatings that appear dark (reddish brown, chocolate, black, and blue-black). Reasons for not abandoning desert varnish as a term are both because of its historical use and because it seems a most apt description for dark surfaces formed in subaerial conditions in hot and cold, and high and low elevation deserts. It must be emphatically stated that this definition of darkness is a macrovisual phenomenon. Close inspection of varnish coatings exposes the heterogeneity of the surface composition.

Desert varnish is a thin sedimentary deposit (~<200 μm thick) and under the microscope, its most notable feature is the presence of micron-sized layers. In typical geological thin sections (30 μm thickness) cut normal to the varnish surface the coatings are opaque. The making of a special ultra-thin section revealed the structure within the coating. This heterogeneity was first observed by Perry and Adams (1978) who showed that desert varnish coatings are composed of alternating light and dark layers. Silicon and oxygen are the primary elements in desert varnish, but oxides including aluminum, manganese, iron, titanium and magnesium are also important, and it is the variability in abundance of these oxides that creates the layers. Dark layers within varnishes are enhanced in manganese. Less laterally uniform variations in composition are also evident, and whole detrital grains can be embedded in varnish coatings. The thickness of layers is also variable and in three dimensions the growth of layers can lead to botryoidal (mounded) structures (Perry and Adams, 1978).

The black desert varnish is not the only type of rock coating draped in controversy; other coating types have also been observed in nature. Silica glazes are also found throughout the world and have been investigated in Hawaii (Curtiss et al., 1985; Farr and Adams, 1984), Morocco (Smith and Whalley, 1988), and Oregon (Farr, 1981). Their clear color is a result of not containing enough oxides to tint them black (manganese) or red (iron).

Recent investigations

It is now widely agreed that desert varnish and silica glazes are true coatings rather than weathering products of an underlying material. Consequently, some source materials for desert varnish must originate from external environments and are introduced by atmospheric transport. Dust can land on rock surfaces and its constituents take part in chemical reactions with some components becoming concentrated and adhered or “glued” together. Limited amount of water is essential for this process. Atmospheric deposits of trace elements are also deposited and have become part of varnish coatings (Thiagarajan and Lee, 2004). Importantly, the chemical composition of desert varnish necessitates that unused elements and materials need to be removed in a never-ending interchange that allows for the concentration and formation of mineral components.

In the past, this enigmatic coating has been ascribed to the action of bacteria or fungi on desert rock surfaces. Microbes are thought to be pervasive in all environments on Earth, and researchers have actively sought a causative link to desert varnish. However, ongoing Martian analog studies in extreme arid conditions such as...
as the Atacama Desert show that bacteria are rarely present on rock surfaces (Jones, 1991). The black top coatings were attributed to biological action, and early investigators speculated that the manganese oxidizing bacteria concentrated the manganese oxides on the top but did not grow as readily underneath rocks. Primarily, these investigations used culture-based studies of microbes, both bacterial and fungal (Hungate et al., 1987). The emphasis was on an exhaustive search for manganese and iron oxidizing microbes. The manganese and iron were thought to have been absorbed from dust by the bacteria and oxidized during biochemical reactions over thousands of years (Dorn and Oberlander, 1981). According to conventional thought, the silicate particles were also obtained from the atmosphere and cemented together by oxides produced by bacteria. Yet, biological mechanisms remain unsatisfactory, and no viable hypothesis has been presented that provides an explanation for key varnish features such as its hardness, a mechanism for binding the heterogeneous components together, a means of producing the lamellar and botryoidal morphologies, and its slow rates of formation (Liu and Broecker, 2000).

While cultures of bacteria were obtainable from the surfaces of varnish-coated rocks, a direct look at those surfaces using electron microscopes, after fixing the surfaces in order to preserve any biology present, rarely revealed the presence of bacteria. This was noted by Jones (1991) in the Atacama and Smith and Whalley (1988) in the Atlas Mountains of Morocco and Perry et al. (2007) in the Mojave Desert.

Incomplete remains of organisms are, however, entombed in desert varnish coatings. The first examination of organic molecules being trapped or complexed in varnish coatings was published by Perry et al. (2003). Other organic compounds may also make a C–O bond or be entombed in the disordered silica matrix. Following the discovery of amino acids, DNA was found using molecular techniques by Perry et al. (2004) and Kuhlman et al. (2006); also several polymorphic compounds are present in varnishes (Perry et al., 2007) and lipids (Schelble et al., 2001). These findings suggest that varnish coatings preserve past biology and as discussed later, may be an important recorder of contemporaneous life in the local environment.

Several investigative techniques including, high resolution electron microscopy has revealed that silica asopal, not clays or metal oxides, is probably the most important mineral present in desert varnish. When there is moisture, depressions on rock surfaces form small dimples to larger indentations in which silicic acid may form, leaving varnish structures in the depressions. Evaporation of water eventually causes concentrations to increase and, eventually, condensation of the silicic acid occurs to produce a gel. During gel formation, surface detrital material and organic compounds are incorporated, and when the gels are dried and dehydrated the components are entombed in a lustrous rock coating (Perry et al., 2006, 2007). Evidence of the occasional action of water is forthcoming from analyses which indicate that despite being formed in arid environments, the coatings have been shown to contain up to 9% water (Perry, 1979). This in itself represented a conundrum until the hypothesis of water-rich silica was put forth.

We can then conclude that silica in desert varnish and silica glazes and even hot-spring silica-rich sinters (see Chapter Sinter) are made by similar processes (Phoenix et al., 2006; Perry and Lynne, 2006). Desert varnish then can be thought of as a silica glaze enriched in oxides.

Hypotheses of formation must stand up to scientific testing, and it has often been said that if we could only make a synthetic coating in the laboratory that we must inevitably understand how coatings are made in nature. Laboratory experiments support the role of silica polymerization. Surprisingly, it turns out to be a relatively easy process to make coatings in the laboratory (Perry et al., 2005). Conversely, several attempts to make coatings using microbes produced no coatings further supporting nonbiological explanations for desert varnish. It cannot be ignored, however, that biological organic compounds may effect the chemical formation of coatings when they are present.

Conclusions

Recent work then indicates that biology is not required for desert varnish formation and the source of the organic components is from outside sources landing on the surface. Importantly, however, the accumulation of environmental products in desert coatings preserves a biological, climatological, and environmental record. Keeping a chronicle of past life is not only of interest on Earth, but similar processes may also be in portent on other planets such as Mars.

Recently, camera images from the Mars Pathfinder landing site have strongly suggested the presence of desert varnish on many Martian rocks (Murchie et al., 2004). Angular, equant, and tabular rocks and large boulders display spectral characteristics suggesting varying levels of ferric minerals. These minerals form part of a desert varnish-like coating that may have formed in the presence of thin films of water when Mars had a moist climate than the present-day climate.

If silica exists in desert varnish-like coatings on Mars, as on Earth, then it may contain chemical signatures of previous life (Perry and Hartmann, 2006). It is possible that on Mars the earliest formed layers in any stromatolite-like sequence may have recorded a wetter and more biologically amenable Martian environment. Moreover, it is likely that Martian desert varnish would be a better preserver of organic matter than its Earth counterpart (Perry and Sephton, 2006). The current Martian environment is much colder and drier than that on Earth. Martian desert varnish records may also extend further back in time. On Earth, desert varnish is a relatively recent rock coating generated in time periods commonly less than 100,000 years; older examples are removed by physical and chemical weathering. Evidence of ancient surfaces and events on Mars suggests that both physical and chemical weathering are less aggressive than on our planet.
Bibliography


Cross-references

**Astrobiology**

**Endoliths**

**Extreme Environments**

**Microbial Silification – Bacteria (or Passive)**

**Sinter**

**DETACHMENT**

**Definition**

Detachment refers to the release and transport of microbial cells and their associated extracellular polymeric substances from an attached microbial biofilm to the fluid compartment bathing the film (Stewart, 1993).

Together with microbial attachment and growth, detachment is a primary process that balances biofilm accumulation and activity. Detachment processes control the (re-)dispersal of cells from biofilms into the planktonic community and hence are crucial for bacterial expansion and the exploration and colonization of new niches (Toutain et al., 2004). Bryers (1988) distinguished several categories of detachment processes: (i) *erosion* is a more or less continuous removal of individual cells or small groups of cells resulting from the moving fluid in contact with the surface of the biofilm, whereas (ii) *abrasion* is caused by the collision of particles. (iii) *sloughing* refers to the release of relatively large particles of biomass, whose size is comparable to or greater than the thickness of the biofilm itself. Furthermore, (iv) *predator grazing* may be considered as another form of detachment process.
Important factors controlling and/or inducing biofilm detachment include the activity of matrix-degrading enzymes, microbially generated gas bubbles, nutrient levels, microbial growth status, availability of multivalent cross-linking cations, fluid shear stress, and contact attrition (Hunt et al., 2004). In addition, there is increasing evidence that bacterial biofilms may actively regulate dispersion processes using cell-to-cell signaling (quorum-sensing). Dissolution of the biofilm matrix and release of bacteria are hypothesized to be triggered when a signal molecule, i.e., an excreted bacterial metabolite, accumulates to a threshold concentration (Hunt et al., 2004; Waters and Bassler, 2005).

### Bibliography


### Cross-references

**Biofilms**

**Extracellular Polymeric Substances (EPS)**

### DIATOMS

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### Synonyms

Bacillariophyta

### Definition

Diatoms (Chromalveolates supergroup, photosynthetic Stramenopiles, Bacillariophyta) are unicellular or colonial eukaryotic algae with unique cell walls composed of amorphous silica and consisting of two parts.

The diatoms (Bacillariophyta) represent an extremely diverse and successful lineage of photosynthetic Stramenopiles (Chromalveolates) with cell walls composed of amorphous silica and consisting of two parts, termed frustules, as their most striking feature. The diatoms are of unicellular organization, but some form colonies. The diatom plastids are derived from red algal secondary symbioses and are golden brown due to the high concentration of the carotenoid fucoxanthin. Diatoms are exceedingly abundant and thought to be the most important group of eukaryotic phytoplankton, responsible for approximately 40% of marine primary productivity (Falkowski et al., 1998). Their high abundance coupled with the resistance of diatom frustules to dissolution in normal waters has resulted in massive sedimentary accumulation and a significant fossil record.

### Cell structure

**Frustule:** The diatom frustule is a highly patterned external wall composed of amorphous silica \(\left[\text{SiO}_2\right]_n\left(\text{H}_2\text{O}\right)\). Silica is often plentiful in natural waters (it is the second most abundant element in the Earth’s crust) and is an energetically inexpensive source of wall material (Falkowski and Raven, 1997). The diatom frustule is constructed of two almost equal parts, termed valves, with the smaller valve (hypothece) fitting into the larger (epitheca). Each theca is typically composed of two parts: the valve (which forms the larger outer surface) and a girdle (circular bands of silica attached to the edge of the valve). The frustule is present in all living diatoms, except following secondary loss in endosymbiotic diatoms living in some dinoflagellates (Tamura et al., 2005) and some foraminifera (Lee and Correia, 2005). Owing to their requirement for silica for cell wall biogenesis, the diatoms play a key role in the biogeochemical cycling of silica (Tréguer et al., 1995). Diatom frustule walls predominate in the sediments of the ocean floor, thus making the diatoms serious players in ocean biogeochemistry over geologically significant timescales (Kemp et al., 2000). Analysis of diatom frustule containing sediments can provide information on past environmental conditions (e.g., Reavic et al., 1998). Fossil diatom frustule deposits are even mined as diatomaceous earth and commercially used as filter material, mild abrasives, and absorbent or insulation materials (Lee, 2008). The precise method of frustule formation is still not fully understood but most models propose the formation of a matrix of organic fibrils and microtubules onto which the silica is deposited. More recently, Sumper and Kröger (2004) have suggested that silica formation takes place in intracellular compartments termed silica deposition vesicles (SDVs).

Two major categories of frustule shapes can be distinguished, that is, discoid or cylindrical cells with radial symmetry in valve view (centric diatoms) and elongated cells with a more or less bilateral symmetry (pennate diatoms). Some pennate diatoms have slits in their frustule that extends between both cell ends, the raphe system. Such diatoms are known as raphid diatoms and they possess the ability to accomplish rapid gliding motility. Other pennate diatoms lacking raphes are nonmotile and termed araphid diatoms (Round et al., 1990; Graham et al., 2009). The motive force for motility may come from...
raphe-associated microtubules and attached motor proteins which drag polysaccharide rods that are liberated from the cell by exocytosis at the raphe fissure (Schmidt, 1997). Mucilage that is secreted from the raphe of some pennate diatoms may also form a stalk used for stable attachment of the cell to substrates.

**Cell division and sexual reproduction:** The regular asexual method of reproduction is by cell division. The valves of the parent cell become the epithecas of the daughter cells with each daughter cell producing a new hypotheca (see Lee, 2008). As a result of cell division, one of the daughter cells is of the same size as the parent cell, and the other is smaller. Once a certain minimal size (about one-third of the maximum size) is reached after several generations, this triggers the start of sexual reproduction whereby gametes are formed. As a result of the fusion of two gametes, a large diploid auxospore (zygote) is formed reestablishing the maximum size of the diatom cell. Vertebrate diatoms are diploid, and gamete production involves meiosis. Centric diatoms are oogamous, producing one or two egg cells per parental cell and form 4–128 sperms per parental cell as the result of mitotic divisions following mitosis (Graham et al., 2009). Centric diatom sperms are flagellates, but there is only a single flagellum (tinsel flagellum, see entry *Algae (Eukaryotic)*). Pennate diatoms are usually isogamous, that is, both gametes are similar in size and neither is flagellate. In addition, all the asexual formation of resting spores may act as a second mechanism of size restoration (Lee, 2008).

**Plastids and storage products:** Plastids are double membrane-bounded organelles usually containing the photosynthetic apparatus or some part of it. The diatom plastids are bounded by four membranes and a chloroplast endoplasmatic reticulum (see entry *Algae (Eukaryotic)*) is present. These are typical features of the plastids of most members of photosynthetic Stramenopiles which are thought to be derived from a single red algal secondary endosymbiosis (McFadden, 2001; Keeling, 2004; Palmer, 2003). The thylakoid membranes within the plastid have the typical structure of the plastids of most photosynthetic Stramenopiles (heterokont algae), grouped into lamellae of three, all enclosed by a girdle lamella. Centric diatoms generally have large numbers of small discoid plastids, whereas pennate diatoms tend to have fewer plastids, sometimes only one. Due to the presence of the brown carotenoid fucoxanthin which is present in most photosynthetic Stramenopiles (see entry *Algae (Eukaryotic)*) the diatom plastids appear brown. Other characteristic accessory pigments are the chlorophylls c1 and c2. Fucoxanthin and chlorophylls are bound within the light-harvesting antenna complexes by Fucoxanthin and chlorophyll a/c-binding proteins (FCP). The FCP proteins are integral membrane proteins localized on the thylakoid membranes within the plastid, and their primary function is to target light energy to chlorophyll a within the photosynthetic reaction centers. Diatoms have chrysolaminarin as a carbohydrate storage product which is dissolved within vacuoles in the cytoplasm, another feature shared with many photosynthetic Stramenopiles. In addition, cytoplasmatic lipid droplets occur. Diatoms are the source of unique highly branched isoprenoid alkanes and steroids which can be used as biomarkers in the water column and in sediments (see entry *Biomarkers (Molecular Fossils)*). An important survival strategy used by diatoms is to produce long-lived resting spores to buffer environmental deterioration (Round et al., 1990), although these only occur in small quantities in sediments.

**Diversity and ecology**

Diatoms are one of the most easily recognizable groups of major eukaryotic algae because of their unique architecturally complex siliceous cell walls. The siliceous material is laid down in certain regular patterns that leave the wall ornamented and these frustule structures are extensively used as taxonomic “fingerprints.” Diatoms are probably the most species-rich group of eukaryotic algae, with over 250 genera of extant diatoms and perhaps as many as 100,000 species occurring in both aquatic and terrestrial habitats (Norton et al., 1996; Sims et al., 2006). Many more diatom species still remain to be described and diatom species may actually number in the millions (Norton et al., 1996). Diatoms comprise the main component of the open-water marine flora and a significant part of the freshwater algal flora. While pennate diatoms are represented in about equal numbers in the freshwater and marine habitats, the centric diatoms are present predominantly in the marine environment (Lee, 2008). Diatoms are probably the most important group of eukaryotic phytoplankton. Their buoyant cells, often augmented by chitin threads or colonial adaptions, enable them to keep within the photic zone better than many other algae. Moreover, some species are able to withstand deep mixing and low light levels (Kilham et al., 1986). Water depth, photon penetration, vertical mixing, and micro-substrate conditions determine the relative importance of each habitat. Diatom productivity follows a seasonal pattern in most lakes, controlled by the variability of climate, nutrient supply, mixing regimes, and in northern latitudes the period of ice cover. Besides planktonic diatoms there are many benthic forms, growing on sediments or attached to rocks or macroalgae, and some species can also be found in soil (Lee, 2008). In freshwaters, the diatoms make up a large part of the periphyton (i.e., organisms attached to submerged vegetation or inorganic substrates), where they are attached to the substrate (e.g., forming biofilms together with cyanobacteria on tufa stromatolites in hardwater creeks, Arp et al., 2010; see Figure 1). Despite an increase in current retards the attachment of diatoms to the substrate, it causes faster growth leading to a higher standing crop in fast-flowing streams than those in slow-flowing streams (Lee, 2008).

Many diatoms exhibit growth habits and forms that trap sediments, and/or promote precipitation of carbonate
Diatoms, Figure 1 Raphid pennate diatoms recovered from tufa stromatolite biofilms of hardwater creeks: (a) diatom assemblage (biofilm sample, e.g., *Navicula* sp., *Gomphonema* sp., *Cymbella* sp.); (b) *Gomphonema* sp. (biofilm sample) with mucilaginous stalk; (c) *Gyrosigma* sp. (biofilms sample); (d) *Planothidium frequentissimum* (pure culture); (e) SEM of *Achnanthidium saprophila* (pure culture; photograph taken by R. Jahn); (f) SEM of *Navicula veneta* (pure culture; photograph taken by R. Jahn); (g) SEM of *Surirella brebissonii* (pure culture; photograph taken by R. Jahn); (h) SEM of *Planothidium frequentissimum* (pure culture; photograph taken by R. Jahn), scale bar LM 20 μm, SEM 5 μm.
forming modern stromatolites (see entries “Microbiallyites, Stromatolites, and Thombolites,” “Microbiallyites, Modern”). Numerous authors have noted the presence of diatoms in living stromatolites in both marine environment (e.g., Golubic, 1976) and limnic and fluvial settings (e.g., Gomes, 1985). Several diatoms are conspicuous in building stromatolites, for example, Mastogloia et al., 1970). The presence of biofilms containing large amounts of solid CaCO₃ is often observed on structures produced within the main body of water by the mucilage produced by biofilm algae, for example, via photosynthesis (Heath et al., 1995).

In warm oligotrophic seas, symbioses between nitrogen-fixing cyanobacteria and diatoms often play a significant role. Richelia, a heterocystous cyanobacterium, and the non-heterocystous Trichodesmium are believed to be the major N₂ fixers in tropical and subtropical oceans (Carpenter and Romans, 1991; Capone et al., 1997; Janson et al., 1999). In the diatom genera Hemiaulus, Rhizosolenia, and Bacteriastrium, Richelia lives as an endosymbiont between the cell wall and the frustule (Fogg, 1982; Villareal, 1994; Janson et al., 1995), with the cyanobacterial partner presumably supplying the eukaryotic partner with nitrogen and/or carbon (Rai et al., 2000). For an extensive bloom of the diatom Hemiaulus hauckii harboring Richelia, it was estimated that the N supply by N₂ fixation by the symbiosis exceeded that of nitrate flux from below the euphotic zone. High abundances of Rhizosolenia-richelia and Richelia-Chaetoceros symbioses have been reported for the subtropical Pacific Ocean (Mague et al., 1974; Gómez et al., 2005); also free trichomes of Richelia have been observed there.

Evolutionary history, fossil record, and classification
Fossil diatoms are known from deposits extending from recent times back to about 180 million years ago (Kooistra et al., 2007). Molecular clock evidence suggests that diatoms appeared no earlier than 240 million years ago (Kooistra and Medlin, 1996). The oldest fossil diatoms frustules are from the Jurassic, and well-preserved, diverse floras are available from the Lower Cretaceous (Sims et al., 2006). The fossil evidence suggests that the first diatoms lived in marine waters and had valves that were radially symmetrical. The main invasion of freshwaters appears to have been delayed until the Cenozoic. Pennate diatoms appear in the late Cretaceous and raphid diatoms in the Paleocene (Sims et al., 2006). Centric diatoms are oogamous and with numerous discoid plastids, pennate diatoms are isogamous with fewer plate-like plastids. These characters previously have led to treat the centric and pennate diatoms as two separate classes. Later, the raphid and araphid pennate diatoms were given equal taxonomic ranking so that three classes were recognized for the diatoms, Coscinodiscophyceae (centric diatoms), Fragilariophyceae (araphid pennate diatoms), and Bacillariophyceae (raphid pennate diatoms) (Round et al., 1990). In molecular phylogenies, however, it became evident that the centric diatoms are not monophyletic, but may represent two lineages to which class rank was given, Mediophyceae and Coscinodiscophyceae (Medlin and Kaczmarska, 2004; Sims et al., 2006).

In turn, the molecular phylogenies show the raphid and araphid diatoms to form a monophyletic lineage, which has been recognized as the class Bacillariophyceae (Medlin and Kaczmarska, 2004; Sims et al., 2006).

Summary
Diatoms (Chromalveolates supergroup, photosynthetic Stramenopiles, Bacillariophyta) are unicellular or colonial eukaryotic algae with unique cell walls, termed frustule, composed of amorphous silica with regular patterns of ornamentation and consisting of two parts. They contain brown plastids derived from red algal secondary endosymbiosis. There are two major types: centric and pennate diatoms. Centric diatoms have a radial symmetry in valve view, containing several small plastids and are oogamous, that is, their gametes are an egg and motile spermatozoids with one flagellum. Centric diatoms have the oldest fossil record and make up two classes, Coscinodiscophyceae and Mediophyceae. Pennate diatoms have elongated cell walls with bilateral symmetry. Those pennate diatoms with a raphe exhibit gliding motility. They contain one or few plastids and are isogamous, that is, their gametes are of equal shape and without flagella. Raphid and araphid diatoms make up the class Bacillariophyceae. Diatoms are probably the most species-rich group of eukaryotic algae. They may be the most important group of eukaryotic phytoplankton in marine and freshwater environments, but are also abundant as benthic forms, growing on sediments or attached to submersed substrates, and can also be found in soils. They form important symbioses with nitrogen-fixing cyanobacteria in tropical seas. Diatom frustules predominate in the sediments of the ocean floor and the analysis of diatom frustule containing sediments can provide information on past environmental conditions. Diatoms produced significant fossil records and their fossil deposits are even mined and commercially
used. Based on fossil evidence, diatoms may be up to 240 million years old.

Bibliography


Cross-references

Algae (Eukaryotic)
Carbon Cycle
Microbial Silification – Bacteria (or Passive)
Microbialites, Modern
Microbialites, Stromatolites, and Thrombolites
Silica Biominalization, Sponges
Stromatolites
Symbiosis
Tufa, Freshwater
DINOFLAGELLATES

Dinoflagellates are a highly diverse group of flagellated protists consisting of both photosynthetic and non-photosynthetic (heterotrophic) taxa in equal proportions (Taylor et al., 2008). Dinoflagellates are an important component of the modern plankton and have been common to abundant in marine and freshwater environments since the Mesozoic. More than 2,000 extant and 2,500 fossil species have been described. Being mostly biflagellated, swimming unicells of typically 10–100 μm in diameter, they can position themselves in the water column and take full advantage of the available light and nutrients. Many genera are indicative of (paleo-) environmental conditions such as water salinity, nutrient levels, and oceanic temperature zones. In modern and ancient sediments, dinoflagellate-derived organic matter contributions can be specified by distinctive steroid biomarkers (dinosterane and related compounds, see entry “Biomarkers (Molecular Fossils)”). Some dinoflagellates, called zooxanthellae, are capable of forming symbioses with a phylogenetically wide range of marine protists and invertebrate animals. For further information, see entries “Algae (Eukaryotic)” and “Protozoa (Heterotroph, Eukaryotic).”

Bibliography

DIVALENT EARTH ALKALINE CATIONS IN SEAWATER

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Synonyms
Divalent cations in seawater

Definition
The most abundant divalent earth alkaline cations in seawater are Mg²⁺, Ca²⁺, and Sr²⁺. These ions and their dynamic change play a major role for the modern oceans. On long geological time scales dynamic changes of divalent cation concentrations in the oceans influence the evolution of life and the climate evolution in the past.

Divalent cations in modern ocean water
Magnesium
Magnesium is the ninth most abundant element in the universe by mass. It constitutes about 2% of the Earth’s crust by mass, and it is the third most abundant element dissolved in seawater and it is the most abundant divalent cation followed by Ca and Sr in seawater (Brown et al., 1992). Mg ions are essential to all living cells, and is the 11th most abundant element by mass in the human body. In seawater (S = 35 psu), Mg is present at approximately 53 mM (mM = millimolar) (Brown et al., 1992). Only the monovalent sodium (Na⁺, 469 mM) is present at higher concentration. Magnesium is about five times more abundant than the third most abundant cation Ca (10 mM). Magnesium concentration is significantly lighter than calcium, so when compared on a weight basis, it is only about 3 times as concentrated (1285 vs. 420 ppm). Magnesium is present in seawater as a free divalent cation with water and other molecules attached to it (Brown et al., 1992; Kastner, 1999). It is estimated that each Mg²⁺ ion has approximately eight water molecules (ligands) tightly bound to it. A small portion (about 10%) of the magnesium is present as a soluble ion pair with sulfate (MgSO₄), and much smaller portions are paired with bicarbonate (MgHCO₃⁻), carbonate (MgCO₃), fluoride (MgF⁻), borate (MgB(OH)₄⁻), and hydroxide (MgOH⁻).

While these ion pairs comprise only a small portion of the total magnesium concentration, they influence seawater chemistry to a large extent. In the case of carbonate (CO₃²⁻), for example, the ion pairing to magnesium stabilizes the carbonate that it is present in far higher concentrations than it would be present in the absence of magnesium. This effect makes seawater a much better buffer in the pH range of 8.0 than it otherwise would be. Without this ion pairing, seawater pH might be significantly higher, and more susceptible to dynamic changes. The average residence time for a magnesium ion in seawater is on the order of several tens of millions of years. That time is substantially longer than that for Ca (~one million years) and aluminum (Al, 100 years), but less than Na (~250 million years). The long seawater residence time of Mg in seawater is a measure of its fairly unreactive chemical behavior indicating that it does get taken out of solution through various biological and chemical processes.

Sources and sinks for magnesium in the oceans
In the present-day ocean, the two most important calciumcarbonate (CaCO₃) polymorphs – low Mg-calcite and aragonite – are the most prevalent carbonates and major sinks for Mg replacing Ca to some extent in the crystal lattice. Mg also precipitates together with Ca directly from the water in some evaporitic settings, as well as by the dissolution and reprecipitation of calcite with magnesium replacing calcium in the crystal structure. Dolomite, a high magnesium type of calciumcarbonate usually does not form in the well-oxygenated modern oceans, although bacterial alteration of seawater is reported to induce dolomite precipitation (Kastner, 1999; McKenzie, 1981) in certain marine anoxic environments.
The amount of magnesium estimated to exist as dolomite deposits suggests the importance of dolomitization as a magnesium sink in the past, despite its low prevalence today (Wilkinson and Algeo, 1989). In general, the geological Mg record shows a general trend of increasing abundance of dolomite in sediments of increasing age (Wilkinson and Algeo, 1989). The relative lack of dolomite formation today might indicate differences in past seawater chemistry, climatic and environmental conditions that are more conducive to dolomite formation.

The interaction of hydrothermal waters with basalt at the mid-ocean ridge (MOR) effectively removes magnesium from the water to the basalt, and in exchange, calcium becomes enriched in the water (Mottl and Holland, 1993). Estimates for the molar exchange rate of Mg to Ca at the MOR are close to unity. These models also predict that interaction at the MOR is a sufficient sink to explain the balance of magnesium in the ocean, as well as being a significant source for calcium.

Marine geological investigations at the mid-ocean ridge estimate a flux rate of the entire ocean volume through the accretion zone every 8 million years, or about $1.75 \times 10^{17}$ g/year, a fraction of the flux rate for rivers of about $4.6 \times 10^{19}$ g/year (Mottl and Holland, 1978). It has also been demonstrated through pore water profiles that magnesium and calcium chemically interact with the underlying crystalline basement resulting in a decrease of magnesium concentration and an increase in calcium concentration with depth below seafloor in the sediment column (Holland, 1984).

### Calcium

Calcium (Ca) is the chemical element with the symbol Ca and atomic number 20. It has an atomic mass of 40.078. Calcium is a soft grey alkaline earth metal, and is the fifth most abundant element by mass in the Earth’s crust. Calcium is also the fifth most abundant dissolved ion in seawater by both molarity and mass, after sodium, chloride, magnesium, and sulfate. Ca is essential for living organisms, particularly in cell physiology, where movement of the calcium ion $Ca^{2+}$ into and out of the cytoplasm functions as a signal for many cellular processes. As a major material used in mineralization of bones and shells, calcium is the most abundant metal by mass in many animals.

**Sources and sinks for calcium in seawater**

Calcium is the second most abundant divalent cation of the major ions in seawater with a concentration of about 410 ppm (Brown et al., 1992). Ca variations in the oceans are often caused by changes in salinity, where the calcium goes up and down just as the salinity and the dynamic changes of the input of rivers, which are often greatly enriched in calcium relative to other ions such as sodium. Calcium is also frequently enriched in hydrothermal vent water released from the ocean bottom. The calcium is dissolved from the hot basalt as the water passes through it, and is released to the ocean. Compared to other conservative cations Ca has a relative short mean residence time of about one million year or less which may reflect its important role in marine biomineralization and other chemical processes. One interesting aspect of calcium in seawater is that the calcium concentration can be higher in deep ocean water than in surface water due to the dissolution of CaCO₃ and the recycling of Ca in the deep ocean. Ca can also locally be depleted in places where precipitation of calcium carbonate is especially rapid. This includes the Bahamas Banks (where oolitic aragonite is precipitated), in parts of the Red Sea, and presumably in some lagoons where calcification is high and the water volume is small.

**Chemical state of calcium in seawater**

In fresh water below pH 11, Ca ions are essentially free. They are bound only to oriented water molecules and move about independently of all other ions in the solution. However, complexation may also occur with other components such as dissolved phosphate, nitrate, or carbonate. This so-called hydration sphere is quite strongly attached to the ion in water, with about eight water molecules tightly attached in the first hydration shell. Beyond this first hydration shell other water molecules are arranged in a second shell in a looser way. All water molecules around the ion are very rapidly exchanging, but those closest to the ion move more slowly and move with it as it moves through the solution.

In seawater, the majority of calcium ions are still free. However, some (~10–15%) are present as an ion pair with sulfate, forming the neutral soluble ion pair CaSO₄. Calcium similarly forms ion pairs with carbonate (CO$_3^{2-}$) and bicarbonate (HCO$_3^-$). While these comprise a small fraction of the total calcium, the calcium carbonate ion pair comprises a fairly large portion of the total carbonate (together with Mg, about two third of the carbonate). These ion pairs consequently tend to lower the free concentration of carbonate, and thereby inhibit precipitation of CaCO₃ and increasing its solubility. In seawater, Ca also forms ion pairs with fluoride, hydroxide, borate, various forms of phosphate, and other less abundant ions. In almost all cases the effect of calcium is smaller than the effect of magnesium on these ions, both because the concentration of magnesium is higher.

**Calcium carbonate in seawater**

A very important aspect of Ca is its supersaturation in seawater. Supersaturation means that given the right circumstances, it will precipitate as solid calcium carbonate. Supersaturation can be described as:

$$K = [Ca^{2+}][CO_3^{2-}]$$

(1)
When \( K = K_{sp}^* \) (the solubility product constant in seawater at any given temperature, pressure, and salinity), the solution is saturated. When \( K \) exceeds \( K_{sp}^* \), the solution is supersaturated and when the product of the concentration of calcium and carbonate is less than \( K_{sp}^* \), the solution is undersaturated, and calcium carbonate can dissolve. In normal seawater, the product of calcium and carbonate is about 3 times the \( K_{sp}^* \) of aragonite and 5 times that of calcite.

The supersaturation (\( \Omega \)) for calcium carbonate in seawater is given by:

\[
\Omega = \frac{[Ca^{++}][CO_3^-]}{K_{sp}^*} \tag{2}
\]

The higher \( \Omega \) is, the more likely precipitation is to take place. At \( S = 35 \text{ psu} \) and 1 atmosphere pressure, the \( K_{sp}^* \) decreases slightly as the temperature rises. Millero (1995) provides a series of long equations for calculating \( K_{sp}^* \) for both aragonite and calcite. For aragonite, the log \( K_{sp}^* \) drops from \(-6.19 \) at \( 25 \)°C to \(-6.44 \) at \( 80 \)°C. In relative terms, the \( K_{sp}^* \) has gone from 1 to 0.91 to 0.55 over this temperature range. Likewise for calcite, the relative \( K_{sp}^* \) has changed from 1 to 0.96 to 0.73 over this range.

**Calcium (magnesium) carbonate precipitate from seawater**

One situation in which calcium carbonate can precipitate involves adding calcium carbonate seed crystals to seawater. In many cases, this action will initiate precipitation of calcium carbonate (and magnesium as well). This precipitation typically does not proceed until supersaturation is gone, but may be stopped by some other processes. Furthermore, precipitation takes place if the supersaturation is pushed to unusually high levels. This can be caused by a rise in pH, a rise in temperature, or more obviously, by a rise in either calcium or carbonate.

However, chemical interaction of various cations, mainly Mg, permit the ocean to be supersaturated. Without these processes, it is unlikely that the ocean could remain supersaturated, and might even make it impossible for corals to maintain skeletons without expending considerable effort to prevent dissolution. Magnesium holds onto carbonate ions and reduces their free concentration, thereby reducing the likelihood of precipitation onto calcium carbonate surfaces. Furthermore, Mg gets onto the growing surface of the crystal, essentially poisoning further precipitation of calcium carbonate due to the higher activation hydration energy of Mg. Note that while both of these processes inhibit precipitation of calcium carbonate, the first actually increases the solubility, whereas the second does not. It is worth noting that the solubility of calcium carbonate in seawater is about 26 times higher than in freshwater at the same temperature. Other processes that inhibit crystal growth in seawater involve both phosphate and organic compounds that get onto the growing crystal like Mg.

**Bicarbonate acidity shift with temperature**

As water is heated, the equilibrium between bicarbonate and carbonate (Equation 3) is shifted toward carbonate.

\[
HCO_3^- \rightarrow H^+ + CO_3^{2-} \tag{3}
\]

\[
Ka^* = \frac{[CO_3^{2-}] [H^+]}{[HCO_3^-]} \tag{4}
\]

\[
[CO_3^{2-}] = Ka^* [HCO_3^-] / [H^+] \tag{5}
\]

\[
pKa^* = - \log Ka^* \tag{6}
\]

This shift toward carbonate is evidenced by the shift in the seawater pKa* for bicarbonate from \( 9.00 \) at \( 25 \)°C to \( 8.68 \) at \( 80 \)°C (calculated from equations provided by (Millero, 1995); the * simply indicates that it is in seawater at a given temperature, pressure, and salinity). From Equation 5 (and related derivations) it is evident that if \( Ka^* \) rises, \([CO_3^{2-}] \) and \([H^+] \) will rise, and \([HCO_3^-] \) will decline.

**Strontium**

**Strontium in seawater**

Strontium, with a molecular weight of \( \sim 87.5 \text{ g/mole} \) is significantly heavier than calcium (40.07 g/mole) or magnesium (24.3 g/mole). In physical size, a strontium ion is about 13% larger than a calcium ion, and 70% larger than a magnesium ion. In seawater (\( S = 35 \text{ psu} \)), Sr is present at approximately 90 \( \mu \text{M} \). By concentration, it is the fifth most abundant cation but is the third most abundant divalent cation. In mass units, it is present in seawater at about 8 ppm. The strontium concentration is largely constant across the oceans, with local variations of only 2–3% relative to calcium (DeVilliers and Nelson, 1999; Bernat et al., 1972). As salinity varies in lagoons and estuaries strontium rises and falls accordingly. It was generally accepted that the strontium concentration was also unchanged with depth, but more recent measurements have shown that the strontium concentration rises with depth due to the presence of *acantharians* that deposit strontium sulfate skeletons, slightly depleting the surface of Sr. As these free floating organisms die and settle to the bottom, the deeper waters become enriched in strontium as the skeletons dissolve (DeVilliers and Nelson, 1999).

Most of the strontium is present as the free ion, with only water molecules attached to it (Millero, 1995). A small portion (about 10%) of the strontium is present as a soluble ion pair with sulfate (\( \text{SrSO}_4^2^- \)), and much smaller portions are paired with bicarbonate (\( \text{SrHCO}_3^- \)), carbonate (\( \text{SrCO}_3 \)), fluoride (\( \text{SrF}^+ \)), borate (\( \text{SrB(OH)}_4^{4+} \)), and hydroxide (\( \text{SrOH}^- \)). The average residence time for a strontium ion in the ocean is on the order of
10–20 million years. That time is similar to magnesium (20–50 million years) and is substantially longer than that for calcium (~1 million year) and aluminum (100 years). It is also substantially lesser than sodium (~250 million years). All three divalent cations are chemically very similar. Size is the primary difference which may cause some chemical differences, especially the solubility of certain strontium salts. Strontium carbonate (strontianite) is less soluble than calcium carbonate, which in turn is less soluble than magnesium carbonate. Interestingly, strontium sulfate (celestite) is much less soluble than calcium sulfate and magnesium sulfate. While none of these are actually saturated in seawater, strontium sulfate is close, at 30% of saturation (McManus et al., 2000). That difference is important, and in fact some organisms use strontium sulfate as a skeleton.

Organisms that use strontium for skeletal formation

Although shells of marine mineralizing species are usually made of calcium carbonate there are few species like Cephalopods, Radiolaria, Gastropods which enrich Sr in their calcium carbonate skeleton. However, there is one unique species Acantharia which is probably the main user of strontium in the ocean forming its skeleton completely out of SrSO₄ instead of CaCO₃ (c.f. Kinsman and Holland, 1969). These free floating unicellular microorganisms are related to radiolaria. They have radiating spines of strontium sulfate that are largely external to the central cytoplasm. Inside the cytosol the spines are connected. Acantharia live in the upper regions of the oceans where they deposit their strontium sulfate skeletons. Presumably, the skeletons of all Sr using species are protected from dissolution due to organic coatings. However, when, for example, Acantharia individuals die and sink, the protection of the strontium sulfate is lost, exposing the strontium sulfate to the open water, resulting in dissolution. They are populous enough that they are an important part of the strontium cycle in the oceans including river and hydrothermal vent. Certain species of radiolaria also use strontium sulfate, despite having silica skeletons. The radiolarian Sphaerozoum punctatum, for example, release flagellated swarmers during reproduction which contain celestite. These crystals are deposited inside a cytoplasmic vacuole, and have a 50–100 nm thick coating of organic material on it. Similar to Acantharia this coating may reduce the likelihood of dissolution of the celestite.

Reconstruction of past and present environmental information from divalent cation ratios (here Mg/Ca, Sr/Ca) and Ca isotope fractionation

Mg/Ca-thermometer

The observation that magnesium was higher in marine carbonates precipitated in warmer water was confirmed for neritic foraminifer shells which are composed of high magnesium calcite (<5% MgCO₃). Another study suggested that pelagic foraminifera, which are composed of low-magnesium calcite (<1% MgCO₃), might also follow this pattern (Savin and Douglas, 1973). Several studies also demonstrated that inorganic carbonates followed the same pattern (c.f. Katz, 1973). This latter point is important because it indicates that the temperature influence could not be entirely biological (Lea, 2003). The underlying basis for Mg/Ca paleothermometry is that the substitution of Mg in calcite is endothermic and therefore is favored at higher temperatures. The enthalpy change for the reaction based on the more recent thermodynamic is 21 kJ/mol which can be shown using the van’t Hoff equation to equate an exponential increase in Mg/Ca of 3%/°C (Lea et al., 1999). Foraminifera shells differ from the thermodynamic prediction in two fundamental ways. First, foraminifera contain 5–10 times lower Mg than predicted from thermodynamic calculations (Lea, 2003). Second, the response of shell Mg to temperature is ~3 times larger than the thermodynamic prediction and inorganic observation, averaging 9 ± 1%/°C. Why the latter effect is so is not known, but it has several important implications for Mg/Ca paleothermometry. First, it increases the sensitivity of this approach. Second, it raises the question of why the response is so much greater in foraminifera and if the augmentation of the response depends on secondary factors that might change over geological time (Lea, 2003).

At present, several planctonic species (e.g., G. sacculifer, G. bulloides, G. ruber and O. universa) have been calibrated by culturing and fit with equations of the form:

\[ \text{Mg/Ca} = (\text{mmol/mol}) = b e^{mT} \]

Where \( b \) is the pre-exponential constant, \( m \) is the exponential constant, and \( T \) is the temperature (Lea et al., 1999). Fitting Mg/Ca-temperature data with an equation of this form has the dual advantage of allowing for an exponential response while also parametrizing, by the use of the natural logarithm. Calibration results for the three species mentioned above indicates exponential constants between 0.085 and 0.102, equivalent to 8.5%, 10.2% increase in Mg/Ca per°C (Nürnberg et al., 1996, Lea et al., 1999, Kisakürek et al., 2009).

Note that the Mg concentration of foraminifer shells, as well as other carbonates, is susceptible to change via dissolution. At present, scientists accept that dissolution alters the Mg/Ca content of foraminifera shells and instead are investigating the degree to which such changes occur and whether correction factors are possible, and the degree to which dissolution affects Mg/Ca ratios.

Results on quaternary timescale

Mg/Ca thermometry has led to a number of important findings for paleoceanographic and paleoclimatic research. These include documenting the history of subpolar Antarctic sea surface temperature (SST) variations (Mashio et al., 1999), tropical Atlantic and Pacific SST changes (Hastings et al., 1998; Nürnberg et al.,...
DIVALENT EARTH ALKALINE CATIONS IN SEAWATER

2000), and changes in bottom water temperature in the Atlantic and Pacific.

Among these Mg/Ca results, the most important are those that are available for the tropics. Past SST changes in the tropics have been a contentious issue because the actual glacial–interglacial changes are relatively small (<5°C) and therefore more difficult to detect. However, applying Mg/Ca-thermometry to a sediment core from the Ontong Java Plateau which lies on the equator in the center of the western Pacific warm pool and covering the last 500,000 years indicate that glacial SST cooling was systematically ~3°C cooler than modern conditions and that this cooling occurred during each of the last five major glacial episodes. Another important observation from Mg/Ca studies is the fact that glacial warming appears to lead ice sheet demise as seen from the oxygen isotope record (δ18O) by ~3,000 years (Lea et al., 2000). This observation suggests a prominent role for the tropics in pacing ice age cycles. However, local changes in salinity may also influence δ18O-records resulting in apparent shifts between Mg/Ca and δ18O-records.

Ca isotope thermometry

The possibility of using calcium isotopes for paleothermometry is a very new idea that is based on the empirical observation of temperature-related fractionation between the isotopes 40Ca and either 44Ca or 42Ca (expressed as δ44/40Ca or δ42/40Ca). This potential paleothermometer has been calibrated in neritic benthic foraminifera (DeLaRocha and DePaolo, 2000), a spinoce tropical planktonic foraminifera (Nägler et al., 2000), and a subtropical planktonic foraminifera (Gussoni et al., 2003). Convincing evidence of the potential utility of this new paleothermometer comes from the study of (Nägler et al., 2000), which demonstrates an increase of 0.24% in shells of the planktic species G. Sacculifer δ44/40Ca/C. Down-core measurements from a core in the tropical Atlantic indicate that shells from glacial intervals are ~0.5–1.0% depleted in δ44/40Ca consistent with colder glacial temperatures (Hippler et al., 2006). The δ44/40Ca-temperature sensitivity is species dependent showing a much lower sensitivity for O. universa of only about 0.02%/°C.

Summary

The divalent alkaline earth cations magnesium, calcium and strontium are key elements for the understanding of inorganic and biologically mediated mineralization and calcification. High resolution measurements of element partitioning and isotope fractionation will help to understand the processes controlling the trace element pathways from a bulk solution to the site of calcification. In particular, the recent findings of Fietzke and Eisenhauer (2006) of temperature dependent Sr isotope fractionation may further help to decipher biocalcification processes.

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Carbon Cycle

Carbonate Environments

Carbonates

Dolomite, Microbial

Isotopes and Geobiology

Salinity History of the Earth’s Ocean

Soda Ocean Hypothesis

DIVERSITY

The number of different species in a habitat; sometimes (falsely) used as a synonym for community structure. See entry “Microbial Communities, Structure, and Function” for further reading.

DOLomite, MICRObial

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Synonyms

Bacterial dolomite; Organogenic dolomite

Definition

Dolomite. Widely distributed rock-forming mineral, CaMg(CO3)2. Usually, white or colorless, but can be yellowish and brown with rhombohedral crystals exhibiting curved, composite faces. It also occurs with massive and granular habit and has rhombohedral {1011} cleavage. Dolomitization. The process of converting calcite in limestones to dolomite by the action of magnesium-bearing solutions.
Introduction
The mineral dolomite is notoriously difficult to synthesize under laboratory conditions at low temperature (less than 50°C) and despite its abundance in the ancient rock record, scarcely forms in modern environments. This apparent departure from the geologists’ guiding principle that the “present is the key to the past,” established by Hutton’s principle of uniformitarianism, is at the heart of the “Dolomite Problem,” which is a twofold issue (e.g., McKenzie, 1991).

The dolomite problem
The first aspect of the problem is the abundance of massive dolomites, comprised of secondary or replacement dolomite, in ancient rock, but their striking absence in modern environments. Secondary dolomite is formed through the process of dolomitization, in which the mineral calcite is replaced by dolomite through interaction with Mg-rich fluids (Pursar et al., 1994; Burns et al., 2000, and references therein). The second aspect of the dolomite problem is the difficulty associated with the synthesis of primary dolomite (precipitation of the mineral directly from aqueous solution) at low temperature. Despite conditions favoring its formation and ample time for precipitation, researchers have been unsuccessful in synthesizing dolomite abiotically under laboratory conditions (e.g., Land, 1998), and attribute this lack of success to slow reaction kinetics (Hardie, 1987; Sibley et al., 1987). Recent research has confirmed long-held assertions (e.g., Nadson, 1928; Neher, 1959) that microorganisms play a fundamental role in the formation of dolomite at low-temperature.

Microorganisms as catalysts for dolomite formation
Vasconcelos et al. (1995) successfully synthesized ferroan dolomite in the presence of a native microbial consortium collected from a modern dolomite-precipitating environment, ushering in extensive scrutiny of microorganisms and microbial processes that facilitate dolomite formation. Modern dolomite-forming environments are isolated, and in those that have been extensively studied, dolomite formation is intimately linked to microbial populations and associated activity, for example, the Coorong region of Australia in coastal lakes (Wright, 1999; Wright and Wacey, 2005), Lagoa Vermelha, Brazil, a hypersaline coastal lagoon (Vasconcelos and McKenzie, 1997), and the Persian Gulf sabkas, evaporitic salt pans (Barbieri et al., 2006). Evidence of primary dolomite precipitation from shallow and deep sea marine sediments, soils, and groundwater has been documented (e.g., Hardie, 1987; Lumsden, 1988; Whipkey et al., 2002; Roberts et al., 2004), but the volume of dolomite in these settings is typically minor, forming as thin beds or nodules. All of these environments and their native microbial communities provide an insight into the populations and the mechanisms by which microorganisms mediate dolomite formation.

Microorganisms implicated in dolomite formation
Whereas a wide variety of microorganisms has been implicated in dolomite formation, the best documented metabolic guild is those bacteria carrying out dissimilatory sulfate reduction under anaerobic conditions. These bacteria utilize organic carbon coupled to sulfate as a terminal electron acceptor for energy, thereby removing sulfate and generating alkalinity. Researchers have inferred their presence from geochemical indicators (e.g., Vasconcelos and McKenzie, 1997), culturing experiments (Warthmann et al., 2000; Wright and Wacey, 2005), and non-culture-based identification. Some of the organisms identified include Desulfostipes saporovans and Desulfosarcina spp. (Wright and Wacey, 2005) and Desulfovibrio brasiliensis (Warthmann et al., 2000).

Two other types of bacterial metabolic pathways have also been shown to mediate low-temperature dolomite formation. Two moderately halophilic aerobic heterotrophs, Halomonas meridiana and Virgibacillus marismortui, produce dolomite under laboratory conditions (Sánchez-Román et al., 2008). These organisms utilize nitrogenated organic matter coupled to oxygen as a terminal electron acceptor, consuming acidity and producing alkalinity. The other bacterial metabolic guild implicated in dolomite precipitation is the sulfide oxidizers, which oxidize H2S, producing sulfate and acidity (Moreira et al., 2004). This interaction has been inferred from geochemical and isotopic data from Lagoa Araruama, Lagoa Vermelha, and Brejo do Espinho, Brazil, and the production of H2S in these environments is produced via bacterial sulfate reduction, also thought to produce microbial dolomites.

Additional metabolic pathways linked to dolomite precipitation are part of the Archaeal domain, and involve either the production or utilization of methane. Anaerobic methane oxidizers, which utilize methane and generate alkalinity, in concert with methanogens, were found to control low temperature dolomite formation in deep-sea sediments as inferred from geochemical and isotopic data (Moore et al., 2004). Isotopic signatures from a range of ancient sediments indicate that the activity of methanogens is linked to dolomite formation (e.g., Mazullo, 2000). Additionally, recent studies have linked methanogenesis to the precipitation of dolomite in shallow groundwater (Roberts et al., 2004). Laboratory experiments identified two methanogenic metabolic pathways associated with dolomite formation, acetoclastic and autotrophic. Acetoclastic methanogens cleave acetate forming methane and carbon dioxide, whereas autotrophic methanogens combine carbon dioxide and hydrogen to form methane. These pathways were associated with Methanosaeta spp. and Methanobacterium spp., respectively (Kenward et al., 2009).

Mechanisms for microbial mediation of dolomite
Although it is clear that the presence of microorganisms under some geochemical conditions promotes dolomite
precipitation, there is still debate as to how microorganisms are involved in facilitating precipitation.

The most important criterion for the precipitation of dolomite is that supersaturated conditions exist. Many of the microbial guilds discussed previously are capable of driving supersaturation of dolomite as a result of their metabolic pathways. Sulfate reduction, aerobic heterotrophic respiration, anaerobic methane oxidation, and acetoclastic methanogenesis produce alkalinity as a by-product of metabolism, which increases supersaturation with respect to carbonate minerals. Sulfate reduction, aerobic heterotrophic respiration, anaerobic methane oxidation, and acetoclastic methanogenesis produce alkalinity as a by-product of metabolism, which increases supersaturation with respect to carbonate minerals. Sulfide oxidation produces acidity, which lowers the saturation state of competing carbonate minerals, thus, favoring the supersaturation of dolomite. Sulfate reduction, aerobic respiration of nitrogenated carbon compounds, autotrophic methanogenesis, and anaerobic methane oxidation increase pH in the system, which also favors supersaturation of the carbonate minerals (Madigan and Martinko, 2005).

Although microbial metabolism can generate solutions supersaturated with respect to dolomite, it has been demonstrated that supersaturation alone does not lead to the precipitation of dolomite and, therefore, microbes likely facilitate dolomite formation by removing additional kinetic barriers present at low temperatures. For example, the sulfate inhibition model asserts that sulfate is a kinetic inhibitor of dolomite precipitation because of the strong neutral complex it forms with magnesium (MgSO₄), essentially decreasing the Mg available to form dolomite (Baker and Kastner, 1981). This was demonstrated at low sulfate concentrations, hence, favoring those microbial interactions which either remove sulfate or occur under these conditions (i.e., sulfate reduction, methanogenesis, and anaerobic methane oxidation). Other researchers have disputed this model due to the fact that many modern dolomites are formed under conditions with sulfate concentrations equal to or above that of seawater (Hardie, 1987) suggesting that sulfate inhibition is not the sole kinetic barrier to dolomite formation. Laboratory studies have shown that at very low or very high sulfate concentrations, dolomite formation is promoted (Brady et al., 1996; Siegel, 1961). Recently, Sánchez-Román et al. (2009) demonstrated that two moderately halophilic aerobic heterotrophic bacteria could promote dolomite precipitation at sulfate concentrations ranging from 0 to 56 mM. Taken together, these data suggest that sulfate may indeed be an inhibitor to dolomite formation under certain conditions, but is likely not the sole kinetic barrier to precipitation.

One common observation in modern microbial dolomites is the intimate association between the precipitated dolomite and the cell wall of the microorganism or extracellular polymeric substances (EPS) produced by the microorganisms. These observations, in combination with evidence of cell-associated mineral nucleation (e.g., Schultze-Lam et al., 1996; Fortin et al., 1997; Douglas and Beveridge, 1998), have lead to the hypothesis that these biological surfaces are involved in the nucleation of dolomite. Laboratory experiments analyzing the charge character of the cell wall or structure, have implicated nucleation directly onto the cell wall (Figure 1; van Lith et al., 2003a; Roberts et al., 2004; Kenward et al., 2009; Bosak and Newman, 2003). Other laboratory investigations mimicking EPS-like conditions or utilizing EPS directly, have shown that nucleation occurs on these substances (Figure 1; Sánchez-Román et al., 2008; Bontognali et al., 2008). Nucleation onto EPS, rather than the cell wall, may benefit the cell by avoiding entombment and is consistent with the fact that cellular material is rarely observed in microbial carbonates (Bontognali et al., 2008).

There are a wide range of potential mechanisms by which the microbial cell wall or EPS may influence mineral precipitation mechanisms and kinetics, including heterogeneous nucleation on the cell wall; enhanced rates of growth via the catalytic influence of organic molecules or surface functional groups; substrate-based coprecipitation; and oriented (epitaxial) aggregation growth (e.g., Fowle and Fein, 2001; Banfield et al., 2000; Chan et al., 2004; Rancourt et al., 2005;
Elhadj et al., 2006; see also Chapters Microbialites, Modern and Extracellular Polymeric Substances (EPS). In particular, Elhadj et al. (2006) demonstrated that small organic molecules, such as those found in cellular material and EPS, influenced calcite growth rates via both electrostatic interactions and their ability to facilitate the release of water molecules around the calcium cation during precipitation. Desolvation of cations has been considered the rate limiting step in many precipitation reactions (Nielsen, 1984) and is consistent with the findings of Brady et al. (1996), who found that dehydration and carbonation of Mg$^{2+}$ was the probable rate-limiting step for dolomite formation at room temperature.

**Microbial signatures preserved in dolomite**

Two primary signatures preserved in ancient rock are commonly used to classify dolomites as microbial in origin: morphology and textures of the rocks and carbon isotopic values. It has been recognized for some time that biogenic processes impact carbonate mineral morphology and, since the identification of aragonite needle dumbbells as biogenic features (Chafetz and Folk, 1984), these features and others have been considered proxies for bacterially mediated carbonate precipitation. Laboratory studies of microbial dolomite have produced dumbbells (Warthmann et al., 2000; van Lith et al., 2003b), spheroids (Sánchez-Román et al., 2008), nanoscale globules (Figure 1b; Sánchez-Román et al., 2008; Bontognali et al., 2008; Vasconcelos et al., 1995), nanoscale rhombs attached to cells (Figure 1a; Roberts et al., 2004), and platy crystal aggregates (Figure 1b; Kenward et al., 2009). Although many of these features are observed in ancient rocks, they are not limited to a biogenic origin and, therefore, not definitive evidence of microbial precipitation.

The composition and isotopic signature associated with carbonate precipitates has been linked with specific microbial communities, including iron reduction, sulfate reduction, and methanogenesis (e.g., Curtis et al., 1986). Those dolomites associated with sulfate reduction are typically low in Fe (due to pyrite precipitation) and $^{13}$C-depleted, whereas methanogenic dolomites tend to have higher Fe abundance and are $^{13}$C-enriched. Potentially, diagnostic δ$^{18}$O$_{dolomite}$ signatures related to temperature (or potentially depth) of precipitation have been difficult to interpret due to the lack of experimental data for low temperature dolomite. Vasconcelos et al. (2005), however, proposed a paleothermometer for dolomite precipitation based on dolomite produced by sulfate reducing bacteria. Despite these efforts, extraordinary fractionation of δ$^{18}$O$_{dolomite}$ has been observed via methanogenic activity that is inconsistent with the current paleothermometer (e.g., Roberts et al., 2004).

**Summary**

For more than a century, the Dolomite Problem has stumped scientists. In recent years, progress towards a solution has been made by understanding the role of microorganisms in precipitating the mineral dolomite in low temperature (less than 50°C) environments. Many microorganisms, in both the Archaeal and Bacterial domains, are involved in these reactions, suggesting that microbial dolomite can form in a variety of biogeochemical environments. Changes in environmental conditions favoring one microorganism over another throughout time, is a potential explanation for the observed changes in abundance of dolomite from the past to the present. The mechanism by which microorganisms actually facilitate precipitation and increase reaction progress, is still debated. However, recent data point to a mechanism involving the microbial cell wall or cellular exudates overcoming kinetic barriers rather than a metabolic or geochemical control.

**Bibliography**


Cross-references

Archea

Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification

Bio signatures in Rocks

Calcite Precipitation, Microbially Induced

Cap Carbonates

Divalent Earth Alkaline Cations in Seawater

Extracellular Polymeric Substances (EPS)

Microbialites, Modern

Microbialites, Stromatolites, and Thrombolites
EARLY EARTH

See entries “Origin of Life,” “Salinity History of the Earth’s Ocean,” and “Critical Intervals in Earth History.”

EARLY PRECAMBRIAN EUKARYOTES

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Definition

Eukaryotes are probably the result of endosymbiosis, and basically three types exist. The basic ones are the simplest eukaryotic cells with a nucleus only (Giardia) (Graczk, 2005). Secondly, protists have mitochondria, which have been proposed to have an alpha-proteobacterial origin (Sagan (Margulis), 1967). Finally, beside mitochondria, cells of eukaryotic algae contain chloroplasts, former single-celled cyanobacteria achieved via endosymbiosis (Mereschkowski, 1905; Wallin, 1920; Sagan (Margulis), 1967). The geological record of protists begins long before the Ediacaran vendobionts. Possibly stem group protists were found in ca. 3 Ga-old rocks (Javaux et al., 2010). Within the Late Mesoproterozoic rocks, the divergence of major eukaryotic clades is observed. Beside the fossil record of early eukaryotes, related biomarkers (steranes) were found in up to 2.7 Ga-old rocks, which may support the early origin of eukaryotes before the Great Oxidation Event (Brocks et al., 1999; Brocks et al., 2003a, b). However, the syngeneity of the biomarkers used for the interpretation has recently been questioned (Rasmussen et al., 2008) and the first appearance of eukaryotic biomarkers is still disputed (Brocks et al., 1999; George et al., 2008; Rasmussen et al., 2008; Waldbauer et al., 2009).

The oldest known single-celled eukaryotes are acritarchs, probably ancestors of dinoflagellates. Acritarchs played a significant role in Proterozoic oceans, and the first record comes from the 3.2 Ga-old Moodies Group of South Africa (Javaux et al., 2010). This finding is surprising, since eukaryotic metabolisms normally need oxygen, and the Great Oxidation Event took place about 700 My later, between 2.32 and 2.45 Ga. A similar discrepancy emerged from the first appearance of eukaryotic biomarkers in 2.7 Ga-old rocks (Brocks et al., 1999). Although often controversially debated, biomarker records may give excellent indications to the diversification of major phylogenetic clades. For instance, the distribution of 24-isopropylecholestane revealed a high abundance of (demo-)sponges in the Cryogenian (McCaffrey et al., 1994; Love et al., 2009), thus suggesting sufficient oxygen in shelf regions already 100 My prior to the Cambrian explosion. Furthermore, early Precambrian eukaryotic life is not only indicated by biomarker findings. In some diagenetically early silicified stromatolites, well-preserved eukaryotic cells have been described. The best material is known from the 1.88 Gy-old Gunflint Chert (Ontario, Canada) (Fralick et al., 2002; Awramik and Barghoorn, 1977). Eosphaera tyleri from these cherts is probably an eukaryotic cell (Kazmierczak, 1979). Beside the single-celled eukaryotes, few larger fossils which are most likely multicellular organisms are known, such as Grypania spiralis from the Mesoproterozoic Gaoyuzhuang Formation (China) and disk-shaped centimeter-sized organisms like Chuaria (Knoll et al., 2006).

In the Cryogenian and Ediacaran, the diversity of eukaryotes, including fungi and lichens, increased significantly. It is obvious that the increase of diversity and size
is related with the Snowball Earth scenarios and increase of oxygen in the atmosphere.

Bibliography


Cross-references

Algae (Eukaryotic)
Biomarkers (Molecular Fossils)
Critical Intervals in Earth History
Ediacara Biota
Geomycology
Lateral Gene Transfer
Origin of Metazoa
Protozoa (Heterotroph, Eukaryotic)
Sponges (Porifera) and Sponge Microbes
Symbiosis

ECOLOGICAL NICHE

Different from the habitat (see entry Habitat) (spatial), the niche defines the function of an organism. See entry “Microbial Communities, Structure, and Function” for further reading.

EDIACARAN BIOTA

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Definition

Ediacaran period: An interval in the history of earth after the Marinoan/Varanger glaciation of the Neoproterozoic Era (see Chapter Snowball Earth), but before the Cambrian radiations. This interval marks the introduction of complex macroscopic organisms, leading to a revolution in the structure and evolution of marine paleocommunities, including the establishment of multi-level trophic structures, coevolutionary predator–prey interactions and infu- nal activity.

Ediacaran biota: A highly distinctive assemblage of Ediacaran-age macroscopic organisms preserved as casts and moulds in siliciclastic, volcanoclastic, and carbonate sediments.

Introduction

Ediacaran biota is a heterogeneous assemblage representing the earliest known communities of macroscopic organisms. Diverse Ediacaran fossil assemblages are coeval with the earliest phosphatized metazoan embryos (Xiao et al., 1998; but see Bailey et al., 2007; Xiao et al., 2007), the earliest undisputed traces of metazoan activities (Jensen, 2003), and the time of eumetazoan crown-group divergence estimated by some (but not all) molecular clocks (Peterson and Butterfield, 2005). Nonetheless, placing the Ediacaran morphologies into a metazoan phylogeny has been an enduring challenge to paleobiologists (Gehling, 1991; Fedonkin, 1992; Jenkins,
Grazhdankin and Seilacher, 2002, 2005; Narbonne, 1992; Seilacher, 1992; Conway Morris, 1993; Runnegar, 1995; Budd and Jensen, 2000; Dzik, 2003; Narbonne, 2005; Fedonkin et al., 2007a). Earlier attempts to classify these organisms, based on superficial similarities with modern taxa, resulted in interpretations relating them with crown-group animals, specifically, colonial hydrozoans, scyphozoans, sessile colonial anthozoans, annelids, primitive arthropods, and echinoderms (Glaessner, 1984). Over time, various members of the Ediacaran biota have been regarded as representatives of a number of groups spanning several Kingdoms including prokaryotic colonies (Steiner and Reitner, 2001); eukaryotic microbial colonies (Grazhdankin and Gerdes, 2007); xenophyophoran protists (Zhubravlev, 1993; Seilacher et al., 2003); intermediates between plants and animals (Pflug, 1972); marine fungi (Petersen et al., 2003); lichens (Retallack, 2007); sponges (Gehling and Rigby, 1996); etenophores (Dzik, 2002); and quasi-mollusks (Fedonkin and Waggoner, 1997; Fedonkin et al., 2007b). Seilacher famously proposed in 1989 and 1992 that Ediacara biota represents an entirely extinct lineage of macroscopic organisms, the Vendobionta, which were based on a “quilted pneu” or air-mattress type modular construction and lacked any significant tissue or organ differentiation. To date, no agreement has been reached concerning either the biological affinities of the Ediacara biota or the degree of its phylogenetic heterogeneity. It does appear, however, that most Ediacaran organisms lack any compelling similarities with metazoans (Seilacher, 1989, 1992; Retallack, 1994; Grazhdankin and Seilacher, 2002, 2005; Narbonne, 2004; Brasier and Antcliffe, 2004).

Morphological disparity
The overall taxonomic diversity of the Ediacaran biota is modest. A little over 250 species have been described, of which half represents valid taxa and the rest either pseudofossils or synonyms reflecting life stage and preservational variations. At least eight high-level taxonomic groups can be recognized demonstrating considerable disparity within the Ediacara biota. (1) Rangeomorpha comprises of fusiform, foliate, and plumose organisms characterized by branched tubular structures (Narbonne, 2004). (2) Frondomorpha are composed of three distinct parts: a large, relatively flattened foliate section, a central stem, and a holdfast or rooting anchor (Laflamme and Narbonne, 2008); the fossil record of these organisms may be biased towards holdfasts that have a much higher preservation potential compared to foliate sections (Gehling et al., 2000; Hofmann et al., 2008). (3) Discoidal structures exhibit dense concentric rugae or broad concentric rings, occasionally with radial furrows (Grazhdankin and Gerdes, 2007). (4) Palaeopascichnida consist of series (sometimes multiple series and even foam-like aggregations) of sausage-shaped or globular chambers that are occasionally branching (Seilacher et al., 2003). (5) Tribachiomorpha, a bizarre group of discoidal organisms with prominent three-fold symmetry (Fedonkin, 1992). (6) Bilateromorpha, as the name says, comprise organisms with a bilaterally symmetric body (i.e., that consists of mirror image halves) (Fedonkin et al., 2007b). (7) Dickinsoniomorpha are flat and thin, at first glance bilaterally symmetric forms that consist of large number of segments, with one segment having a distinctive crescent shape and superficially resembling an arthropodal head-shield; the segments of the two sides do not line up perfectly along the midline, however, but are offset, are alternate, a kind of symmetry known as glide reflection (as opposed to mirror reflection symmetry) (Ivantsov, 1999, 2001, 2004, 2007). (8) Petalonamidae, an imperfectly defined group dominated by serially quilted body plans, most exotic to mainstream biology; their mouldic preservation within sandstone is intriguing in that the specimens appear in variously curved and oriented shapes, approximately resembling internal moulds of pots and troughs (Pflug, 1972; Grazhdankin and Seilacher, 2002) (Figure 1).

Patterns of distribution
Ediacaran fossils have been documented globally within a discrete and well constrained interval of geological time between 575 and 544 Ma. Within this range, there is a clustering of three taxonomically distinct fossil assemblages: the Avalon-, Ediacara- and Nama-type biotas (Waggoner, 2003). The Avalon-type biota consists of rangeo- and frondomorphs. The Ediacara-type biota is the most diverse assemblage characterized by discoidal structures, frondo-, tribachio-, bilatero-, and dickinsoniomorphs. The Nama-type biota is dominated by petalonamidae. Palaeopascichnida are unique in that they occur in all three assemblages. The Avalon-, Ediacara-, and Nama-type biotas have been interpreted as evolutionary lineages, or biogeographic provinces, or preservational artefacts (Waggoner, 1999, 2003; Narbonne, 2005; Shen et al., 2008). It remains the case, however, that the Avalon-, Ediacara-, and Nama-type biotas were each confined to a certain habitat. These assemblages thus represent three principal ecotypes on a global scale, with Avalon-type biotas distributed in quiet waters on muddy substrates; Ediacara-type biotas inhabiting microbial substrates in shallow marine wave- and current-agitated settings; and Nama-type biotas found in extremely shallow-water river-mouth bar shoals (Grazhdankin, 2004) (Figure 2). Within each ecotype, species distribution and abundances exhibit extreme heterogeneity and patchiness on a population scale (Clapham and Narbonne, 2002; Clapham et al., 2003; Droser et al., 2006; Droser and Gehling, 2008). This may result from a variety of factors, including competitive interactions, initial distribution of colonists, history of disturbance, or underlying heterogeneity of the abiotic environment.

Preservation
Ediacaran organisms lack hard skeletons, yet the fossil preservation of their soft parts has almost no counterpart
Ediacaran Biota, Figure 1  Morphological disparity of the Ediacaran biota. (a) *Palaeopascichnus delicatus*, a member of Palaeopascichnida (hyporelief). (b) *Nemiana simplex*, a member of Psammocorallia, a group of radially symmetrical sand bodies, with smooth hemispherical lower surface (hyporelief). (c) *Yorgia waggoneri*, a member of Dickinsoniomorpha (hyporelief). (d) *Ventogyrus chistyakovi*, a member of Petalonamae (hyporelief). (e) *Kimberella quadrata*, a member of Bilateromorpha (hyporelief). (f) *Charniodiscus concentricus*, a member of Frondomorpha (vertical preservation in sandstone). (g) *Rangea schneiderhoehni*, a member of Rangeomorpha (hyporelief). (h) *Cyclomedusa davidi*, a discoidal structure (hyporelief). (i) *Tribrachidium heraldicum*, a member of Tribrachiomorpha (hyporelief). (a), (c–i) Southeast White Sea area, Russia. (b), Podolia, Ukraine. Scale bar, 10 mm.
to the classic examples in Phanerozoic (Conway Morris and Grazhdankin, 2005) (Figure 3). The preservation of Ediacaran fossils in coarse-grained sandstones might suggest that the organisms had an unusually tough integument (Seilacher, 1992), comparable with chitin of fungal cell walls (Retallack, 1994, 2007). A unique histology of Ediacaran bodies, such as presence of biomaterials indigestible for contemporary microorganisms or possible absence of degrading enzymes such as collagenase is sometimes invoked (Runnegar, 1991; Dzik, 1999). However, examples of Ediacaran fossils encrusted with aggregations of microcrystalline pyrite suggest that the Ediacaran tissues were not immune to microbial degradation after death, and that their decay fueled reduction of seawater sulfate. The most widely accepted view is that Ediacaran taphonomy represents a non-uniformitarian mode of preservation, associated with extensive pre-Cambrian microbial mat cover; certainly many of these fossils occur on microbially bound bedding surfaces characterized by finely-wrinkled, shagreen texture, and other microbially induced sedimentary structures (Gehling, 1991, 1999; Seilacher, 1999). Microbial mats and biofilms may have provided an adhesive surface to anchor benthic organisms during storms or inundation events, as well as protected the substrate from erosion. Storm deposits could be subsequently recolonized by a new microbial mat that would in turn reduce particle and fluid mixing between oxic and anoxic layers in the sediment thereby facilitating early diagenesis. Sediments sealed by microbial mats would effectively act as closed dynamic systems of physical, chemical, and biochemical processes (see Chapter Sediment Diagenesis – Biologically Controlled). A smothered microbial mat itself could provide a template for precipitation of early diagenetic minerals and act as detailed “death-masks” of the associated Ediacaran corpses.

**Biogeochemical perturbations**

The sudden appearance and global dispersal of densely populated macroscopic communities of Ediacaran organisms would have had global ecological repercussions, and the Ediacaran period is noted for pronounced biogeochemical perturbations. Prior to appearance of the Ediacaran biota (~580 Myr ago), $\Delta^{34}S$, the difference between coeval carbonate-associated sulfate and pyrite was conspicuously low while the ratios of “highly reactive iron” (i.e., the iron that is geochemically and biologically active during early sediment diagenesis) to total iron were high (Fike et al., 2006; Canfield et al., 2007), both indicating low ambient oxygen (at least locally).
Following the appearance of the Ediacaran biota, highly reactive iron fell to essentially modern levels, $\Delta^{26} \text{Sr}$, $\delta^{13} \text{C}_{\text{org}}$, and $\delta^{13} \text{C}_{\text{carb}}$ briefly dropped to all time lows, and $\delta^{13} \text{C}_{\text{org}}$ fell to less than 10 to 20% PAL. The Ediacaran biota evolved immediately in response to the permissive ecology (Fike et al., 2006; Canfield et al., 2007). Alternatively, the rise of oxygen in Ediacaran time could be the consequence of a new-found sink for organic carbon, namely the production of abundant organic-carbon-adsorbing clay minerals -- a consequence of newly evolved, soil-forming, terrestrial ecosystems (Kennedy et al., 2006). This, of course, echoes observations of pedogenic features (pertaining to processes of soil formation) in proximity to some Ediacaran fossils (Retallack, 1994, 2007). Thus, some biogeochemical perturbations during the Ediacaran period may have been the consequence of biotic evolution, rather than its cause (Butterfield, 2007).

### Summary

Ediacaran biota is a geobiological phenomenon. From the evolutionary point of view it looks like an assortment of multiple experiments in multi-cellularity and/or macroscopic size. In the ecological perspective, the Ediacaran biota witnessed what is arguably the earth’s most profound shift in ecosystem structure over the past 4 billion years, from an archaic world of limited morphological diversity and extreme evolutionary stasis to one of high diversity and rapid Phanerozoic-like turnover (Butterfield, 2007). The fossilized Ediacaran biota is seen through a unique taphonomic window, a product of intimate short-time relationships between macroscopic organisms and microbial substrates. Irrespective of whether the Ediacaran biota is related to Metazoa that define the Phanerozoic, it represents a key step in the origin of the modern biosphere.

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Biofilms
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Critical Intervals in Earth History
Mat-Related Sedimentary Structures
Microbial Degradation
Sediment Diagenesis – Biologically Controlled
Snowball Earth

ENDOLITHS

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Definition
Endoliths: Organisms, growing in the interior of rocks. They are cryptoendoliths when growing within structural cavities, chasmoendoliths when inhabiting fissures and cracks of the rock, and euendoliths when actively penetrating rocky substrates (Golubic et al., 1981).

Endolithically growing organisms
The endolithic growth form occurs in a variety of organism groups comprising cyanobacteria, red and green algae, fungi, lichens and nonphotoautotrophic bacteria. Most endolithic habitats (if not all) accommodate several of these organism groups and are accompanied by a large variety of nonphotoautotrophic bacteria (McNamara et al., 2006).

Biogeography
Polar climate
Probably the best known endolithic communities, first described by Friedmann and Ocampo in 1976, are those of the Beacon Sandstone in the dry valleys of southern Victoria Land, Antarctica. In his studies, Friedmann (1977, 1978) specified cryptoendolithic lichens forming multicolored zones several millimeters wide under the surface of sandstone rocks as well as chasmoendolithic lichens mainly growing in granitic rock substrates. Fertile stages of both allowed them to be identified as Buellia sp. (Friedmann et al., 1980) as well as species of the genera Lecidea and Acarospora (Friedmann, 1982). At a later stage, two euaryotic communities (one lichen and one green algae – Hemichloris – dominated) plus three cyanobacterial communities (a red Gloeocapsa, a Hormathonema-Gloeocapsa and a Chroococcidiopsis community) were distinguished at this site (Friedmann et al., 1988).

Within a coastal desert at Marble Point, near the frozen Ross Sea, Friedmann (1982) identified heavy chasmoendolithic growth within weathered marble, consisting of filamentous green algae and cyanobacteria, being accompanied by colonies of colorless bacteria.

After the first discovery of endoliths in Antarctica, chasmoendolithic algae were also detected in other parts of the continent, such as the Vestfold Hills, Princess Elisabeth Land and Mawson Rock, and Mac. Robertson Land, where Broady (1981) found a variety of green algae and cyanobacteria within quartz stones, charmokites, gneisses and other rocks. Small endolithic colonies with cyanobacterial, bacterial and fungal components growing within a gypsum crust on the surface of sandstone boulders were discovered by Hughes and Lawley (2003) on Alexander Island, Antarctic Peninsula.

The structure of endolithic communities collected from Granite Harbour at the Ross Sea coast was studied by De Los Rios et al. (2003, 2005) utilizing scanning electron microscopy with backscattered electron imaging (SEM-BSE). They describe an intimate contact between the organisms and the surrounding substrate and in a subsequent study, combined with molecular analyses (De Los Rios et al., 2007), they were able to distinguish two different types of endolithic biofilms.

An extensive distribution of a chasmo- and cryptoendolithic cyanobacterial community, dominated by the unicellular cyanobacterium Chroococcidiopsis sp., was reported from granite rocks of an area extending at 600 m elevation at Mt. Falconer, in the McMurdo Dry Valleys (Büdel et al., 2008; Figure 1a–c). The authors found that community to be highly active due to regular dewfall on the rock surface. Radiocarbon dating indicated that the community is by far younger than those reported from a 1,000 m higher elevation by Bonani et al. (1988).

Besides Antarctica, cryptoendolithic habitats were also discovered in the Canadian High Arctic (Omelon et al., 2006), hosting an assemblage of cyanobacteria, algae, fungi and heterotrophic bacteria within sandstone rocks.

Arid and semiarid climate
Endolithic cyanobacteria are known to occur in a variety of habitats within hot and tropical regions...
throughout the world. On the hillsides of the Negev Highlands, Friedmann et al. (1967) and later Danin and Garty (1983) observed both chasmoendolithic and coccoid euendolithic cyanobacteria within different rock types. A widespread and relatively uniform distribution of endolithic algae and cyanobacteria within sandstones was discovered by Bell et al. (1986), along a transection covering both temperate and semidesert biomes of northern Arizona, southern Utah, and western New Mexico. Büdel and Wessels (1991) identified chasmoendolithic, cryptoendolithic, and hypolithic cyanobacteria within semiarid to arid regions of Africa, North America, Australia, and...
Europe. The genus *Chroococcidiopsis* was found to occur almost worldwide within the cryptoendolithic habitat of hot deserts (Büdel, 1999; Weber et al., 1996, Figure 1c). Even within halite rocks in the hyperarid core of the Atacama Desert, this genus was observed to grow together with heterotrophic bacteria (Wierzchos et al., 2006).

In recent studies, endolithic cyanobacteria were also identified by means of molecular methods, like the endolithic communities within travertine deposits in Yellowstone National Park, which turned out to contain a variety of different cyanobacteria, including several new lineages (Norrис and Castenholz, 2006). The endolithic communities in limestone from a Maya archaeological site only inhabited photosynthetically inactive bacteria, with cyanobacteria being restricted to the epilithic growth form (McNamara et al., 2006).

Descriptions of endolithic lichens within hot and tropical regions are much rarer. Wessels and Schoeman (1988) observed the endolithically growing *Lecidea* aff. *sarcogynoides* Koerb. on Clarens sandstone within the Golden Gate Highlands National Park, South Africa. The endolithic Sonoran Desert lichen *Verrucaria rubrocincta* Breuss was found to occur locally in abundance in calcite plates of open desert pavements (Bungartz et al., 2004).

**Temperate climate**

Endolithically growing organisms were first described by Bachmann (1890), who recognized the endolithic growth of *Verrucaria calciseda* DC. and also other species (e.g., *Sarcogyne pruinosa* Smft., *Aspicilia flavida* Hepp.) somewhat later (1892), by studying microscopical thin sections of local limestone samples. The chasmoendolithic growth of lichens, like *Aspicilia gibbosa* Ach., along mica minerals was first detected by Bachmann (1904), whereas Diels (1914) described a completely chasmoendolithic formation of algae inhabiting dolomite rocks in the Alps.

In a recent study, Hoppert et al. (2004) investigated the colonization of limestone in the Dachstein area of the Alps after continuous retreat of two glaciers, observing the endolithic hyphae of black fungi (Aureobasidiomycetes) as first colonizers 2 years after exposition of the rock, whereas the first green algae appeared between 5 and 12 years after exposure. Exposure times between 30 and 100 years resulted in macroscopically visible biofilms being dominated by endolithically growing lichens.

In contrast to that, endolithic growth below temperate-zone cliffs of the Niagara Escarpment, Canada, was only present under 6% of the surface area (Matthes-Sears et al., 1997), whereas the majority of the surface was covered by epilithic growth.

**Aquatic habitats**

Even in the coastal areas of marine environments, a variety of endolithically growing organisms are known to occur. At the limestone coasts of the northern Adriatic near Rovinj, Schneider (1976) identified a variety of cyanobacteria and lichens. Le Campion-Alsumard (1979) described ten different species of cyanobacteria, which were often associated with green algae, fungi, and bacteria, revealing bathymetric distribution patterns up to 80 m below sea level in the region of Marseille.

In Shark Bay, western Australia, lithified stromatolites, which were formed by the coccolid cyanophyte *Entophysalis major* Ercegovčić, were described to be colonized by a thin green layer of predominantly epilithic thalli of the cyanobacterium *Hornamothemena violaceo-nigrum* Ercegovčić, which sent short endolithic filaments about 50 µm deep into the substrate (Golubic, 1983). Papineau et al. (2005) utilized molecular techniques to characterize a diverse community consisting of bacteria and archaea with only a small fraction of cyanobacteria within this endolithic habitat.

Recently, endolithic microbial communities have been discovered even in the extremely acidic (pH = 1) geothermal environment of Yellowstone National Park (Walker et al., 2005), where an acidophilic eucaryotic red alga (*Cyanidium* sp.) and actinobacteria (*Mycobacterium* spp.) made up the vast majority of the community. In contrast to that, geothermal rocks in the area of Rotorua, New Zealand, were found to be inhabited by a variety of endolithic cyanobacteria with the red algal genus *Cyanidium* occurring but playing only a minor role (Gaylarde et al., 2006).

In a pH-neutral and oxygen-rich creek in the midland area Spessart (Germany), the cyanobacterium *Synechococcus elongatus* Näg. was found to be growing cryptoendolithically within the bleached sandstone (Büdel et al., 1991, Figure 1d).

**Advantages of endolithic growth**

**Light**

It appears quite obvious that the overlying rock substrate effectively shields light from the endolithic organisms. Matthes et al. (2001) experienced an exponential decrease of light intensity in limestone with increasing depth and, using a normal photosensor, they measured 1% of the incoming light 1 mm within the dry substrate. Friedmann and Ocampo-Friedmann (1984) found that in a dry stage about 0.1% of the incoming light reached the upper level of the lichen zone, compared to about 1% in a wet stage. Weber et al. (1996) however explain that due to light dispersion and reflectance within the rock, scalar irradiance, measuring light intensity at an angle of nearly 360°, has to be investigated. By inoculating sterile quartz sand with cyanobacterial cultures, we were able to show that at higher light intensities, colonies of *Chroococcidiopsis* sp. (strain 90.1 and 90.5; culture collection of Department of Plant Ecology and Systematics, University of Kaiserslautern) and *Nostocopsis lobatus* (strain 92.1) settled significantly deeper within the substrate (Spearman’s rho, strain 90.1: \( n = 48, r_s = 0.442, p = 0.002 \); strain 90.5: \( n = 48, r_s = 0.752, p < 0.001 \); strain 92.1: \( n = 30, r_s = 0.494 \),
Temperature

In the cold deserts of Antarctica, Friedmann and Ocampo-Friedmann (1984) reported the endolithic environment to be much less affected by rapid temperature oscillations and coherent freezing and thawing cycles that occur at the rock surface as a result of the frequent gusts of katabatic winds. Friedmann (1980) measured temperature differences up to 7 K between the rock surface and 3 cm within the rock, while Friedmann et al. (1981) registered more balanced and up to 3 K higher temperatures within the lichen zone, as compared to the rock surface.

In the High Arctic, Omelon et al. (2006) measured only minor differences (0.1–0.3 K) between the mean temperature values and no major differences between the minimum and maximum temperature values (0–1.1 K) at the rock surface and 5 mm below on a south-facing outcrop.

Similarly, Weber et al. (1996) measured the absolute temperature maxima to be only 1°C lower within the endolithic habitat of a South African sandstone plateau, as compared to the rock surface.

In summary, the temperature effect seems to be important in some Antarctic habitats where the endolithic growth prevents the organisms from frequent freezing and thawing. In warm climates, however, no major effect can be concluded from the minor temperature differences.

Humidity

Both in hot and cold deserts, porous rocks like sandstone are expected to function as a substantial water reservoir that allows the organisms to be active over a far longer timespan than on the rock surface (Friedmann et al., 1981; Weber et al., 1996). The latter authors measured water content values between 4% and 79% in the upper 3 cm of the sandstone at the end of the dry season, and found these water contents to be positively correlated with the N- and C-content of the sandstone samples (Spearman’s
Chlorophyll content (mg chl \( \text{m}^{-2} \))

Growth characteristics and weathering patterns of endoliths, Table 1

Sollas described the chemical weathering effect of the endolithic lichen *Verrucaria rupestris* Schrad. (now *Verrucaria muralis* Ach.) growing on limestone. Bachmann (1892) observed that fungal hyphae and even fruiting bodies (e.g., apothecia) were able to grow within hard limestone rocks, and concluded that the hyphae secrete a substance dissolving the rock. In 1916, he was the first to propose the widely accepted weathering mechanism within carbonate rocks: the active organisms release CO\(_2\), which forms carbonic acid (H\(_2\)CO\(_3\)) in a humid environment that in turn, together with the calcium carbonate of the rock, forms water-soluble bicarbonate.

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3
\]

\[
\text{H}_2\text{CO}_3 + \text{CaCO}_3 \leftrightarrow \text{Ca}^{2+} + 2\text{HCO}_3^-
\]

During the following decades there have been several descriptions of weathering patterns on limestone (e.g., Degelius, 1962; Le Campion-Alsumard, 1979; Danin et al., 1983; Gehrmann et al., 1992) and even stromatolites were observed to be weathered by the partly epi- and endolithically growing cyanobacterium *Hormatethena violaceo-nigrum* (Golubic, 1983). Weber et al. (Respiration-Induced Weathering Patterns of Two Endolithically Growing Lichens) studied the weathering patterns of two endolithic lichen species (*Hymenelia prevostii* (Duby) Krempelh. and *H. coerulea* (DC.) Massal., Figure 1e and f) in the Limestone Alps and observed the same general weathering pattern. However, *H. prevostii* revealed to develop more than twofold the biomass compared to *H. coerulea* and to modify a significantly wider zone within the substrate (Figure 1e and f; Table 1).

Pohl and Schneider (2002), however, described an overall protective effect of endoliths on carbonate rocks, since after primary colonization they did not observe a continuous loss of material but an internal recycling within the biofilm. Together with the coauthors, they even saw the upper substrate layer, which is usually impregnated by extracellular polymeric substances (EPS) of cyanobacteria, as a protective shield and recognized a stabilizing effect by the tightly woven internal cellular network of the organisms.

Weathering traces on silicate rocks (garnets) were also recognized already a long time ago (Bachmann, 1911), but weathering marks on quartz crystals were unknown.

**Grazing organisms**

A less frequently named potential advantage is the endolithic shelter against grazing organisms. Schneider (1976) observed intense grazing of epilithic algae and cyanobacteria along the limestone coast of the Adria by gastropods, whereas endolithic organisms were shielded within the substrate. Tretiach (1995) described frequent grazing marks on epilithic lichens, whereas endolithic lichens that were particularly frequent in deeply shaded humid habitats revealed hardly any grazing marks by arthropods, gastropods or other small animals.

Therefore, in certain habitats, endolithic growth may be an effective shelter against grazing organisms, whereas it is not considered to be a major controlling factor.

**Weathering mechanisms and biosignatures**

Weathering features connected with the endolithic growth of organisms were observed as early as in 1880, when Sollas described the chemical weathering effect of the endolithic lichen *Verrucaria rupestris* Schrad. (now *Verrucaria muralis* Ach.) growing on limestone. Bachmann (1892) observed that fungal hyphae and even fruiting bodies (e.g., apothecia) were able to grow within hard limestone rocks, and concluded that the hyphae secrete a substance dissolving the rock. In 1916, he was the first to propose the widely accepted weathering mechanism within carbonate rocks: the active organisms release CO\(_2\), which forms carbonic acid (H\(_2\)CO\(_3\)) in a humid environment that in turn, together with the calcium carbonate of the rock, forms water-soluble bicarbonate.

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at that time. A distinctive weathering pattern of sandstone was discovered by Friedmann (1982), who described a dissolution of the cementing substance along the growth zone of endolithic lichens in the Antarctic cold desert. A very similar weathering pattern was observed by us (Weber et al., 1996) in the South African Tshipise Sandstone, where endolithic cyanobacteria caused the exfoliation of the substrate along the growth zone (Figure 1g).

Such an exfoliation of sandstone was also described by Wessels and Schoeman (1988) for the endolithically growing lichen Lecidea aff. sarcogyroides. Since the silicate weathering could not be explained by an acidification as that of limestone, the driving mechanism was unknown until recently, when we could show that an alkalinization process during photosynthesis was responsible (Büdel et al., 2004). This alkalinization was shown to be responsible for a dissolution of the binding material, etch marks on the quartz grains, and a precipitation of calcium carbonate within the studied sandstone (Figure 1h), which resulted in the previously described exfoliation of the sandstone.

The formation of minerals, which could serve as biosignatures within rock deposits was already described in 1916 when Bachmann explained the deposition of calcium oxalate by endolithic cyanobacteria within limestone. Friedmann and Weed (1987) observed the formation of quartz rhinds on top of the endolithic organisms within the Beacon Sandstone, South Africa. This dense crystalline layer above the organisms was also recognized by us, but as stated earlier, we also observed the precipitation of calcium carbonate within the growth zone of the cyanobacteria (Figure 1h; Büdel et al., 2004).

The preservation of cell structures in the form of fossil endoliths is known from the silicified stromatolithic crusts of the 1,500–1,700 Ma old Dahong Yu Formation in China (Zhang and Golubic, 1987). But also ooid assemblages of several Eothyella species within 800 Ma old rocks were found to be comparable to modern ooids of the Bahama Bank and the Arabian Gulf (Knoll et al., 1986). Body fossils of endolithic bangiacean rhodophytes were found in sediments that are 420 Ma old (Kamirezak and Golubic, 1976), whereas Wierchhos et al. (2005) described the recent formation of cell-shaped structures within the sandstones of Antarctica. The latter authors propose these structures to be important markers in the search for former life on Mars. Golubic and Schneider (2003) as well as Banfield et al. (2001) point out that the activity of endolithic organisms left a rich fossil record of boring and mineralization patterns behind, which reveal the nature of palaeoenvironmental conditions under which they lived.

Conclusions

Endolithic growth is realized by a variety of organisms, such as cyanobacteria, algae, lichens, fungi, and bacteria, and occurs in various habitats all over the world. The advantages of endolithic growth are multifarious, like the shield from high light intensities or grazing organisms or the occurrence of more favorable temperature and humidity conditions, with their relevance being variable within the different habitats. The endolithic organisms are known to have weathering effects on both calcareous and siliceous substrates with different underlying processes. The weathering marks and also newly formed minerals can serve as biosignatures, which allow to draw conclusions on endolithic growth in former times or on other planets.

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Endosymbiosis is a close, prolonged physical and/or metabolic association between distinct organisms, one of which lives inside the body of the other, either within its cells or in extracellular spaces. Please refer to entry “Symbiosis” for further reading. See also entries “Cyanobacteria,” “Algae (Eukaryotic),” “Diatoms” and “Fungi and Lichens” for particular forms of endosymbioses.

Exoenzymes – an overview
Figure 1 illustrates the most relevant microbial exoenzymes and their substrates. Their catalytic mechanisms as well as selected review articles describing the respective enzymes in detail are shown in Table 1. Figure 2 summarizes the impact of microbial enzymes on environmental processes and their application in biotechnology; both aspects are discussed in detail in the following paragraphs.

Exoenzymes and their impact on environmental processes
Prokaryotic and eukaryotic microorganisms are one of the main driving forces of biogeochemical cycles as they can decompose virtually all natural compounds, thereby exerting a lasting effect on biosphere and climate (Madigan et al., 2008). Archaea, bacteria, and fungi are the only living agents that are capable of regenerating essential elements such as carbon, sulfur, nitrogen, and iron by decomposing dead organic matter. Moreover, some microorganisms are able to degrade plastics, particularly biopolymers, and highly complex xenobiotics such as crude oil, and thus contribute significantly to the cleanup or detoxification of environmental pollutants (bioremediation; Knackmuss, 1996).
Symbiotic interactions between microorganisms and animals (e.g., termites and ruminants) owe a great debt to the beneficial activities of microbial exoenzymes (Madigan et al., 2008). For example, a varied intestinal flora of bacteria, fungi (and protozoa), enable herbivores to digest cellulose and other plant polysaccharides. A vital part of human nutrition thereby directly stems from this exoenzymatic activity in the intestinal tracts of livestock and other ruminants, converting low-energy plant foods into high-energy foods such as milk, dairy products, and meat. On the other hand, uncontrolled microbial growth together with the production of hydrolytic enzymes such as proteases, lipases, and cell-wall degrading enzymes also destroys vast amounts of food and can even lead to food-borne infections or poisoning.

In deleterious host-pathogen interactions, exoenzymes produced by the pathogens may act as virulence factors (Madigan et al., 2008). Prominent examples for such pathogenic enzymes are (a) hyaluronidases and coagulases produced by human and animal pathogens that degrade the intercellular matrix component hyaluronic acid or dissolve fibrin clotting, respectively, enabling them to invade and colonize host tissues, or (b) pectinases and cellulases produced by plant pathogens that break up cell walls and facilitate invasion and degradation of living plants.

**Exoenzymes – nature’s biotechnology tools**

Microbial enzymes have served humankind since prehistoric times in the traditional production and conservation of food such as wine and cheese. However, enzymes as molecular tools were not discovered until the second half of the nineteenth century and have since been extensively used in various industries (Damhus et al., 2008). Classical chemical transformation processes have various inherent drawbacks, for example, poor product yields, high energy costs, harsh and hazardous reaction conditions and unwanted, partly harmful by-products. In
contrast, enzymatic reactions proceed under mild conditions are highly specific and involve very fast reactions. Moreover, enzymes contribute to sustainable development as they can be isolated from microorganisms which are cultured using primarily renewable resources (Damhus et al., 2008).

Modern advancements in biotechnology, especially in the field of molecular genetics and protein engineering, have brought the application of a wide variety of biocatalysts to many different industries (Figure 2 and Table 2): (a) amylases, proteases, lipases, and pectinases are used in the food industry, ranging from traditional applications in wine and beer production, cheese ripening, baking, but also for fruit juice clarification and sweetener production; (a) xylanases, cellulases, and amylases are employed in the paper industry for bleaching, recycling, and coating; (c) lipases, proteases, amylases, and cellulases are added to laundry and automated dishwashing detergents to improve cleaning performance and reduce energy and water consumption; (d) in the textile industry cellulases, laccases, and peroxidases are used for bio-bleaching of jeans fabrics and cellulases/proteases for bio-polishing of cotton fabrics, wool and silk products; (e) amylases and cellulases are employed for biofuel production from starch- and cellulose-containing raw materials.

Summary
Microorganisms are able to degrade all naturally occurring organic compounds and also certain xenobiotic substances that persist in oxic environments and thereby deserve to be considered omnipotent. To this end, archaea, bacteria, and fungi secrete specific enzymes, the so-called “exo enzymes” into the immediate surroundings that act to break down their substrates in part or even up to complete mineralization. Exoenzymes are thus of great importance for elemental recycling processes within biogeochemical cycles, yet they also contribute substantially to symbiotic or pathogenic interactions between microbes and higher organisms and are furthermore useful catalysts for various biotechnological and industrial applications.
**Bibliography**


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**Exoenzymes, Figure 2** Microbial enzymes: ecological impact (left panel) and biotechnological applications (right panel). The top panel shows a colony of *Streptomyces* sp. degrading carboxymethyl cellulose incorporated in the agar and stained with Congo-red.
Exoenzymes, Table 2  Exoenzymes, producing organisms, and their applications in biotechnology

<table>
<thead>
<tr>
<th>Enzyme (class)</th>
<th>Source organism (examples)</th>
<th>Application/industry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase (hydrolases)</td>
<td><em>Rhizopus</em> sp. <em>Bacillus</em> sp.</td>
<td>Starch saccharification, washing powder, baking industry, biofuel production</td>
</tr>
<tr>
<td>Catalase (oxidoreductases)</td>
<td><em>Micrococcus</em> sp. <em>Aspergillus</em> sp.</td>
<td>Analytical applications, textile industry, conservation of egg-products</td>
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<tr>
<td>Cellulase (hydrolases)</td>
<td><em>Trichoderma</em> sp. <em>Clostridium</em> sp.</td>
<td>Textile industry, paper industry, biofuel production, washing powder</td>
</tr>
<tr>
<td>Chitinase (hydrolases)</td>
<td><em>Enterobacter</em> sp. <em>Streptomyces</em> sp.</td>
<td>Mosquito control, control of plant pathogenic fungi</td>
</tr>
<tr>
<td>Glucose-isomerase (isomerases)</td>
<td><em>Bacillus</em> sp. <em>Arthrobacter</em> sp.</td>
<td>Starch saccharification, soft drinks</td>
</tr>
<tr>
<td>Glucose-oxidase (oxidoreductases)</td>
<td><em>Trichoderma</em> sp. <em>Saccharomyces</em> sp.</td>
<td>Baking industry, conservation of egg-products, Sweets (prevention of sugar crystallization)</td>
</tr>
<tr>
<td>Invertase (hydrolases)</td>
<td><em>Basidiomyces</em> sp. <em>Aspergillus</em> sp. <em>Burkholderia</em> sp.</td>
<td>Textile industry (jeans bleaching), Washing powder, cheese production</td>
</tr>
<tr>
<td>Lipase (hydrolases)</td>
<td><em>Aspergillus</em> sp. <em>Clostridium</em> sp.</td>
<td>Fruit juice clarification, textile industry</td>
</tr>
<tr>
<td>Pectate-lyase (lyases)</td>
<td><em>Streptomyces</em> sp. <em>Aspergillus</em> sp.</td>
<td>Fruit juice clarification, coffee- and cacao-production</td>
</tr>
<tr>
<td>Pectinase (hydrolases)</td>
<td><em>Aspergillus</em> sp. <em>Clostridium</em> sp.</td>
<td>Textile industry (jeans bleaching)</td>
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<tr>
<td>Peroxidase (oxidoreductases)</td>
<td><em>Bacillus</em> sp. <em>Aspergillus</em> sp. <em>Bacillus</em> sp.</td>
<td>Baking industry, washing powder, meat tenderizer</td>
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<tr>
<td>Protease (hydrolases)</td>
<td><em>Aspergillus</em> sp. <em>Bacillus</em> sp. <em>Klebsiella</em> sp.</td>
<td>Starch saccharification</td>
</tr>
<tr>
<td>Pullulanase (hydrolases)</td>
<td><em>Bacillus</em> sp. <em>Clostridium</em> sp.</td>
<td>Baking industry, paper industry, textile industry, animal food additive</td>
</tr>
</tbody>
</table>


**Cross-references**

- Aerobic Metabolism
- Archaea
- Bacteria
- Biogeochemical Cycles
- Carbon Cycle
- Extracellular Polymeric Substances (EPS)
- Fermentation
- Fungi and Lichens
- Microbial Biominalization
- Microbial Degradation
- Symbiosis

**Definition**

*Extracellular polymeric substances (EPS)* are molecules having a range of sizes, compositions, and chemical properties that are produced and secreted by bacteria and other microorganisms, and contribute to the cell adaptability, resiliency, and functional roles in environments.

*Capsule* refers an extracellular matrix that immediately surrounds a cell.

*Biofilms* refer to attached microbial cells surrounded within a matrix of EPS.

**Introduction**

It has been realized for a long time that microbial organisms may exist as free-living individuals as well as in groups, attached to surfaces or to each other, and that under many conditions cells often prefer to attach to surfaces. Under the fluctuating, often less-predictable conditions of natural systems, such attachment may confer advantages and a certain degree of stability to cells. It is now realized that most bacteria and other microorganisms (e.g., cyanobacteria, archaea, diatoms) exist as attached “biofilms.” The biofilm consists of microbial cells that have attached to a surface (or aggregated), and have surrounded themselves within a gelatinous matrix of EPS, and may exhibit varying degrees of community structure (Decho, 1990). Biofilm formation appears to be a common microbial process that occurs under a wide range of conditions and environments, and whose influences span aquatic, terrestrial, the epi- and
endo-biont communities of plants and animals, and as well as disease and health processes (Stoodley et al., 2002).

The EPS matrix

One major change that occurs upon attachment to a surface and the formation of a biofilm is the secretion of EPS. The EPS consist of a wide range of molecules that confer different physical/chemical properties and adaptations for cells. The EPS matrix consists of molecules having a range of molecular sizes and conformations. The physical structure of natural EPS is poorly understood but may contain waterchannels, physical forms ranging from tight, dense gels to looser, dispersed slimes, whose EPS diffusivities (i.e., the ability of molecules to diffuse through EPS) may vary considerably. This provides cells with the potential capability to bind and sequester important ions, nutrients, and other molecules, and create strong biogeochemical gradients over small (i.e., micrometer) spatial distances. Cells may additionally surround themselves with EPS capsules. Together, EPS form the three-dimensional architecture from which cells may structure interactions, and even construct organized macrostructures.

EPS is an operational designation that refers to the milieu of relatively “large” molecules contained in the extracellular matrix in proximity to cells. In the past, cold-ethanol precipitations were used to separate the large polysaccharide-rich fraction of EPS from other molecules in the extracellular matrix. It is now realized, however, that a host of other molecules (e.g., proteins, lipids, and even DNA, etc.) may be present in the extracellular matrix. It is now realized that in natural biofilms, the polysaccharide fraction represents only a (small) subset of a more diverse EPS matrix. It is now realized that in some natural systems, a large portion of the EPS cannot be defined as a protein, carbohydrate, or lipid, and hence does not conveniently fit into classical chemical categories.

Initially, EPS was largely considered to be a matrix of “exopolysaccharides” (i.e., extracellular polysaccharides); and implied that the terms EPS and exopolysaccharides were synonymous. However, this was, in part, based on the specific carbohydrate-focus of investigators at the time, and further due to an artifact of carbon-rich culture conditions that were needed to grow bacteria and produce abundant quantities of exopolysaccharide. Much has been learned about polysaccharide chemistry from these important seminal studies (for an early review see Sutherland, 1982).

Currently, it is assumed that EPS contains proteins that may act as both structural components for the EPS matrix and as extracellular enzymes. More recently, deoxyribonucleic acids (DNA) have been abundantly found in the EPS of certain bacteria grown in culture, and in EPS extracted from natural microbial communities. While it is likely that some of this DNA may originate from the lysis of bacteria cells, there is growing evidence that bacteria may actively secrete DNA as part of the EPS. This has important implications for understanding DNA as solely an information molecule. Also, recent interest has centered on the occurrence of very refractory forms of proteins, called “amyloid proteins” in EPS. These heavily cross-linked proteins have been previously linked to disease processes in humans. In the EPS matrices of microbial communities, such amyloid proteins may provide a refractory “rebar” to resist degradation of the EPS.

Careful examinations of the EPS matrix are now being conducted using specialized equipment such as atomic force microscopy (AFM), nuclear magnetic resonance (NMR) spectroscopy, and confocal scanning laser microscopy (CSLM) in order to image and characterize the matrix under in situ conditions (Figure 1).

Roles of EPS in geobiology

A great deal of interest is developing in the role of EPS as it influences the broader area of geobiology. This interest centers on the role of biologically produced molecules in mineral formation. Certain molecules may directly or indirectly influence how minerals are formed through precipitation events.

The role of EPS in this process may be twofold (Dupraz et al., 2009). When EPS is newly secreted by microorganisms, certain functional groups (e.g., carboxyls) on the EPS may efficiently sequester Ca^{+2} ions by forming bidentate bridges between adjacent EPS molecules, and thus inhibit complexation with available carbonate anions, and precipitation. However, partial degradation

Extracellular Polymeric Substances (EPS), Figure 1 Image showing the abundant extracellular polymeric substance (EPS) secretions (green) surrounding cyanobacteria and heterotrophic bacteria (blue) within a stromatolite microbial mat. The large round objects (red) are calcium carbonate sediments, called ooids. Image was collected using a confocal scanning laser microscopy (CSLM) (scale bar = 10 μm).
or steric-inhibition (i.e., blocking the binding) of these functional groups may allow Ca++ ions to accumulate locally reaching saturation levels in hydrated pockets within the EPS. Then, certain functional groups on EPS molecules may serve as a potential nucleation site to form a unidentate binding of a Ca++ cation, and allowing the cation to complex with a carbonate anion to initiate precipitation of aragonite (CaCO₃), a process which initiates the biogeminal formation of calcium carbonate. This interest has been fueled by exciting studies of biologically mediated mineral formation that occurs within early oceans (Arp et al., 2001) and the important role of CaCO₃ in global carbon flux. The secretion of EPS by microbial flora likely developed early in the evolution of life, as evidenced by their roles in stromatolite formation, and broader microbial mat processes. Evidence supporting the presence of EPS in fossil microbial mats and other types of microbial-based fossils are now being found.

The presence of EPS in sediment systems strongly influences the physical stability of the sediments against resuspension by wave action and currents. Different physical forms of EPS, ranging from highly soluble (in water) to cohesive gels, have different (i.e., quantitative) stabilizing effects on sediments (Underwood and Paterson, 2003). Additionally, the presence of EPS contributes to the optical properties (i.e., reflectance, scattering, and absorbance) by sediments.

In open aquatic systems such as oceans, microorganisms often form aggregated communities, also called “marine snow,” which occur suspended within the water column. Here, the EPS are especially important in the cohesion of cells and molecules into aggregates. Smaller non-cell containing forms of EPS, called “transparent exopolymer particles” (TEP) may aggregate into larger marine snow (Passow et al., 2001). These aggregation processes become especially pronounced during the later stage of phytoplankton blooms in oceans and lakes, and during extreme examples called “excessive mucilage events,” such as those which occur periodically in the northern Adriatic Sea and other areas. Ultimately, the aggregation process eventually sends the microbial cells, EPS, and their sorbed ions and molecules to the sediments. This sedimentation process collectively carries with itself large quantities of carbon and important trace elements, and contributes to the global flux of these elements in oceans.

Conclusion
EPS are extracellular polymeric substances that are produced and secreted by microorganisms, and may vary in their physical state, chemical properties, and functional roles to cells. The EPS are often produced in association with a biofilm. The production of EPS influences many processes that have broader biological and geobiological importance. For example, EPS gels alter the physical stability and optical signatures of sediments and seawater, and are involved in biogeminal precipitation, and the global flux of carbon and other elements within oceans. Finally, biofilms and their EPS are prominent components of extreme’ environments ranging from cold Antarctic epipontic communities to anhydrophilic hypersaline ponds to the high temperatures/pressures of hydrothermal vent systems.

Summary
It is now realized that most bacteria and other microorganisms (e.g., cyanobacteria, Archea, diatoms) exist as attached “biofilms.” The biofilm consists of microbial cells that have attached to a surface (or aggregated), and have surrounded themselves within a secreted gelatinous matrix of EPS. EPS is an operational designation that refers to the milieu of relatively “large” molecules contained in the extracellular matrix in proximity to cells. The EPS confer different physical/chemical properties and provides cells with the potential capability to bind and sequester important ions, nutrients, and other molecules, and create strong biogeochemical gradients over small (i.e., micrometer) spatial distances. Together, EPS form the three-dimensional architecture from which cells may structure interactions, and even construct organized macrostructures. A great deal of interest is developing in the role of EPS as it influences the broader area of geobiology. This interest centers on the role of biologically produced molecules in mineral formation.

Bibliography

Cross-references
Bacteria
Calcite Precipitation, Microbially Induced
Detachment
Endoliths
Definition

The word “extreme” comes from the Latin word “extremus,” the superlative of “exter” (= on the outside) (Rothschild and Mancinelli, 2001). Whereas there is no general agreement on how to define an extreme environment, the term is commonly used for any setting that exhibits life conditions detrimental or fatal to higher organisms with respect to its physicochemical properties. Thus, extreme environments differ in one or more aspects from those which humans consider as “normal,” moderate conditions with circumneutral pH, temperatures between 20°C and 35°C, pressures around 0.1 MPA (1 atm), and adequate concentrations of nutrient and saline. It should be considered, however, that such definition represents an anthropocentric view and that what is extreme and what is normal from a microbial perspective remains questionable.

If not completely uninhabitable to life, extreme environments typically harbor specially adapted organisms, the so-called extremophiles. Most extremophiles are unicellular organisms, that is, protists, bacteria, and archaea. As a rule, extreme environments show a low diversity of multicellular organisms and only few animals are able to withstand the harsh conditions of particular extreme environments. Terms describing extremophiles usually combine an environment-specific prefix with the suffix “-phile” (Greek word for “loving”). Replacing the suffix “-phile” by “-tolerant” implies that an organism tolerates rather than requires the respective conditions, and actually has its optimum at more moderate conditions. Prefixes may be combined for organisms that thrive in more than one extreme (e.g., “thermoacidophiles”). Such organisms are termed polyextremophiles.

Extreme environments can be categorized into several classes.

Acidic environments

Environments below pH 5, including, for example, sulfuric pools, geysers, and areas polluted by acid mine drainage are called acidic environments. Organisms dependent on acidic conditions below pH 5 (pH 7 = neutral) are called acidophiles. Examples are the bacterial genus Acidithiobacillus (used in biominering, see entry), Ferroplasma acidiphilum, a sulfuric-acid producing archaean involved in acid mine drainage (see entry Acid Rock Drainage), the red alga Cyanidium caldarium (the most heat and acid tolerant alga known), the green alga Dunaliella acidophilica, the fungus Trichosporon cerebriae, and the archaeum Picrophilus (Rothschild and Mancinelli, 2001). The major challenge for these organisms is to maintain their internal cellular pH at a constant, circumneutral level, that is, around pH 7. Individual strategies encompass, inter alia, (i) reinforcement of the cell membrane, (ii) secretion of extracellular polymeric substances (EPS) (see entry “Extracellular Polymeric Substances (EPS)”) to limit proton diffusion into the cell, (iii) secretion of buffer molecules capable of sequestering protons, including, for example, basic amino acids (lysine, histidine and arginine), and (iv) the ability to actively pump protons out of the cell (for reviews, see Baker-Austin and Dopson, 2007; Rothschild and Mancinelli, 2001).

Alkaline environments

Environments above pH 9 include, for example, hydrothermal springs (Figure 1a) and soda lakes (e.g., Lake Van, Mono Lake, Figure 1b) are called alkaline environments. Organisms dependent on alkaline (high) pH values > pH 9 are termed alkaliphiles (Horikoshi, 1998). Examples are the bacterium Bacillus alcalophilus (Horikoshi, 1998) and the archaeon Natronococcus occultus (Tindall et al., 1984). Like acidic habitats, alkaline environments pose particular requirements for the inhabiting organisms, specifically the ability to maintain internal pH at a level not higher than ~pH 8.5 (Horikoshi, 1998). Strategies to achieve this include, inter alia, (i) buffering cell cytoplasm using polyamines rich in amino acids with positively charged side groups (lysine, arginine, and histidine), (ii) introducing acidic compounds to protect the cell from the alkaline environment (e.g., teichoic acid, acidic amino acids, and uronic acids), (iii) Na+ ion channels actively driving the transfer of protons into the cell, (iv) synthesis of specific enzymes resistant to high pH. The enzymes of alkaliphiles found a variety of industrial applications. For instance, alkaline proteases, protein-hydrolyzing enzymes, are widely used in the detergent industry.

Hypersaline environments

Environments with salt concentrations greater than that of seawater (35%; Figure 1, see also Chapters “Saline Lakes”, “Deep Biosphere of Salt Deposits”) are called hypersaline environments. Organisms dependent on salt concentrations greater than 35% (sea water) are called halophiles. Halophiles are known from the Archaea, Bacteria, and Eukarya (Grant et al., 1998; Gunde-Cimerron et al., 2005). Eukaryotes are represented, for instance by the green alga Dunaliella, and even animals occur in hypersaline environments, although in low species diversity (e.g., the brine shrimp Artemia, and the salt fly Ephydra). In the bacterial domain, halophiles represent many different taxonomic groups, whereas
Examples and features of extreme environments. (Images courtesy of J. Reitner and G. Arp)

(a) A volcanic hot spring on Iceland, providing an environment for thermophilic bacteria and archaea. The spring bottom shows a temperature zonation of brown and black microbial mats. (b) Mono Lake, California, is an excellent example for both a hypersaline and an alkaline environment. Being a terminal lake, Mono Lake has no outlet to the ocean, and dissolved salts in the runoff have increased the water’s alkalinity (pH ~10) and salt concentration (70%).

(c) Underwater view of the ~2 m deep hypersaline Lake 21 on Christmas Island (Republic of Kiribati). Kiritimati is a raised atoll, with a rather arid climate. Periodically flooding with sea water and subsequent evaporation cause the salinity of numerous inland lakes to increase to values about 120%, four times that of sea water. The lake bottom is covered with microbial mats dominated by Cyanobacteria at the surface.

(d) A calcifying microbial mat from the same setting as in (c), showing a complex layering of differently pigmented microbes. In the deeper parts of the mat, carbonate precipitation proceeds under anaerobic conditions, ultimately producing massive microbialites.

(e) The Bonneville Salt Flats in northwestern Utah, USA, are a remnant of the ancient glacial Lake Bonneville. Up to 1.8 m, thick salt crusts provide an extremely hypertonic environment for halo- and xerophilic microorganisms.

(f) Lake Clifton, 120 km south of Perth (Western Australia), provides a fragile ecosystem where the growth of a large thrombolite reef (see “Microbialites”) is sustained by calcium carbonate rich aquifers water and strong seasonal trends in salinity (salinity 30–80%). Notably, these modern thrombolitic microbialites coexist with a diverse invertebrate fauna.
those in the archaeal domain fell into a single order (Halobacteriales) and family (Halobacteriaceae; DasSarma and DasSarma, 2008). The most significant challenge for halophiles is to prevent the loss of water from the cell into the saline environment, and the accumulation of excess salt concentrations within the cell. The latter may give rise to the collapse of proteins and macromolecules, because the electric charges of the salt ions disturb the electrostatic interactions within biomacromolecules. Halophiles basically use two strategies to tackle these challenges. (i) Halophilic bacteria and algae accumulate organic compatible solutes, polar, highly soluble molecules uncharged at physiological pH, such as amino acids and their derivatives to counterbalance the osmotic pressure of the surrounding medium (Galinski, 1993; Oren, 1999). (ii) Halophilic archaea, thriving at NaCl concentrations higher than 1.5 M, use the so-called “salt-in strategy.” These most halophilic organisms concentrate K⁺ ions within the cell in order to balance osmotic pressure. Their proteins are particularly rich in acidic amino acids, thus enhancing resistance against structural collapse. For more information, see entry “Halobacteria – Halophiles.” Several reports provided evidence that viable extremely halophilic microorganisms were enclosed in ancient salt sediments. Please refer to “Deep Biosphere of Salt Deposits” for details.

Extremely hot environments
Environments periodically or consistently above 40°C are called extremely hot environments (Stetter, 1998). Depending on the temperature, these settings harbor thermophiles (growth optimum 45–80°C, Martinko and Madigan, 2006) or hyperthermophiles (growth optimum above 80°C). (Organisms dependent on moderate temperatures between 10°C and 50°C (optimum 30–40°C) are called mesophiles.) Whereas thermophiles have a fairly broad ecological amplitude, including, for example, hot waters, sun-heated soils, and waste dumps, hyperthermophiles mostly occur at water-containing, geothermally heated terrestrial and marine springs and sediments (Figure 1a). As a rule, prokaryotes are able to grow at higher temperatures than eukaryotes (limit ~65°C). Accordingly, hyperthermophilic life is mainly represented by deeply branching bacteria, (e.g., Aquifex, Thermotoga) and archaea (e.g., Sulfolobus, Methanothermus; Stetter, 1998). The highest commonly accepted temperature for life has been reported for the hyperthermopiezophilic archaeon Methanopyrus kandleri (122°C; Takai et al., 2008). The challenge for organisms living in hot environments lies in avoiding thermal degradation of cellular biomacromolecules, which may, for example, result in unfolding of proteins. Individual strategies encompass stabilization of proteins by introducing additional disulfide bridges, ionic interactions, hydrogen bonds, and hydrophobic interactions. Another alternative is to introduce the proline residue at particular sites into proteins (the proline rule; Imanaka, 2008). Because many scientists believe that prebiotic molecules and the first living organisms originated in hot environments, hyperthermophiles may provide insight into the early stages of life on Earth. Please refer to “Hydrothermal Environments, Terrestrial,” “Hydrothermal Environments, Marine,” “Hot Springs and Geysers,” and “Origin of Life” for further reading.

High-pressure environments
Environments under extreme hydrostatic or petrostatic (rock) pressure are high-pressure environments. Organisms thriving in these settings are called piezophiles (formerly barophiles). Broadly spoken, piezophiles organisms depend on pressures greater than atmospheric pressure (0.1 MPa, Yayanos, 1998), although organisms living, for example, in shallow coastal waters are commonly neither considered as piezophiles nor as extremophiles. Based on their optimal growth pressure, piezophiles have further been classified as piezotolerant (0.1–10 MPa; 10MPa ~ 1,000 m water depth), piezophilic (10–50 MPa), and hyperpiezophilic (>50 MPa) bacteria and archaea (Fang and Bazylinski, 2008). Whereas, as a rule, the relative growth rate of these organisms decreases with pressure, the upper pressure limit of life has not yet been determined (Yayanos, 1998; Schrenk et al., 2010). High pressures affect biological systems by making the structures (e.g., membranes) more compact, as opposed to the effects of high temperature. Pressurization hinders any process resulting in a positive volume change, and vice versa. For example, if a reaction is accompanied by a volume decrease of 300 mL mol⁻¹, it is enhanced more than 200,000-fold by applying a pressure of 100 MPa (~10,000 m water depth; Abe et al., 1999). Likewise, hydrostatic pressure has been shown to exert a considerable influence on many protein–protein interactions, the efficacy of enzymatic catalysis, replication, and translation. Piezophiles have therefore evolved specific adaptations, for example, in terms of membrane lipid composition and cell division. Pressure has also a significant effect on microbial-mediated redox reactions, and metabolic versatility appears to be a specific adaptation to deep environments (Fang and Bazylinski, 2008; Lauro and Bartlett, 2008). For detailed reading, please refer to entry “Piezophilic Bacteria.”

Extremely cold environments
Environments with temperatures below 5°C for prolonged periods of time, such as cold polar regions, glaciers, sea-ice, deep-sea sediments, and permafrost soils are considered extremely cold environments. Organisms capable of growth and reproduction in these settings are called psychrophiles (cryophiles). They depend on low temperatures (<0°C to 20°C) and have a growth optimum below 15°C (Morita, 1975). The lowest temperature limit for life seems to be around −20°C and has been reported for bacteria living in permafrost soil and in sea-ice (D’Amico
Extremely dry environments

Environments without free water are considered extremely dry environments and they include hot and cold deserts, and some terrestrial endolithic habitats (see also Chapter "Endoliths"). Organisms dependent on very dry environments are termed xerophiles, whereas those tolerating only temporary desiccation are referred to as "xerotolerant." Most organisms thriving in very dry environments are actually xerotolerants, which rely on at least periodically available free water. A strategy to cope with prolonged periods of dryness is to enter the state of anhydrobiosis, which is characterized by little intracellular water and no metabolic activity. Organisms that can become anhydrobiotic are found among bacteria, yeast, fungi, plants, and even animals such as nematodes and the brine shrimp Artemia salina (Rothschild and Mancinelli, 2001). The terms “xerophile” and “xerotolerant” are often used to include organisms thriving under conditions of low water activity $a_w < 0.80$. The $a_w$ is a measure of the amount of water within a medium that an organism can use to support growth. It represents the ratio of the water vapor pressure of the substrate to that of pure water under the same conditions and is expressed as a fraction (pure water, $a_w = 1$; saturated NaCl, $a_w = 0.75$). The lowest $a_w$ value recorded for growth to date was reported for the spoilage mould Xeromyces bisporus ($a_w = 0.61$; see Grant, 2004 for a review). According to this definition, the most xerophilic organisms known, thrive in foods preserved by some form of dehydration or enhanced sugar levels, and in hypersaline environments where water availability is limited by a high concentration of salts (Grant, 2004). Whereas the former are dominated by xerophilic filamentous fungi and yeasts, high-salt environments are almost exclusively populated by prokaryotes. For the strategies employed by these organisms to cope with the osmotic stress exerted by these environments see above, and entry “Halobacteria – Halophiles.”

High-radiation environments

Environments exposed to high doses of ionizing radiation are high-radiation environments. Ionizing radiation is radiation with sufficient energy to ionize molecules, most commonly ultraviolet (UV) radiation and natural radioactivity. When such radiation passes through (living) matter, ions and free radicals are produced that react rapidly and modify molecules (Cox and Battista, 2005). Ionizing radiation is therefore potentially detrimental for life, mainly due to DNA damage resulting from the generation of reactive oxygen species and the hydrolytic cleavage of water. Organisms that are capable of withstanding high doses of ionizing radiation are called radioreistant. There is no clear pattern of evolution among ionizing-radiation-resistant species, and they occur scattered over the three domains of life. Well-studied microbial examples are the archaeon Thermococcus gammadotolerans (Jolivet et al., 2003) and the bacterium Deinococcus radiodurans (Cox and Battista, 2005). Strategies to adapt to high doses of ionizing radiation involve (i) increasing the numbers of genome copies, (ii) tight spatial arrangement of nucleoids, (iii) accumulation of efficient radical scavengers, (iv) delaying DNA replication until damage repair has been completed, and (v) improved enzymatic genome-repair process. For further reading, please refer to entry “Radioactivity.”

Summary

“Extreme environment” refers to any setting that exhibits life conditions detrimental or fatal to higher organisms with respect to its physicochemical properties, in particular pH, temperature, pressure, saline concentrations, and radiation. Major classes of extreme environments encompass acidic (pH < 5), alkaline (pH > 9), hypersaline (salinity > 35%), pressurized (> 0.1 MPa), hot (> 40°C), cold (<5°C), dry ($a_w < 0.80$), and high-radiation environments. These environments typically harbor specially adapted organisms, the so-called extremophiles. These can be classified into acidophiles, alkaliophiles, (hyper-) thermophiles, psychrophiles, xerophiles, and radioreistant organisms.

Bibliography


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**Cross-references**

- Acid Rock Drainage
- Algae (Eukaryotic)
- Alkalinity
- Archaea
- Bacteria
- Chroococcidiopsis
- Deep Biosphere of Salt Deposits
- Deep Biosphere of the Oceanic Deep Sea
- Diatoms
- Endoliths
- Fungi and Lichens
- Halobacteria – Halophiles
- Hot Springs and Geysers
- Hydrothermal Environments, Marine
- Hydrothermal Environments, Terrestrial
- Origin of Life
- Permafrost Microbiology
- Radioactivity (Natural)
- Saline Lakes
- Soda Lakes
- Terrestrial Deep Biosphere
FE(II)-OXIDIZING PROKARYOTES

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Synonyms
Fe-oxidizers; Fe(II)-oxidizing prokaryotes/microorganisms; Ferrous iron-oxidizing prokaryotes/microorganisms; Iron-oxidizers; Iron-oxidizing prokaryotes/microorganisms.; Iron(II)-oxidizing prokaryotes/microorganisms

Definition
Fe(II)-oxidizing prokaryotes. Diverse species of the prokaryotic domains Bacteria and Archaea have the ability to oxidize Fe(II), ferrous iron, to Fe(III), ferric iron. The electrons obtained from the oxidation of Fe(II) are utilized for energy generation in aerobic or anaerobic respiration and/or for assimilative reduction reactions.

Introduction
Aerobic neutrophilic Fe(II)-oxidizing bacteria were among the first environmentally relevant prokaryotes that were discovered and studied in the nineteenth century. At that time, eye-catching ochre deposits in ponds and slowly running waters had attracted the attention of microbiologists. Microscopic analyses of such ochre deposits revealed the prevalence of twisted stalks and/or sheaths that were covered with iron minerals. Bacteria were suggested to be responsible for the production of these specific iron-covered structures, and subsequently the first aerobic Fe(II)-oxidizing prokaryotes were described: Gallionella ferruginea and Leptothrix ochracea (reviewed by Emerson, 2000; Canfield et al., 2005). In contrast, the anaerobic oxidation of Fe(II) by phototrophic bacteria (Widdel et al., 1993), nitrate-reducing bacteria (Straub et al., 1996), and archaea (Hafenbradl et al., 1996) was not discovered until the end of the twentieth century. Aerobic and anaerobic Fe(II)-oxidizing prokaryotes play decisive roles in the biogeochemical cycle of iron. In comparison to other physiological groups, only few species of Fe(II)-oxidizing prokaryotes are available in pure culture. The capability to oxidize Fe(II) is apparently phylogenetically widespread because the known species belong to diverse phyla of the Bacteria and the Archaea. The mechanism(s) of prokaryotic Fe(II)-oxidation are largely unknown (Canfield et al., 2005).

Geochemical aspects of Fe(II)
Iron is the fourth most abundant element in the Earth’s crust, and depending upon the geochemical conditions, it occurs either in soluble forms or in a variety of minerals (Cornell and Schwertmann, 2003). In natural habitats, Fe(III) minerals are reduced to Fe(II) mainly by the metabolic activities of prokaryotes. The concentration of dissolved Fe(II) is controlled by precipitation-dissolution reactions and by adsorption processes: Fe(II) precipitates with carbonates, phosphates, or sulfides and tends to adsorb to soil particles and mineral surfaces (Cornell and Schwertmann, 2003; Canfield et al., 2005). Chemical reoxidation reactions of Fe(II) depend on the pH and on the presence of appropriate oxidants. The reaction rate of Fe(II) with molecular oxygen is very low at acidic pH values, and hence Fe(II) is fairly stable in the presence of oxygen under acidic conditions. With increasing pH, the reaction rate of Fe(II) and molecular oxygen increases, and at fully aerated conditions Fe(II) is rapidly oxidized to ferric iron (Emerson, 2000; Canfield et al., 2005). In the absence of molecular oxygen, Fe(II) can be chemically oxidized at appreciable rates only by nitrite or Mn(IV). However, Mn(IV) minerals are insoluble, and the environmental significance of these nondiffusible oxidants is therefore limited (Canfield et al., 2005). It is thus
speculated that anaerobic Fe(II)-oxidizing prokaryotes are important catalysts for the reoxidation of Fe(II) in anoxic environments.

**Fe(II) as electron donor in aerobic or anaerobic respiration**

Dissimilatory Fe(II)-oxidizing prokaryotes utilize Fe(II) as electron donor in aerobic or anaerobic respiration, i.e., they gain energy by coupling the oxidation of Fe(II) to the reduction of molecular oxygen or to the reduction of nitrate. As in the oxidation of Fe(II) to Fe(III), only one electron can be obtained, dissimilatory Fe(II)-oxidizing prokaryotes have to oxidize large quantities of Fe(II) to gain enough energy for maintenance and growth. Today, three physiological groups of prokaryotes are known to grow with Fe(II) as electron donor: acidophilic aerobic, neutrophilic aerobic, and neutrophilic nitrate-reducing prokaryotes. Due to energetic considerations, the oxidation of Fe(II) with nitrate at low pH values should also yield enough energy for prokaryotic growth. However, acidophilic Fe(II)-oxidation coupled to the reduction of nitrate has not been reported so far (Kappler and Straub, 2005; Appelo and Postma, 2007). The dissimilative oxidation of Fe(II) leads to the formation of Fe(III) minerals which are well soluble under acidic conditions but barely soluble at neutral pH. Hence, neutrophilic Fe(II)-oxidizing prokaryotes have to cope with a virtually insoluble metabolic end product.

**Acidophilic aerobic Fe(II)-oxidizing prokaryotes**

A variety of autotrophic and heterotrophic prokaryotes that thrive in environments of low pH (<3) were described to grow by dissimilatory aerobic oxidation of ferrous iron (Johnson, 2007). At acidic pH values, Fe(II) is stable in the presence of molecular oxygen, and hence prokaryotes do not need to compete with the chemical oxidation. However, the energy gain of acidophilic aerobic Fe(II) oxidation is low because the redox potential of the redox pair Fe$^{3+}$/Fe$^{2+}$ is with +770 mV rather high. The difference to the redox potential of the redox pair O$_2$/H$_2$O (> +1,000 mV below pH 3) allows therefore only little energy generation (Kappler and Straub, 2005; Appelo and Postma, 2007). Nevertheless, representatives of different phyla of the Bacteria (e.g., Acidithiobacillus ferrooxidans, Sulfbacillus acidophilus) and the Archaea (Acidianus brierleyi, Sulfolobus metallicus) exploit Fe(II) in aerobic respiration. Moreover, three species of the genus Leptospirillum are obligate aerobic Fe(II)-oxidizing bacteria, i.e., Fe(II) is the only electron donor and molecular oxygen the only electron acceptor utilized (Johnson, 2007). All other acidophilic Fe(II)-oxidizing prokaryotes make use of alternative electron donors (e.g., hydrogen, sulfur) and sulfur or Fe(III) as alternative electron acceptors. Species of acidophilic Fe(II)-oxidizing bacteria that also grow by dissimilatory reduction of Fe(III) were shown to catalyze the cycling of iron in laboratory experiments (Johnson et al., 1993).

**Acidithiobacillus ferrooxidans** was the first acidophilic aerobic Fe(II)-oxidizing bacterium that was isolated (Temple and Colmer, 1951); it belongs to the Gammaproteobacteria and is so far the best studied of all acidophilic prokaryotes. However, details of the flow of electrons from Fe(II) to molecular oxygen are still debated. Apparently, Fe(II) is oxidized on the cell surface at the outer membrane. Subsequently, a variety of membrane-bound and periplasmic cytochromes plus the periplasmic copper protein rusticyanin transfer the electrons to a cytochrome c oxidase. There the electrons are used together with protons to reduce molecular oxygen to water (Johnson, 2007; Castelle et al., 2008).

**Neutrophilic aerobic Fe(II)-oxidizing bacteria**

So far, only few representatives of the Alpha-, Beta-, and Gammaproteobacteria are described to grow by dissimilatory aerobic oxidation of Fe(II) at neutral pH. This physiological group gains more energy than their acidophilic counterparts because the difference between the redox potentials of the relevant redox couples is greater at neutral than at acidic pH values (Fe(III)/Fe(II) > +100 mV; O$_2$/H$_2$O > +820 mV; Kappler and Straub, 2005; Appelo and Postma, 2007). However, in oxic environments of neutral pH Fe(II) has a half-life in the order of few minutes and is chemically oxidized to ferric iron (Canfield et al., 2005). This competition with the chemical oxidation is likely the major reason why neutrophilic aerobic Fe(II)-oxidizing bacteria thrive preferentially in microoxic niches or oxic/anoxic boundaries. Laboratory studies showed that the activity of Fe(II)-oxidizing bacteria contributes up to 90% to the formation of Fe(III) under such conditions. Typical habitats for neutrophilic aerobic Fe(II)-oxidizing bacteria include groundwater springs, freshwater, and marine hydrothermal vents, wetlands, and plant rhizospheres (Emerson, 2000). Due to their production of conspicuous iron-encrusted twisted stalks or sheaths, Gallionella ferruginea and Leptothrix ochracea were the first Fe(II)-oxidizing bacteria that were already discovered in the nineteenth century. Only during the past few years, additional morphologically inconspicuous aerobic Fe(II)-oxidizing bacteria were isolated, e.g., Ferritrophicum radicicola and Sideroxydans paludicola (Canfield et al., 2005; Weiss et al., 2007). According to physiological studies, several of the novel strains are obligate aerobic Fe(II)-oxidizing bacteria, i.e., Fe(II) is the only electron donor and molecular oxygen the only electron acceptor utilized (Weiss et al., 2007).

**Neutrophilic anaerobic Fe(II)-oxidizing nitrate-reducing prokaryotes**

Under anoxic conditions, bacteria (Alpha-, Beta-, Gamma-and Deltaproteobacteria) and archaea (Archaeoglobales) are capable of oxidizing Fe(II) with nitrate as the terminal electron acceptor. At pH 7, all redox pairs of the nitrate reduction pathway can accept electrons from Fe(II), because their redox potentials are more positive than that
of the iron redox couple (Straub et al., 1996). Nitrate-dependent Fe(II) oxidation was reported for diverse habitats such as rice paddy soil, activated sewage sludge, freshwater or marine sediments (Canfield et al., 2005). The first observation of this metabolism was made with a freshwater enrichment culture: Fe(II) was oxidized to Fe(III) and nitrate was concomitantly and stoichiometrically reduced to molecular nitrogen (Straub et al., 1996). As Fe(II) was the sole electron donor supplied, Fe(II) oxidation coupled to nitrate reduction definitely supported cell growth in this enrichment culture. The situation is not so clear in most pure cultures of Fe(II)-oxidizing nitrate-reducing bacteria (e.g., Acidovorax sp. strain BoFeN1 or Thermomonas sp. strain BrG3) because they need an organic co-substrate for growth. In such strains it is questioned whether Fe(II) oxidation is beneficial and supports cell growth or whether Fe(II) is just oxidized in a rather unspecific side reaction. All known species are able to utilize alternative electron donors (e.g., hydrogen, organic acids) and alternative electron acceptors (nitrite, oxygen, ferric iron). Geobacter metallireducens, isolated as Fe(III)-reducing bacterium, couples the oxidation of Fe(II) to the reduction of nitrate to ammonium; it is unclear if G. metallireducens obtains energy by this process (Lovley et al., 2004; Canfield et al., 2005). Ferroglobus placidus is so far the only representative of the domain Archaea that grows by coupling the dissimilatory oxidation of Fe(II) to the reduction of nitrate to nitric oxide and nitrogen dioxide. Alternatively, F. placidus utilizes hydrogen, sulfide, acetate, or monoaromatic compounds as electron donor and thiosulfate or Fe(III) as electron acceptor (Hafenbradl et al., 1996; Lovley et al., 2004).

**Fe(II) as electron donor for assimilative reduction reactions**

Various groups of prokaryotes are able to reduce oxidized inorganic compounds for the synthesis of biomass. Such assimilative reduction reactions require a source of electrons (Canfield et al., 2005). Phylogenetically and physiologically different groups of chemotrophic and phototrophic prokaryotes have the ability to exploit Fe(II) as source of electrons for assimilative reduction reactions such as the fixation of carbon dioxide. Chemotrophic acidophilic and neutrophilic, aerobic and anaerobic Fe(II)-oxidizing prokaryotes have been discussed before because they oxidize the major fraction of Fe(II) during respiratory energy generation. In contrast, phototrophic Fe(II)-oxidizing prokaryotes utilize light as energy source and oxidize Fe(II) exclusively for assimilative purposes. The assimilative oxidation of Fe(II) leads to the formation of Fe(III) which is barely soluble at neutral pH. Neutrophilic phototrophic Fe(II)-oxidizing bacteria therefore have to cope with a virtually insoluble metabolic end product.

**Anaerobic phototrophic Fe(II) oxidation**

Anoxygenic phototrophic bacteria were the first prokaryotes discovered that oxidize Fe(II) in the absence of molecular oxygen (Widdel et al., 1993). So far, only few cultures of Fe(II)-oxidizing anoxygenic phototrophic bacteria were established either from freshwater or from marine sediments. They are affiliated with the purple sulfur (Thiodictyon sp. strain F4), the purple non-sulfur (e.g., Rhodobacter sp. strain SW2, Rhodovulum rubiginosum), or the green sulfur bacteria (Chlorobium ferrooxidans). All strains have the ability to utilize alternative electron donors such as hydrogen, reduced sulfur compounds, or organic acids (Canfield et al., 2005). The discovery of anoxygenic phototrophic bacteria that directly catalyze the oxidation of Fe(II), provides an alternative or additional explanation for the origin of Precambrian banded iron formations (Widdel et al., 1993; Kappler and Straub, 2005).

**Summary**

Phylogenetically diverse species of the prokaryotic domains Bacteria and Archaea have the ability to oxidize Fe(II) during aerobic or anaerobic respiration and/or for assimilative reduction reactions. Only one electron can be obtained from the oxidation of Fe(II) to Fe(III) and Fe(II)-oxidizing prokaryotes have to oxidize large quantities of Fe(II) to sustain cell metabolism. Due to the complex geochemistry of iron, acidophilic and neutrophilic Fe(II)-oxidizing prokaryotes have to cope with different constraints: (1) Acidophilic Fe(II)-oxidizing prokaryotes gain less energy in respiration than their neutrophilic counterparts; (2) Neutrophilic Fe(II)-oxidizing prokaryotes have to cope with a virtually insoluble metabolic end product. It is discussed that anoxygenic phototrophic Fe(II)-oxidizing bacteria contributed to the generation of Precambrian banded iron formations.

**Bibliography**


**Introduction**

Reduction of Fe(III) by prokaryotes has been known since the beginning of the twentieth century but was not considered to be of importance. At that time, only few bacterial strains were known to reduce small amounts of Fe(III) during fermentation. In addition, it was misleadingly presumed that prokaryotes cause reduction of Fe(III) mainly indirectly by producing sulfide, releasing organic compounds, lowering the redox potential, or decreasing the pH. This perspective changed notably with the discovery of dissimilatory Fe(III)-reducing bacteria, i.e., bacteria that utilize Fe(III) as terminal electron acceptor in an anaerobic type of respiration (Balashova and Zavarzin, 1979, 1980; Lovley and Phillips, 1988; Myers and Nealson, 1988). In order to gain energy for maintenance and growth by this type of respiration, significant amounts of ferric iron have to be reduced. Additionally, it became clear that neither lowering the redox potential nor decreasing the pH is sufficient to cause Fe(III) reduction. Furthermore, it was demonstrated that only very few organic compounds (e.g., fructose, cysteine) were able to reduce Fe(III) chemically at significant rates. In the last 20 years, numerous dissimilatory Fe(III)-reducing prokaryotes were identified and it is now generally accepted that this physiological group of prokaryotes has a strong influence on the iron geochemistry of most environments. Because many Fe(III)-reducing prokaryotes also have the ability to catalyze the reduction of Mn(IV) or Mn(III) to Mn(II) they additionally influence the geochemistry of manganese (Lovley et al., 2004; Canfield et al., 2005; Kappler and Straub, 2005).

**Evolutionary consideration**

The ability to reduce Fe(III) occurs widely within the domains *Bacteria* and *Archaea*. Most known Fe(III)-reducing prokaryotes that grow by Fe(III) reduction belong to the phylum Proteobacteria with representatives in each of the five subdivisions *Alpha*- (e.g., *Acidiphilium ruhrum*), *Beta*- (e.g., *Ferrribacterium limneticum*), *Gamma*- (e.g., *Shewanella oneidensis*), *Delta*- (e.g., *Geobacter sulfurducens*), and *Epsilonproteobacteria* (e.g., *Sulfurospirillum barnesi*). Several Gram-positive bacteria that belong to the phylum Firmicutes (e.g., *Thermoanaerobacter siderophilus*) and some separate bacterial lineages like *Geothrix fermentans* or *Geovibrio ferrireducens* also have the ability to reduce ferric iron (Lovley et al., 2004). Fe(III) reduction in the domain *Archaea* was discovered by testing pure cultures that had originally not been isolated with Fe(III): Representatives of the phyla Euryarchaeota (e.g., *Pyrococcus furiosus*) and Crenarchaeota (e.g., *Pyrobaculum islandicum*) showed reduction of ferric iron (Vargas et al., 1998). The great phylogenetic diversity of Fe(III)-reducing prokaryotes with representatives in all deeply branching phyla, along with iron isotope studies, supports the hypothesis that Fe(III) reduction was an early mode of energy metabolism. Further geochemical evidences...
suggest that other major electron acceptors such as sulfate, nitrate, or molecular oxygen were not available on early Earth (Lovley et al., 2004; Canfield et al., 2005; Johnson et al., 2008).

Geochemical aspects of Fe(III)
Iron is the fourth most abundant element in the Earth’s crust and Fe(III) minerals may account for a few percent dry weight of rocks, soils, and sediments. Hence, in most anoxic ecosystems Fe(III) minerals are the dominant electron acceptors for bacteria and archaea. Today, 16 different Fe(III) oxides, hydroxides, or oxide hydroxides are known and for simplicity they are often collectively termed Fe(III) oxides. They are all composed of Fe together with O and/or OH and differ in composition and crystal structure. Widespread Fe(III) oxides include ferrihydrite, goethite, hematite, lepidocrocite, and magnetite (Cornell and Schwertmann, 2003). In contrast to other electron acceptors such as oxygen, nitrate, or sulfate, Fe(III) oxides are poorly soluble at neutral pH. The solubility of Fe(III) oxides at neutral pH depends furthermore on the type of oxide, the degree of crystallinity and the crystal size, and may range from 10^{-9} M (e.g., ferrihydrite, low crystallinity, small crystals) to 10^{-18} M (e.g., goethite, high crystallinity, large crystals). At circumneutral pH, Fe(III)-reducing prokaryotes therefore have to cope with a virtually insoluble electron acceptor. With increasing acidity, the solubility of Fe(III) oxides increases and ferric iron is well soluble at pH values below 2.5 (Cornell and Schwertmann, 2003). Concomitant with the change in pH and Fe(III) oxide solubility, the redox potential of the transition between ferric and ferrous iron changes significantly. The redox potential of the redox pair Fe^{3+}/Fe^{2+} is +770 mV only at acidic pH values. At neutral pH, with poorly crystalline Fe(III) oxides (ferrihydrite, lepidocrocite) as the oxidant, the redox potential ranges between +100 and −100 mV, while the redox potential of more crystalline Fe(III) oxides (goethite, hematite, magnetite) may be as low as −300 mV (Canfield et al., 2005; Straub et al., 2001). In respect of solubility and energetics, acidophilic and neutrophilic Fe(III)-reducing prokaryotes cope with entirely different substrates.

Reduction of Fe(III) by fermenting bacteria
The first Fe(III)-reducing prokaryotes that were studied in laboratory cultures belonged to the domain Bacteria and reduced only small amounts of Fe(III) during fermentative growth on organic substrates such as glucose or malate. This physiological group comprises of a variety of bacteria and includes anaerobes (e.g., Clostridium pasteurianum, Vibrio spp.), facultative anaerobes (e.g., Escherichia coli, Paenibacillus polymyxa), and aerotolerants (e.g., Lactobacillus lactis, Lactococcus lactis). Accordingly, Fe(III) reduction by fermenting bacteria was observed under both anoxic and oxic culture conditions. For fermenting bacteria, Fe(III) reduction is only a minor pathway for electron disposal and research on this type of metabolism fell behind efforts to understand dissimilatory Fe(III) reduction. The mechanism(s) of electron transfer to Fe(III) during fermentation is unknown and it is also unclear whether this process yields energy for growth. Thermodynamic calculations suggest that Fe(III) reduction during fermentation results in a slightly greater energy yield than fermentation alone but experimental evidences are ambiguous (Lovley, 1991; Canfield et al., 2005). So far no representative of the domain Archaea was described to reduce Fe(III) during fermentative growth.

Dissimilatory Fe(III)-reducing prokaryotes
Dissimilatory Fe(III)-reducing prokaryotes utilize Fe(III) as terminal electron acceptor in anaerobic respiration, i.e., they gain energy by coupling the oxidation of an electron donor to the reduction of Fe(III). As in the reduction of Fe(III) to Fe(II) only one electron can be disposed, dissimilatory Fe(III)-reducing prokaryotes have to reduce large quantities of Fe(III) to gain enough energy for maintenance and growth. Numerous strains of phylogenetically dispersed, dissimilatory Fe(III)-reducing prokaryotes have been isolated into pure culture or have been detected by molecular techniques in a wide range of pristine or contaminated habitats, including freshwater and marine sediments, wetlands, soils, aquifers, the deep subsurface, deep subterranean thermal water, hydrothermal vents, and hot springs (Lovley et al., 2004). This widespread occurrence correlates well with the ubiquitous presence of Fe(III) minerals. Consistent with the great variety of ecosystems inhabited, pure cultures of Fe(III)-reducing prokaryotes show a wealth of physiological qualities in respect to oxygen concentration (strict or facultative anaerobes), temperature (growth reported between 4 and 121°C), pH (growth reported between pH 1 and 9), and salinity (freshwater and marine species). In addition, autotrophic growth with carbon dioxide as sole carbon source and fixation of molecular nitrogen was described for some species of Fe(III)-reducing prokaryotes. No prokaryote characterized so far, depends solely on Fe(III) as terminal electron acceptor for growth. In the absence of Fe(III), prokaryotes either grow by fermentation (e.g., Thermotoga maritima) or utilize alternative electron acceptors. Common alternative electron acceptors include oxygen (e.g., Pantoea agglomerans), nitrate (e.g., Geobacter metallireducens), manganese (e.g., Deferribacter thermophilus), reduced sulfur compounds (e.g., Desulfuromusa kysingii), and fumarate (e.g., Desulfuromonas acetoxidens). Furthermore, some species transfer electrons to heavy metals (e.g., uranium, chromium), graphite electrodes, humic substances, or chlorinated compounds (e.g., tetrachloroethene, trichloroacetic acid). The vast majority of Fe(III)-reducing prokaryotes utilizes fermentation end products such as acetate, ethanol, or hydrogen as electrons donors. Only few prokaryotes are known to couple the dissimilatory reduction of Fe(III) to the oxidation of sugars (e.g., Acidiphilium...
Acidophilic Fe(III)-reducing bacteria

A variety of autotrophic and heterotrophic bacteria that thrive in environments of low pH (<3) can grow by dissimilatory Fe(III) reduction (Johnson, 2007). All known species are facultative anaerobes, and most of them synthesize cell compounds required for dissimilatory Fe(III) reduction only in the absence of oxygen. In contrast, some species of acidophilic Fe(III)-reducing bacteria (e.g., Acidiphilium cryptum) constitutively express all genes necessary for Fe(III) reduction (Johnson, 2007). This is surprising because the redox potential of the redox pair O₂/H₂O reaches values above +1,000 mV below a pH value of 3 (Appelo and Postma, 2007). Hence, cells gain more energy by aerobic respiration than by utilizing Fe(III) as terminal electron acceptor (redox potential of the redox pair Fe³⁺/Fe²⁺: +770 mV).

Several species of acidophilic Fe(III)-reducing bacteria (e.g., Acidithiobacillus ferrooxidans, Sulfolobus acidophilus) also grow by oxidizing Fe(II) with molecular oxygen; such prokaryotes were shown to catalyze the cycling of iron in laboratory experiments (Johnson et al., 1993). So far no representative of the domain Archaea was described to grow by dissimilatory Fe(III) reduction at acidic pH values.

Neutrophilic Fe(III)-reducing prokaryotes

The majority of dissimilatory Fe(III)-reducing prokaryotes that were isolated and characterized thrives at circumneutral pH and has to cope with a poorly soluble electron acceptor under natural conditions. In addition, the redox potential of the redox pair Fe(III)/Fe(II) is much lower (100 to −300 mV, depending on the type of ferric iron oxide) than the redox potential of the redox pair O₂/H₂O (+820 mV) relevant for aerobic respiration. Therefore, at neutral pH, Fe(III) is a less valuable electron acceptor in terms of energy generation than molecular oxygen. Similarly, prokaryotes gain more energy from the respiration with nitrate or manganese as terminal electron acceptors. Hence, respiratory Fe(III) reduction at neutral pH occurs only under anoxic conditions in the absence of more favorable electron acceptors (Canfield et al., 2005). In some studies on neutrophilic Fe(III)-reducing prokaryotes, only chelated forms of ferric iron such as Fe(III) citrate or Fe(III) NTA (nitritoltriacetate) were applied. Caution is necessary in the interpretation of results from such studies because chelated Fe(III) forms are soluble at circumneutral pH, have redox potentials that are higher than those of non-chelated Fe(III) minerals, and may react rather unspecifically with electron-releasing cell compounds (Straub et al., 2001).

Most research on the biochemistry of microbial Fe(III) reduction focuses on members of the genera *Shewanella* and *Geobacter*, two genera with significant differences in their basic physiology. *Shewanella* species are facultative anaerobes that belong to the *Gammaproteobacteria*. They grow very well in nutrient-rich medium and oxidize lactate or pyruvate incompletely to acetate. In contrast, species of the genus *Geobacter* belong to the *Deltaproteobacteria* and most of them are described as anaerobes. The most common electron donors utilized by *Geobacter* species include acetate, ethanol, and hydrogen. Despite differences in basic physiological features, various c-type cytochromes play a significant role in intracellular electron transfer processes in both *Shewanella* and *Geobacter* species.

Mechanisms of prokaryotic Fe(III) reduction

Considering the phylogenetically widespread ability of prokaryotes to reduce Fe(III) and the great prokaryotic physiological diversity, it is not surprising that Fe(III)-reducing prokaryotes developed different strategies to cope with Fe(III) as electron acceptor. According to experimental observations, prokaryotes developed the following strategies to transfer electrons from the cell to the surface of Fe(III) minerals: (a) Physical contact between cell and mineral surfaces allows for direct delivery of electrons; an attachment of cells to Fe(III) minerals has been repeatedly reported and seems to be the prerequisite for the delivery of electrons to Fe(III) in some Fe(III)-reducing prokaryotes such as *Geobacter metallireducens*. (b) Iron chelators increase the solubility of ferric iron; some evidence indicates that *Shewanella alga*me* and Geothrix fermentans* produce and release Fe(III) chelators specifically during growth with Fe(III) minerals. (c) Electron-shuttling compounds accomplish the transfer of electrons from the cell to the mineral; diffusible electron-shuttling compounds help to bridge the spatial distance between cells and ferric iron (Lovley et al., 2004; Kappler and Straub, 2005). Reduction of Fe(III) by electron shuttling compounds is of particular importance in natural habitats where prokaryotic cells and Fe(III) minerals are not evenly distributed and Fe(III) might not be directly/physically accessible for prokaryotic cells. Naturally occurring electron-shuttling compounds that were shown to sustain prokaryotic growth include humic substances (Lovley et al., 1996) and sulfur compounds (Straub and Schink, 2004). Note that prokaryotes which reduce Fe(III) only via naturally occurring electron-shuttling compounds are no Fe(III)-reducing prokaryotes in a strict sense as their actual electron acceptor is the shuttling molecule and not Fe(III).

Summary

Phylogenetically diverse species of the prokaryotic domains *Bacteria* and *Archaea* have the ability to reduce Fe(III) during fermentative or respiratory growth by the transfer of electrons. Fe(III)-reducing prokaryotes have been recovered or detected in a great variety of pristine or contaminated habitats, and their widespread occurrence correlates well with the ubiquitous presence of Fe(III) minerals. Only one electron can be disposed
by the reduction of Fe(III) to Fe(II) and hence Fe(III)-
reducing prokaryotes have to reduce large quantities 
of Fe(III) to sustain cell metabolism. Due to the complex 
geochemistry of iron, acidophilic and neutrophilic Fe(III)-
reducing prokaryotes have to cope with different 
iron species: Fe(III) minerals are soluble only below 
a pH value of 3. At circumneutral pH, Fe(III) is only 
poorly soluble and significantly less energy can be gained 
by Fe(III) respiration compared to aerobic respiration. 
However, Fe(III) is the dominant electron acceptor 
in many anoxic habitats and prokaryotes developed different 
strategies for its utilization.

Bibliography
Lovley, D. R., 1991. Dissimilatory Fe(III) and Mn(IV) reduction. Microbiological Reviews, 55, 259–287.

Cross-references
Anaerobic Transformation Processes, Microbiology
Archaia
Bacteria
Fe(II)-Oxidizing Prokaryotes
Fermentation
Geobacter
Isotope Fractionation (Metal)
Isotopes and Geobiology
Metals, Acquisition by Marine Bacteria
Microbial Degradation
Nitrogen
Shewanella
Siderophores

FERMENTATION
Fermentation is a route of obtaining energy from the oxidation of organic substrates using an endogenous electron acceptor (usually an organic compound). The energy yield in fermenting systems is limited when compared to respiration. Nevertheless, fermentation still allows growth under conditions when external, inorganic electron acceptors are lacking, or when iron as part of the electron carriers in the respiratory chain is not available and therefore an electron transport chain cannot be synthesized. Common fermentation substrates are sugars, which are present in large amounts in the environment, for example, as photosynthesis products and constituents of plant cell wall material. Microbial sugar fermentation pathways encompass lactic acid fermentation, alcoholic fermentation, and propionate fermentation, according to the major metabolic products generated. Another important fermentation pathway is acetoclastic methanogenesis, that is, fermentation of acetate to CO₂ and CH₄, which is performed by some methanogenic archaea (see entry “Methane, Origin”). Hydrogen is another common product of microbial fermentation processes.

For further details and references, please refer to entry “Microbial Degradation.”

FLUORESCENCE IN SITU HYBRIDIZATION (FISH)
FISH (fluorescence in situ hybridization) is a hybridization technique employing reverse complementary
fluorescently labeled probes to detect and localize only those parts of the chromosome, a gene or its transcript with which they show a high degree of sequence similarity. Results are mostly evaluated by either fluorescence microscopy or cell sorting methods like flow cytometry. Depending on target gene, probe chemistry, labeling, and combination with other analytical tools, a wide range of different FISH applications can be employed for advanced in situ analysis of community structure, dynamics, localization, activity, function and interactions of different cellular species.

Introduction
Antonie van Leeuwenhoek’s ingenious improvement of the microscope in the seventeenth century revolutionized biology as this initiated novel ways to explore our world and ultimately led to the discovery of a new dimension of life forms, the microorganisms. However, since traditional microscopy reveals mainly structural features, its usefulness to advanced biological research reached its limits with the beginning of the molecular era. Fortunately, new possibilities for advanced microscopy applications emerged with the parallel development of two techniques: (1) in situ hybridization techniques (ISH) employing labeled gene probes targeting specific regions of a particular gene, and, (2) fluorescence microscopy. Fluorescence in situ hybridization (FISH) makes use of reverse complementary gene probes labeled with fluorochromes that are hybridized to specific regions on a chromosome (e.g., a specific gene) of gene transcripts (e.g., rRNA), and are then viewed by fluorescence microscopy or evaluated by other cell sorting methods like flow cytometry. FISH revolutionized nearly all disciplines in microbiology, in particular environmental research fields like microbial ecology, environmental biotechnology and geomicrobiology, since this enabled a cultivation-independent exploration of whole cells of culturable as well as not yet cultured microorganisms in their natural, complex environment (Amann et al., 1995; Amann and Fuchs, 2008). However, along with the development of other novel molecular tools and the growing comprehension of the enormous complexity of the biology of microorganisms, the first generation of FISH tools reached its limits as well, especially for applications on oligotrophic ecosystems characterized by a low amount of cells with often low activities in a strongly confounding background, which is often the case in geomicrobiology. Different modifications of the first generation FISH protocol as well as different combinations with other analytical tools were undertaken in order to meet the increasing demands with respect to, e.g., improved targeting of cells with low ribosome content or probe-repelling cell wall chemistry, alternative strategies to increase probe specificities or signal intensities, advanced image analysis, elucidation of activity and function by targeting other genes than the 16S rRNA gene and combining with different analytical tools, and finally cell enrichment of probe-targeted cells for, e.g., subsequent identification by sequencing (Wagner et al., 2003; Wagner et al., 2006; Amann and Fuchs, 2008; Orphan, 2009; Wagner, 2009). The development of novel FISH protocols in combination with other advanced analytical tools and microscopes is most likely to continue to play a crucial role in microbial ecology and geomicrobiology research. Only microscopy-based tools can provide us with the possibility to visualize the morphotype, growth characteristics, spatial distribution, and interactions of different taxa on a single cell level within complex communities. Furthermore, despite all progress in nucleic acid–based approaches like PCR and the “omics” approaches, only microscopy-based tools can link isolated extracted DNA/RNA markers, with intact, individual cells in their natural environment, and thus also exclude molecular biases often observed with nucleic acid extraction and PCR (Table 1). The greatest benefit will be reached when combining different FISH tools with various methods such as cultivation and molecular approaches. Ultimately, a systematic approach can be undertaken to reveal discrepancies between different tools caused by extant biases of each tool in terms of sensitivity and specificity, so that a validated, advanced insight into the true diversity, dynamics, function, activities, and relationships in the microbial world can be obtained.

Sample preparation, fixation and storage of samples
In contrast to nucleic acid–based tools, which simply require either addition of preservative agents or instant freezing of biomass and storage in sterile tubes for subsequent nucleic acid extraction, preparation of samples for FISH requires usually more consideration and effort, especially for long-term storage of samples of unknown microbial composition and/or employing certain types of FISH protocols that may disrupt the original structure of the sample.

Preparations for standard applications
1. Sampling strategy: While FISH may principally work equally well on nearly any kind of optimally fixed cells with high ribosome content, irrespective of cell titers or the nature of the environmental sample, our abilities to detect, visualize, and quantify the cells may be obstructed by tedious, time-consuming screening of samples with either too low or too high cell counts, confounding background caused by autofluorescence of the background or unspecific binding of probes or labels to nontarget cells/substances (Amann and Schleifer, 2001). Some of these problems can be minimized by considering how the sample should be optimally treated already during sampling, for example, by concentration, dilution, embedding, cell extraction, or particular consideration on chemical–physical conditions to avoid cells getting exposed to stress.

2. Fixation time and protocols: The purpose of the fixation is to maintain the original microbial composition
of the samples as well as the morphological integrity of cells during the different hybridization steps (elevated temperature, exposure to enzymes, detergents and osmotic gradients) and prevent cell losses, while simultaneously making the cells permeable for the probe(s) (Amann, 1995). Unfortunately, there is not one plain fixation protocol for all cell types for all different FISH protocols. Different fixation protocols have been developed to cope with the complexity of archaea, bacteria, and Eukarya with regard to their cell envelope packages and the type of probes to be employed for a particular FISH protocol. A suboptimal fixation protocol may make cells impermeable for probes or even cause cell lysis, especially during longer storage times or when exposing the cells to certain procedures such as incubation with enzymes prior to the FISH procedure. Apart from the selection of an optimal fixative reagent, it is also relevant to pay attention to the concentration of the fixative reagent and the incubation time and conditions. Fixation times may range from a few min up to >24 h, depending on cell type and FISH protocol, and are generally followed by a washing step for removal of the fixative reagent. The two most common fixation procedures are based on either aldehydes (e.g., formalin, paraformaldehyde (PFA), glutaraldehyde) or on alcohols (e.g., ethanol, methanol). In the majority of cases, Gram-negative cells within the domain Bacteria are usually fixed with 4% PFA, and Gram-positive cells within the domain Bacteria with ice cold 96% ethanol (Amann, 1995). To a certain extent, some Gram-negative species will also tolerate EtOH fixation, and some Gram-positive species (general exceptions are, e.g., Actinomycetes and Streptococci) may also tolerate PFA fixation (especially if the incubation time is short, see, e.g., de los Reyes et al., 1997). The previously mentioned fixation protocols may also work well for certain Archaea and Eukarya species, but in some cases, especially for certain Archaea species (Nakamura et al., 2006) or eukaryotes like fungi (Baschien et al., 2001; Tsuchiya and Taga, 2001; Weber et al., 2007) or protozoa, see, e.g., Fried et al. (2002); Petroni et al. (2003); Fried and Foissner (2007); Weber et al. (2007), other fixative reagents or post treatments with enzymes like chitinase or endo-isopeptidase are more suitable. When dealing with unknown samples, it is therefore recommended to explore the suitability of different fixation protocols. Irrespective of the fixation protocol used, the cells must be fixed as soon as possible with optimally prepared fixation solutions, preferably already during the sampling occasion to avoid population shifts and for optimal evaluation of true probe signal intensities as these

### FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

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<th>Diagnostic possibilities</th>
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<th>Methodological strengths/weaknesses:</th>
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<tr>
<td></td>
<td>Preparation of biomass prior to analysis</td>
<td>Only with multiplex PCR Yes</td>
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<td>Long-term storage of sample</td>
<td>Fixation of cells No</td>
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**a** Including different FISH protocols and advanced microscopy tools.

**b** In certain cases, samples may be stored at −80°C and the fixation omitted (Yilmaz et al., 2010b).

**c** Only with certain FISH protocols like mRNA-CARD FISH, RING-FISH, gene-FISH, and in situ RCA.

**d** Depends on the FISH protocol and how this is combined with other analytical tools and appropriate references.

**e** Exception, FISH protocols based on PCR.

**f** Depends on concentration/dilution procedure.
may in certain cases also serve as an indicator of activity (see, e.g., Wagner et al., 1995; Molin and Givskov, 1999; Rossetti et al., 2007).

3. Storage of fixed cells: Fixed samples (in wet or dry state, such as on a microscope slide or on a membrane filter) can be stored at room temperature or at +4°C for shorter periods. For optimal long-term storage at −20°C, fixed cells are generally dissolved in a 1:1 (v/v) solution with a 50% end concentration of ethanol in order to avoid freezing (Amann et al., 1994). Cells concentrated on a membrane filter are normally stored dry at −20°C (e.g., Teira et al., 2004). So far, no systematic studies on the maintenance of cell integrity or ribosome content in fixed cells after longer storage periods exist, but it is generally recommended that once the cells have been fixed, quantitative studies should be performed within days to avoid biases caused by unknown deterioration of cells or decrease of ribosome content.

Preparations for omics-based applications
For genomic or proteomic applications, unfixed biomass is desirable to minimize possible sequencing biases introduced by cross-linking and to avoid possible cell lysis difficulties, so that FISH targeted cells can be enriched by, e.g., flow cytometry and subjected to, e.g., whole genome sequencing (Stahl and Amann, 2001). However, other multicopy ribosomal genes or even stable multicopy RNA molecules have been used as targets for FISH probes (23S-rRNA, 28S-rRNA, precursor rRNA, intergenic spacer region). These alternative gene targets may overcome some of the problems occasionally encountered when targeting the 16S rRNA gene, such as low information content and limited probe accessibility or information about activity status (Trebesius et al., 1994; Oerther et al., 2000; Schmid et al., 2001; Schonhuber et al., 2001; Zimmermann et al., 2001; Peplies et al., 2004). However, despite this, the 16S rRNA gene has remained the main gene target in FISH applications, especially for environmental systems with unknown diversity, since the 16S rRNA gene is nowadays routinely sequenced in most studies so that the amount of retrieved 16S rRNA gene sequences has thus come to outrange all other databases of other gene sequences.

**Principle of FISH:** The FISH procedure generally consists of the following steps (Adapted from Manz et al., 1992 and Amann, 1995):

1. Optional: For certain cases an additional post-treatment of the fixed/non-fixed biomass may be needed for increased permeabilization of probes through rigid cell envelope packages, or for removal of confounding background, by, e.g.:
   - Incubation with enzymes like lysozyme, protease, proteinase K, mutanolysine, endoisopeptidase, chitinase
   - Removal of wax or chemical precipitates by chemical solvents
   - Use of detergents (SDS) or other chemicals like hydrochloric acid
   - Physical treatment like exposure to microwave radiation
   - Addition of chemicals to avoid dissolution of essential compounds

2. Successive dehydration in an EtOH series (50%, 80%, 96%, each step between 1–3 min).

3. Hybridization of the fixed sample with the probe on a microscope slide or in solution under stringent conditions (Figure 1). Stringent conditions are achieved by keeping the hybridization temperature constant (+46°C) but varying the formamide concentration for different probes (Stahl and Amann, 2001).

4. Subsequent washing with preheated buffered washing solutions at a slightly higher temperature (+48°C). After this, the hybridized samples can be evaluated or stored at dark prior to evaluation (for a shorter period at room temperature or at +4°C, for longer periods, at −20°C).

5. Optional: Counter stain with, e.g., an appropriate nucleic acid stain like DAPI (4′,6-diamidino-2-phenylindole) or SYBR Green (e.g., Wagner et al., 1994, or Engel et al., 2003).
6. Evaluation is achieved by:

Visualization:
- Standard epifluorescence microscopy, or confocal laser scanning microscope for three-dimensional reconstruction (Wagner et al., 1998).
- Combination with other microscopes like electron microscopy (e.g., Gerard et al., 2005), RAMAN spectroscopy combined with a microscope (Wagner, 2009), or nanoSIMS (Behrens et al., 2008; Orphan, 2009).

For digital image analysis of FISH images, various software packages are available, such as CMEIAS, COMSTAT, and daime (Heydorn et al., 2000; Daims and Wagner, 2007; Zhou et al., 2007; Daims, 2009).

Other options:
- Membrane-based evaluation (e.g., dot blot, Wagner et al., 1994).
- Combination with other analytical tools (e.g., screening of uptake of radioactive tracers or stable isotopes (Nielsen et al., 2005; Orphan, 2009) or other FISH protocols targeting non-ribosomal genes or their transcripts for exploring activities or functions.
- Reverse hybridization by microarray technology, where multiple probes are deposited on a support material to hybridize a labeled sample with the probes (DeSantis et al., 2005; Loy and Bodrossy, 2006; Taylor et al., 2010).
- Cell sorting or probe signal intensity evaluation by flow cytometry (Behrens, 2003; Amann and Fuchs, 2008), RING (Recognition of IGdividual Genes)-FISH (Zwirglmaier et al., 2004b), or magneto-FISH (Orphan, 2009).
- Whole genome amplification (Orphan, 2009; Yilmaz et al., 2010b).

**Basic equipment and reagents for standard FISH applications:**
- Hybridization oven (46°C)
- Water bath (48°C)
- Polypropylene screw top tubes, filter paper
• Reagents (for chemical composition, check, e.g., Amann, 1995):
  − NaCl
  − Tris/Phosphate buffer
  − EDTA
  − SDS
  − Formamide
  − 50%, 80%, 96% EtOH
  − Reagents/equipment for improved probe accessibility

• Labeled probes
• Counterstains like nucleic acid stains (e.g., DAPI (4′,6-
  diamidino-2-phenylindole) or SYBR Green)
• Microscope slides (possibly coated with, e.g., poly-
  l-lysine (Sigma-Aldrich, www.sigmaaldrich.com/sigma-
  aldrich/home.html) or a gelatine-chromium sulphate
  solution (Amann, 1995)
• Mounting medium of appropriate pH (e.g., from
  vectorlabs.com/; various mounting media from
• Some form of fluorescence microscope.

FISH probes (probe design, probe evaluation,
probe databases)

For a proper interpretation of FISH experiments, probes
need to be optimally designed and handled. Several
different types of softwares (e.g., the ARB software
package, (www.arb-home.de) and Primrose (www.
bioinformatics-toolkit.org/index.html)) and detailed
guidelines for probe design for particularly FISH experi-
ments exist (Amann, 1995; Amann and Schleifer, 2001;
Stahl and Amann, 2001), and (Hugenholtz et al., 2001).
Below follows an updated summary of some of those
guidelines, focusing mainly on applications with the
ARB software package:

1. Construction of a molecular data base: Prior to all
probe design, a comprehensive data base of all relevant
cOMPlete gene sequences of the target group and rele-
vant nontarget groups should be collected for a thor-
uous evaluation of phylogenetic relationships. Partial
sequences may also be included, but these should
be handled more critically due to their limited value
for a full evaluation of all potential probe targets.
Sequences can be downloaded from different catego-
ries of databases: (1) all kinds of gene sequences:
gov/), (2) regularly updated and curated ribosomal
gene sequences: RDP (http://rdp.cme.msu.edu/),
SILVA (www.arb-silva.de/), Green Genes (http://
greengenes.lbl.gov/cgi-bin/nph-index.cgi); the ribo-
somal internal spacer collection (http://egg.umh.es/
rissc/); (3) functional genes: Fun Gene (http://
fungene.cme.msu.edu/index.jsp). Principally, most
softwares that can align sequences, perform phylo-
genetic evaluations, and design biomarkers like PCR
primers can be used for FISH probe design as well.

2. Hierarchic probe design strategy: Where possible, it
is recommended to design a hierarchic probe set
consisting of several probes targeting different phylo-
genetic levels of the target organism/group (see, e.g.,
Amann and Schleifer, 2001; Amann and Fuchs,
2008). If the different probes are labeled with different
fluorochromes and combined in one FISH experiment
on a mixture of cells with different phylogenetic rela-
tionships, different overlaps of the probes will produce
different color combinations depending on the taxo-
nomical relationships. This facilitates a straightforward
top to bottom identification of the taxonomical status
(e.g., from phylum down to species level) of different
cell species in a complex sample (Amann, 1996).

3. General rules for standard oligonucleotide probe
design:

(a) Select the appropriate gene sequences of the
desired target group. Depending on bioinformatic
software, design the probe according to the
recommended procedure. For example, if using
the ARB software (Ludwig et al., 2004), select
from the menu “probe design” an appropriate PT
server, then define appropriate parameters such as
length of probe (average length of probes for stan-
dard oligonucleotide probes is between 15–25
nucleotides), minimum group and maximum non-
group hits, GC content, melting temperature range,
range of nucleotide positions, and maximum hair-
pin bonds.

(b) The selected probe should be completely specific
(complementary) to a region of the selected target
sequences and have at least one mismatch to the
same target region in nontarget sequences. Opti-
mally, the mismatch should be centralized in the
nontarget sequences in order to maximize the
destabilizing effect of the mismatch.

(c) Perform probe match to evaluate the specificity of
the probe (e.g., in the bioinformatic software
package ARB (www.arb-home.de), the RDP Probe
Match tool (http://rdp.cme.msu.edu/probematch/
search.jsp), the probeBase probeCheck tool (http://131.130.66.200/cgi-bin/probecheck/content.
pl?id=home) or general data bases like the National
Center for Biotechnology Information (www.ncbi.
nlm.nih.gov/).

(d) Unfortunately, there is no such thing as a perfect
probe that only targets the target group as the
course of the evolution of gene sequences is a ran-
don process. During a recent review Amann and
Fuchs (2008) showed that it is more advisable to
evaluate group coverages as the percentage of group members that can be identified relative to the total number of members in the target group. With this approach, three groups can be distinguished: true positives; false negatives; and false positives. Parallel to the constantly growing amount of gene sequences, the coverage of each probe target group is ultimately going to change and possibly demand more complex probe sets. For example, in the early days of the FISH technology, only one probe was needed to target all recognized species in the Bacteria domain (Amann et al., 1990). However, a decade later, Daims et al. (1999) showed that a mixture of three different Bacteria domain targeting probes were needed to cover all so far (up till 1999) recognized sublineages.

(e) Evaluate the melting temperature of the probes (e.g., as described by (Hugenholtz et al., 2001) or the probeBase, www.microbial-ecology.net/probebase/). Optimally, the probe should have a melting temperature > 57°C. If the melting temperature is < 57°C, the temperature can be increased by shifting the position of the probe or expanding the length of the probe.

(f) Check probe accessibility. A reoccurring problem is the probe accessibility. The general explanation for is that certain regions of the secondary structure of the ribosomal gene are inaccessible for probes. Unfortunately, the accessible and inaccessible regions of the ribosomal molecules appear to vary from species to species, so that only precise estimations of probe accessibilities can be made for those species where a systematic probe accessibility mapping has been performed (see, e.g., Fuchs et al., 1998; Behrens, 2003a, 2006b). The general recommendation is therefore to design probes on different target positions and then to simply explore on a trial-and-error experimental basis which of the selected probes produce high probe signals. However, Yilmaz et al. (2006) showed that principally all positions on the ribosome molecule may yield satisfying probe signals if a sophisticated approach based on a thermodynamics-based probe design is employed. With this approach, FISH experiments of any given probe can be mathematically simulated. Based on this, theoretical predictions on, e.g., the probe accessibility, the formamide denaturation profile, and the potential of mismatch discriminations can be made (for further details see Yilmaz et al., 2010a). Based on this, further optimizations of the probe accessibility can be made such as modifying the length of the probe or the experimental hybridization conditions (e.g., increase of the hybridization time up to 96 h).

(g) Check for potential self-complementarity of the probe which may initiate duplex formations between the probe and the target (e.g., caused by hairpins or dimers).

(h) Naming of probe. There are three types of nomenclatures for probes: (1) the short name (e.g., EUB 338), which is often constructed by a shortage of the name of the target group (e.g., EUB for Eubacteria), followed by a number that denotes the first position of the probe on the target gene (e.g., nucleotide position 338 on the E. coli gene); (2) a name based on a comprehensive nomenclature to denote main features of the probe such as the target gene, target group, target group level, 3’ end of the probe, and probe length (Alm et al., 1996); and (3) the probeBase accession number (e.g., pB-00159 for probe EUB 338) as assigned by the online probe database ProbeBase (www.microbial-ecology.net/probebase/).

4. Labeling of probes: The first rRNA-targeted oligonucleotide probes for FISH were radioactively labeled, developed by microautoradiography and evaluated by light microscopy (Giovannoni et al., 1988). Unfortunately, the work with radioactively labeled probes restricted the applicability of FISH. However, the applicability of FISH increased as soon as the first fluorescently labeled rRNA-targeted oligonucleotide probes were reported (DeLong et al., 1989). Probes are nowadays generally covalently labeled at the 5’ end of the oligonucleotide, and the most commonly used fluorochromes for FISH are sulfoindocyanine fluorochromes like Cy3 and Cy5, fluorescein, ALEXA probes, rhodamine, and Texas red. The criteria for selecting appropriate fluorochromes are based on the capabilities of the available epifluorescence microscope and on the nature of the cells and their surrounding environment, where the aim is to avoid a confounding background and unspecific binding of fluorochromes to nontarget cells, and/or to minimize bleaching of probe signals. During the last decade, several different approaches have been used to increase the probe signal intensities in those cases where standard labeled probes produce too weak fluorescent signals. A simple strategy was to employ mixtures of monolabeled probes targeting different sites of the ribosomal gene (e.g., Amann et al., 1990; Krumholz et al., 1990; Morris et al., 2002; Sunamura and Maruyama, 2006). Another strategy is to employ alternative labeling procedures of oligonucleotide probes, such as polynucleotide FISH (e.g., Trebesius et al., 1994), horseradish peroxidase (employed in catalyzed reporter deposition (CARD-FISH), e.g., Pernthaler et al., 2002a; Wendeberg, 2010) or various types of multiple labeling of probes (Amann and Schleifer, 2001) such DOPE-FISH (Stoecker et al., 2010).

5. Purchase and handling of probes: Probes are chemically synthesized and can be purchased with or without label. In earlier times, probes had to be manually labeled (Amann, 1995), but nowadays labeled probes can be easily purchased from most providers (e.g., Biomer, Invitrogen, MWG, SIGMA) of molecular reagents like PCR primers at a rather low cost (starting
from approximately 50 USD). Probes are normally shipped in lyophilized stage and must be dissolved in either sterile water or an appropriate buffer (depending fluorochrome chemistry) to a stock solution of normally 100 pmol/µl. It is recommended to store aliquots of working solutions in individual tubes in dark at −20°C, and to avoid excessive freeze-thawing of the aliquots. For long time storage or transport at room temperature it is recommended to lyophilize the probes.

6. Evaluation of probes:
   (a) For any purchased probe, it is recommended to check for the probe's concentration, length, homogeneity, and the completeness of the labeling, to avoid unwanted competition between labeled and unlabeled probes for target sites as this may reduce the probe signal intensity. This can be done as described by Stahl and Amann (2001), or as recommended by the provider who performed the labeling.
   (b) Newly designed probes are generally first tested under non-stringent conditions (i.e., by excluding formamide in the hybridization buffer). If positive probe signals are obtained in reference strains as expected, stringent conditions of the probe should be determined by an increasing formamide series, as described in Amann and Schleifer (2001). Probe signal intensities can be quantified by digital image analysis with, e.g., the daime software, as described by (Daims et al., 2009).
   (c) Combination of probes. The great advantage of FISH over standard PCR applications is that several differently labeled gene probes can be simultaneously combined and thus enable a simultaneous identification of different probe-targeted species within a complex sample. The general guidelines for optimal combination of different probes are:
      • Probes with identical formamide concentrations for optimal stringencies (at which the probe hybridizes to the specific target but not to nontarget groups) can be combined in the same hybridization experiment.
      • Probes with different formamide concentrations for optimal stringencies cannot be combined in the same hybridization experiment. Instead, successive hybridizations must be performed for each probe, starting with the probe with the highest formamide concentration.
   (d) Most standard epifluorescence microscopes are equipped with filter sets that allow separate recording of at least two to three differently labeled probes and one UV-based nucleic acid stain like DAPI (4',6-Diamidino-2-phenylindol). Such recorded images can then be merged into one image with image analysis software packages. However, recent developments based on, e.g., advanced confocal laser scanning microscopes with multichannel image analysis, special tools such as spectral imaging or FISH protocols employing quantum dot labeled probes allow the simultaneous combination of a considerably higher amount of differently labeled probes (e.g., Ainsworth et al., 2006; Anderson, 2010; Bentolila et al., 2006).

7. Probe bases: Different attempts to construct databases with FISH probes have been made during the last two decades. However, the probeBase (www.microbial-ecology.net/probebase) is currently the most regularly updated data base for ribosomal targeted probes, encompassing 2,568 different probes targeting species within Archaea, Bacteria, and, to some extent, Eukarya (further probes targeting protists and cyanobacteria can be retrieved from www.sb-roscoff.fr/Phyto/Databases/RNA_probes_introduction.php) for FISH as well as for ~16 different microarray applications (version September 2010). Here, many of the so far published probes can be easily searched by different criteria (target organism, target sites, probe name, probe sequence, probeBase accession number, references). Detailed information about the probe chemistry are listed and links are provided for online probe matches to other databases like the ProbeCheck http://131.130.66.200/cgi-bin/probecheck/content.pl?id=home, Green Genes (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi), or RDP (http://rdp.cme.msu.edu/). Furthermore, up to 150 ribosomal gene sequences retrieved from own clone libraries can be uploaded to screen for probes targeting these sequences (Loy et al., 2007, 2008).

8. Controls: For a valid evaluation of probe signals, three different categories of controls are needed: (1) Positive controls where probes are employed that should or should not overlap with the intended target gene. (2) Negative controls, to explore the impact of confounding background caused by, e.g., autofluorescence, and of nonspecific binding of fluorochrome or probe to target cells as well as nontarget cells. To achieve this, two different negative controls are recommended: exclusion of probes during the hybridization experiment, and application of negative reference probes like the NONSENSE probe (Loy et al., 2002) or the non-EUB probe (reverse complementary to the EUB probe, (Wallner et al., 1993). (3) All experiments should be performed on appropriate optimally fixed strains carefully selected to serve as positive as well as negative controls.

9. Other probe types: Note that some of the rules applied for design and handling of standard oligonucleotide probes may not be applicable to other probe types, like polynucleotide probes, PNA FISH probes, or CARD FISH probes.

General guidelines for evaluation and presentation of FISH results
For optimal interpretation of FISH experiments, several parameters should be considered, as outlined below. Omitting any of these principles may lead to false interpretations of FISH results.
Checklist for optimal FISH experiments and evaluation of results:

1. Were optimal sampling strategies, fixation protocols, and storage conditions employed?
2. Were appropriate reference strains used simultaneously in parallel with the FISH experiments? If strains are lacking, clones based on CLONE-FISH (Schramm et al., 2002) may also be used to experimentally evaluate the FISH experiments.
3. Were the microscopical investigations performed with optimal microscope settings?
4. Were appropriate probes/probe combinations (positive as well as negative controls) used under stringent conditions?
   (a) If the probe signals were positive, explore if nonspecific probe signals can be unequivocally excluded or if the identity of the probe signal can be confirmed with other measures.
   (b) If the probe signals were negative on the sample subject to investigation but otherwise positive with positive controls, explore if this is caused either by cell losses during the hybridization procedure, low cell numbers in the original sample, or low activity (and thus low ribosome content and probe signal intensity).
5. If quantifications of FISH images were performed, explore if the amount of sample applied to the microscope slide was appropriate. A too high or too low cell concentration may result in an over- or underestimation of cell numbers. Evaluate also if the amount of microscope slide fields investigated was high enough to ensure statistical evaluations of error ranges. For further evaluation of quantification, spiking of sample may be recommendable (SPIKE-FISH, Daims et al., 2001).
6. When presenting FISH results, it is recommended to describe results as “cells targeted by probe X,” instead of uncritically identifying the probe signals with the intended target group of the probe. This is especially important when applying probes to samples with unknown microbial composition and the applied probe is known to target false-positive as well as false-negative species and no further means have been undertaken to confirm the identity by, e.g., appropriate combinations of hierarchic FISH probe sets or subsequent sequencing (Amann and Fuchs, 2008).

Drawbacks of oligonucleotide FISH and the benefits of novel FISH technologies

The first generation of FISH protocol based on oligonucleotide probes revolutionized nearly all disciplines in microbiology. However, along with applications of this protocol on different ecosystems and species, many of the limits of the first-generation FISH protocol became obvious:

1. Successful detection dependent on ribosome content (minimum 370 ± 16S rRNA molecules per E. coli cell (Hoshino et al., 2008).
2. Confounding background caused by auto-fluorescing compounds like photosynthetic pigments, proteins like cofactors (e.g., F420 for methanogens), other parameters in the surrounding environment (e.g., precipitates, fibers, minerals, humic substances); or by unspecific absorption of fluorochrome label.
3. Probe technical problems (e.g., probe permeabilization problems, probe accessibility in the ribosomal gene, probe specificity).
4. Limited information content of the 16S rRNA gene for taxonomical resolution of closely related species.
5. Limited possibilities to confirm true identity and explore phylogenetic relationships and identify novel gene groups or species.
6. Limited information on in situ physiology in terms of:
   (a) Activity status (high ribosome content may reflect exponential growth stage, but this appears to be species dependent and needs to be explored for each particular species group (Wagner et al., 1995; Molin and Givskov, 1999; Rossetti et al., 2007);)
   (b) Function, since the ribosomal gene does not reveal information about function and since the genotype is not always linked to the phenotype (e.g., many functional microbial groups are polyphyletic, such as methanogens, sulphate reducers, denitrifiers, cyanobacteria, spirochaetes, reductive dechlorinators);
   (c) Limited possibilities to monitor gene expressions (mRNA transcripts) or proteins.
7. Applications limited to prokaryotic and eukaryotic cells. However, the first notes on successful FISH applications on bacteriophages (personal communication, K. Zwirglmaier, D. Scanlan, A. Millard, University of Warwick, UK) was recently made with the Recognition of individual genes [RING]-FISH protocol (Zwirglmaier et al., 2004b).

To overcome these limitations, several different strategies have been employed, ranging from modifying different parts of the procedures of the first-generation FISH protocol, searching for alternative gene targets, probe chemistries and labeling procedures, to combining FISH with other markers (e.g., activity stains, enzymes, isotopes), analytical procedures, and advanced microscopes or other analytical tools for evaluation of several different parameters for a holistic interpretation of in situ microbial ecology. Today, at least four categories of advanced FISH strategies can be recognized, which can be combined in a multitude of ways for various novel applications.

Category I – increasing the probe signal intensity:
- Incubate sample prior to fixation with substrates or stress-promoting compounds like chloramphenicol (Kalmbach et al., 1997; Ouvrney et al., 1997).
- Employ non-fluorescent labeling of probes for increased detection of cells in, e.g., a strongly autofluorescing background based on, e.g., radiolabels (Giovannoni...
Employ alternative probe design based on helper probes for oligonucleotide probes to increase probe accessibility (Fuchs, 2000).

Employ alternative probe chemistries for increased hybridization efficiencies and specificities based on peptide nucleic acid probes (PNA) (Perry-O’Keefe et al., 2001), or locked nucleic acid (LNA) probes (Kubota et al., 2006a).

Employ FISH probes targeting the precursor 16S rRNA and/or in nucleotide or probe quenching (Behrens, 2003; Behrens et al., 2004; Wagner et al., 2003).

Employ self-ligating probes to overcome background autofluorescence and avoid washing steps (Sando and Kool, 2002).

Employ PCR-generated probes to generate polynucleotide probes (Trebesius et al., 1994; Zimmermann et al., 2001; Meisinger et al., 2007; Pernthaler, 2002; Preston et al., 2002; Moraru et al., 2010).

Employ modified fluorescent labeling technologies based on horse radish peroxidase labeled probes and catalyzed reporter deposition (CARD)-FISH (Pernthaler et al., 2002a, b; Hoshino et al., 2008; Pavlekovic et al., 2009); multiple labeling of probes (Amann and Schleifer, 2001; Stoeckler et al., 2010) or quantum dot conjugated probes (Bentolila et al., 2006).

Category II – exploring activity or function:

Combine FISH with rRNA dot slot to quantify cellular rRNA content (Ravenschlag et al., 2000).

Employ FISH probes targeting the precursor 16S rRNA gene and intergenic spacer regions (Oerther et al., 2000; Schmid et al., 2001; Muter et al., 2010).

Combine FISH with different activity indicating stains like redox dyes (Nielsen et al., 2003), halogenated thymidine analogue bromodeoxyuridine (BrdU) (Pernthaler et al., 2002), exo-enzyme labeled probes (Xia et al., 2007), or antibodies to target cell surface markers or proteins (Currin et al., 1990; Lubeck et al., 2000; Gmür and Lüthi-Schaller, 2007).

Incubate sample with radioactively labeled substrates or stable isotopes to explore specific uptake of labeled substrates by micro-autoradiography (Nielsen et al., 2005); RAMAN spectroscopy (Wagner, 2009), or nano-SIMS (Behrens et al., 2008; Orphan, 2009).

Target functional genes or even the mRNA gene by CARD-FISH (Wagner et al., 1998; Pernthaler and Amann, 2004; Orphan, 2009; Pilhofer et al., 2009), two-pass TSA-mRNA FISH (Kubota et al., 2006b), or by alternative labeling (Coleman et al., 2007).

Target functional genes. For this, there are two categories: (1) PCR-generated probes like polynucleotide FISH (Moraru et al., 2010) or (2) amplification of the target sequence like in situ PCR (Hodson et al., 1995; Rodríguez-Castaño, G., 2008; Tani et al., 1998), in situ loop-mediated isothermal amplification (Maruyama et al., 2003); recognition of individual genes [RING]-FISH (Zwirglmaier et al., 2004b; Pratscher et al., 2009; Hauer et al., in preparation), in situ rolling-circle amplification (Maruyama et al., 2005; Hoshino et al., 2010), cycling primed in situ amplification (CPRINS, Kenzaka, 2005; Tamaki et al., 2005), circularizable probes (Zhang et al., 2006), circularizable probes (Zhang et al., 2006; Hoshino et al., 2010), peptide nucleic acid assisted rolling circle amplification FISH (Smolina et al., 2007), and polynucleotide FISH (Moraru et al., 2010).

Category III – combining FISH with advanced cameras, microscopes, or other analytical tools:

• CCD camera (Amann and Schleifer, 2001); confocal laser scanning microscopy (Wagner et al., 1998), and advanced digital image processing (Daims, 2009; Daims and Wagner, 2007)

• Electron microscopy (Kenzaka et al., 2005; Ishidoshiro et al., 2005)

• Spectral imaging (Ainsworth et al., 2006)

• RAMAN spectroscopy (Wagner, 2009)

• nanoSIMS (Behrens et al., 2008; Orphan, 2009)

• Atomic force microscopy (Huang et al., 2009)

Category IV – enrichment of FISH targeted cells for subsequent analyses, and combination with high-throughput technologies:

• Automatic cell sorting and enrichment techniques based on flow cytometry (Amann and Fuchs, 2008)

• Magneto-FISH (Orphan, 2009; Stoffels et al., 1999)

• RING-FISH (Zwirglmaier et al., 2004a)

• Whole genome amplification (Yilmaz et al., 2010b)

Many of these improvements minimize at least some of the drawbacks observed with the first generation FISH protocol; however, these new approaches do also introduce other drawbacks. For example, CARD-FISH has replaced the standard FISH protocol in several cases because it produces stronger probe signals (Pernthaler et al., 2002a, b) and just recently it was shown that it can be combined with polynucleotide probes targeting functional genes in a subsequent step (Moraru et al., 2010). Nevertheless, compared to standard oligonucleotide FISH, CARD-FISH is a rather time-consuming FISH protocol and allows only the application of one probe per hybridization experiment. Furthermore, as peroxidase can be found in nearly all organisms, there may be a risk for unspecific probe reactions. Fortunately, the peroxidase can be denatured prior to the FISH procedure, however, optimal denaturation conditions appear to be species dependent and may thus require individual optimizations (Pavlekovic et al., 2009). Similarly, the recent developments of various protocols to target non-ribosomal genes
like various functional genes and mRNA transcripts, or combining with various analytical tools, present different advantages as well as disadvantages. For example, considerable higher costs, advanced equipment, tedious protocols, loss of probe specificity, less flexibility in terms of combination with other probes or protocols or on various organisms, problems with adequate preservation of gene target due to high decay rate (e.g., precursors or mRNA), or unpredictable cross-reactions (e.g., when incubating a complex community with radioactive substrates or stable isotopes). However, irrespective of these drawbacks that may eventually be overcome in future, unequivocal interpretations of FISH experiments in the environment is the unknown biodiversity and multitude facets of dynamics and interactions, which paradoxically seem to grow with each advancement in the science of microbiology. Thus, it appears likely that the development of novel FISH technologies will continue in parallel with the development of novel tools and our increased understanding of microbial diversity.

**Different FISH applications in geomicrobiology**

The fields explored in geomicrobiology are extremely diverse, ranging from the deep biosphere to the atmosphere surrounding our planet Earth, and from there to the outer space. The conditions for the different ecosystems will therefore vary tremendously so that different sampling technologies and modifications of FISH protocols must be employed in order to achieve successful FISH results. So far, FISH has not been employed in geomicrobiology as often and as successfully as in other microbiological fields like environmental biotechnology (e.g., wastewater treatment plants, Nielsen et al., 2009) or in medical sciences (e.g., Bridger et al., 2010). This is because many of the ecosystems explored in geomicrobiology are often exposed to extreme conditions and generally contain low amounts of usually novel microorganisms with slow or unknown growth rates. Furthermore, special considerations must be made in terms of interpreting results with regard to the risk of contamination of samples during sampling (e.g., during drilling experiments) and to the impact of geochemical conditions on so far published FISH protocols. Because of these obstacles, many of the recent advanced developments of FISH protocols have actually been driven by microbial ecologists and geomicrobiologists (Orphan, 2009). Below follow some examples of how FISH has been employed for some of the most widely explored ecosystems in geomicrobiology (see also images in Figure 2):

**Aquatic systems:** In general, successful FISH experiments can be performed on aquatic systems, since at least one of the main parameters for good microbial growth and dispersion of cells and nutrients, water, is abundant. Nevertheless, several parameters may introduce several biases, e.g., low nutrient status and/or naturally occurring autofluorescing particles (Vesey et al., 1997). Aquatic systems with high nutrient content such as contaminated lakes or even anthropogenic systems like wastewater treatment plants will contain greater amounts of highly active bacteria than oligotrophic systems like aquifers and thus produce stronger FISH probe signals. For improved FISH on oligotrophic systems, different approaches have been undertaken:

**Aquifers/ground water/drinking water:** These ecosystems are generally dominated by planctonic cells in rather low cell numbers due to extreme conditions like oligotrophy, different redox states (e.g., anaerobic conditions), or toxic compounds (e.g., heavy metals, chlorinated drinking water or contaminated aquifers). Due to this, the general turn-over activities and thus also the ribosome content in the cells are generally extremely low. For adequate FISH evaluation, samples often need to be concentrated (e.g., by filtration) and confounding precipitates or debris removed (e.g., by cell extraction, Caracciolo et al., 2005). Early studies reported that standard FISH protocols may work well on certain aquatic samples like lakes and bottled water (Pernthaler et al., 1998; Glöckner et al., 2000; Watanabe et al., 2000; Flies et al., 2005; Loy et al., 2005); however, more recent studies report that either rainbow FISH (Sunamura and Maruyama, 2006) or CARD-FISH are more recommendable on drinking water or groundwater (Sekar et al., 2003; Nielsen et al., 2006; Wilhartitz et al., 2007; Meisinger et al., 2010a).

**Ocean:** Similar to the freshwater systems mentioned above, oceanic ecosystems are generally dominated by planctonic cells in rather low cell numbers. However, in contrast to the conditions in the aquifers in the subsurface, the cells in oceans are often exposed to more dynamic conditions. Despite this, the ribosome content in the cells and the turn-over activities may be rather low at least for certain species – depending on location and other conditions such as oxygen concentration, light frequencies, redox conditions, nutrient conditions, flux rates. For adequate FISH evaluation of cells, samples often need to be concentrated (e.g., by filtration) and confounding precipitates or debris must be removed (e.g., by cell extraction). Early studies demonstrated that standard FISH protocols may work well (Murray et al., 1998; Glöckner, 1999; Cottrell and Kirchman, 2000; Morris et al., 2004); however during the last decade, most studies have tried to increase probe signal intensities by modifying standard FISH protocols or applying other FISH tools like polynucleotide FISH (DeLong et al., 1999; Pernthaler et al., 2002; Preston et al., 2002) or CARD-FISH (Pernthaler et al., 2002a; b; Ishii et al., 2004; Teira et al., 2004; Herndl et al., 2005). In addition to this, various combinations with different activity targeted studies based on incubation with chloramphenicol (Ouerverney and Fuhrman, 1997), radioactive substrates like thymidine (Pernthaler et al., 2002) or bromodeoxyuridine (BrdU, Pernthaler and Pernthaler, 2005), or other substrates such as amino acids...
Fluorescence In Situ Hybridization (FISH), Figure 2 (Continued)
Fluorescence In Situ Hybridization (FISH), Figure 2 Demonstration of different images from different ecosystems produced by different FISH techniques.

(Ouverney and Fuhrman, 2000; Cottrell, 2000; Cottrell, 2003), have been undertaken.

Soil/sediments/subseaﬂoor: Soil and sediments are rather difﬁcult environments for successful FISH studies, as these habitats are extremely diverse, complex, and subject to harsh and ﬂuctuating conditions in terms of, e.g., water content and redox conditions. Thus, more than most other ecosystems, the biosphere of microorganisms in soil is often characterized by micro-niches which may be exposed to dramatic changes with regard to space and time. In addition to this, many substances in soil or sediments produce strongly confounding background such as humic compounds, mineral precipitates or other microscopic particles of unknown character. For successful FISH experiments, two different main approaches have been made (for a review, see Schmid et al., 2006; Schmid et al., 2007): (1) Spatial separations of cells or micro-niches by approaches such as embedding and sectioning of soil columns or by confocal laser scanning microscopy of soil aggregates, different cell extraction procedures such as density centrifugation or cation exchange membranes (McDonald, 1986; Smith and Stibrley, 1994; Caracciolo et al., 2005; Kurola et al., 2005; Lunau et al., 2005; Bertaux et al., 2007), or flow cytometry (Kalyuzhnaya et al., 2006; Podar et al., 2007). (2) The second approach employed a variety of different FISH protocols. The early studies reported generally only partial success or even contradictory results between FISH and other analytical tools (Hahn et al., 1992; Ludwig et al., 1997; Zarda et al., 1998; Llobet-Brossa et al., 1998; Christensen, 1999; Hristova et al., 2000; Ravenschlag et al., 2000; Janvier et al., 2003; Kobabe et al., 2004; Rusch and Amend, 2004; Flies et al., 2005; Knittel et al., 2005). However, during the last years, most studies have now replaced the standard FISH tool with CARD-FISH and nanoSIMS (Ishii et al., 2004;
Mussmann et al., 2005; Schippers et al., 2005; Tal et al., 2005; Orphan 2009).

Karst regions/rock/deep biosphere: During the last decade, our understanding of the role of microorganisms and their interactions with rocks and minerals over geological timescales has expanded considerably. The interaction of minerals with water and microbes do not only influence soil fertility, transport compounds, and pollutants through the subsurface, but they also contribute to dissolution of rocks or precipitation of various mineral formations like speleothems in caves and may thus even play a key role in global climate change (Northup and Lavoie, 2001; Brown and Lee, 2007; Cockell and Herrera, 2008). Several attempts have been made to screen for microbial life in rocks and set up validated guidelines for this (Barton et al., 2001; Barton et al., 2006; Tobin et al., 1999). The reoccurring concern is to not only prove extant microbial processes (Orphan and House, 2009) but also current cellular-based in situ activities in often carbon-poor systems. Since it may be difficult to retrieve sufficient amounts of RNA or other activity biomarkers from these systems due to low cell counts and activities, FISH, in particular in combination with advanced tools like nanoSIMS, may play a crucial role for demonstrating whole cells with targetable genes (Orphan, 2009). Despite these obstacles, standard FISH as well as CARD-FISH protocols have been successfully applied on, e.g., microbial mats in association with water and energy-rich substances for life based on chemolithoautotrophy in rocky environments or caves (Engel et al., 2003; Macalady et al., 2006; Meisinger et al., 2007; Meisinger et al., 2010b). However, certain types of microbial mats may be strongly hampered by confounding background caused by various mineral precipitates (Engel et al., 2010; Koebberich, 2008). Rigid environments like calcified biofilms or speleothems thus demand sophisticated modifications of standard FISH protocols as well as CARD-FISH (e.g., Shiraiishi et al., 2008).

Extremophiles in various geological environments: Extremophiles thrive in extreme ecosystems which are characterized by extreme physical or geochemical conditions. Such conditions may pose a particular challenge for FISH experiments. So far, FISH has only been practiced in certain types of extremophlic ecosystems with mixed success, e.g., in hot springs (Nubel et al., 2002; Simbahan et al., 2005; Nakagawa et al., 2006; Weidler et al., 2008), polar regions (Junge et al., 2004), halophilic systems (Rossello-Mora et al., 2003; Maturrano et al., 2006), mines/bioleaching systems (Schrenk et al., 1998; Edwards et al., 1999; Bond et al., 2000; Takai, 2002; Baker et al., 2004), hydrothermal vents (Harmsen et al., 1997; Schrenk et al., 2003; Nakagawa et al., 2006), and methane hydrate seeps (Boetius and al, 2000).

Summary

FISH (fluorescence in situ hybridization) is a valuable tool for in situ identification of microbial community structure, cell morphology, dynamics and spatial distribution. If combined with different analytical tools additional information of activity, function and interactions of different species on single cell level can be performed. However, for successful FISH experiments, it is crucial to optimize and validate all steps throughout the FISH protocol, ranging from proper sampling technique and fixation, selection of appropriate gene markers, hybridization conditions and relevant controls, to optimal detection and evaluation strategies.

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Foraminifera (from ancient Greek “hole bearers”, refers to the pores that early observers recognized in the calcitic tests) are amoeboid, eukaryotic protists with a large network of very thin cytoplasm extrusions (reticulopodia). Most species produce a genetically fixed shell (test), made of organic matter, agglutinated particles, calcite, aragonite, high-magnesium calcite, or opaline silica. The tests may be either simple bowls or tubes, or branched, coiled, substructured, and ornamented in diverse complexity. Several thousand species are found living in modern oceans, and this number still increases. Several times more extinct species are recovered from the fossil record. Foraminifera are considered an order (sometimes a class) in paleontology, subdivided by the structure and composition of modern and fossil tests. They are ranked among the “Granuloreticulosa” in biology, characterized by (1) their branching and anastomosing networks of reticulopodia, (2) a rapid bidirectional transport of intracellular granules and membrane domains driven by specific tubulin assemblies, and (3) a complex alternation of sexual and asexual reproductive cycles. Genetic investigations led to the definition of the first rank group “Foraminifera” within the Supergroup “Rhizaria”. Independent from such rankings, they range among the most common, diverse, and ubiquitous groups of single celled, test building eukaryotes on earth.

Earth history
The earliest fossils presumed for the foraminiferal lineage reach back into the Mesoproterozoic, despite severe problems in defining even Ediacaran remainders (Dong et al., 2008). The Cambrian provides already several foraminiferal families with organic-walled or agglutinated tests (Benton, 1993). A significant eukaryote radiation occurs in the Ordovician, coincident with increasing nutrient supply, enhanced primary production, and the propagation of planktonic metazoan stages. Such amplified planktonic food chains sponsored increased flux rates of organic matter at the sea floor, and thus the radiation of planktotrophy (Nützel et al., 2006). The calcitic fusulinids start in the Silurian (Loeblich and Tappan, 1988), and extend into the first rhizarian, rock forming mass deposition of biogenic shallow water carbonates, from the Pennsylvania to the Permian. The fusulinid clade develops all principal morphological adaptations known from modern benthic foraminifera sheltering phototrophic symbionts, thus these mass deposits should result from host–symbiont interactions (Anderson and Lee, 1991). The structure of modern marine primary production was based on the evolution of diatoms, coccoliths, and modern dinoflagellates in the Triassic to Jurassic (Falkowski et al., 2004). Restructuring and enhancing marine primary productivity to an elevated level is recorded by the boost of rotaliid foraminifera, modern metazoans, and increasing bioturbation (Martin et al., 2008). One tribe of calcitic foraminifera, the globigerinids, completely adapted to this new planktonic food chain, and started to enter a purely pelagic live style in the Middle Jurassic. The boost of rock forming biogenic carbonate (chalk) and silica (diatomite) production from these new planktonic systems, however, was delayed to the Cretaceous in the marine realm, and to the Eocene for lacustrine diatomites. The first major radiation of planktonic foraminifera occurred during the mid-Cretaceous. The density structure of the upper water
column and the formation of deeper water masses were altered by plate tectonic activities, a major long-term rise of global sea level, and an overall increase of global temperatures (Leckie, 1989; Martin et al., 2008). Following the massive mass extinction of planktonic foraminifera at the K/T boundary, symbiosis with phototrophic endosymbionts was one key factor for the radiation of Cenozoic lineages (Norris, 1996). The diversity of benthic foraminifera, and thus presumably their ecological demands and adaptations, were influenced more modestly. The first appearance of foraminiferal tests build up by opaline silica occurs in the Miocene, by the benthic foraminiferal suborder Silicoloculinina (Loeblich and Tappan, 1988). Enhanced terrestrial silica weathering and riverine silica input by grassland expansion offers a causally driven correlation, similar to marine diatom diversification (Falkowski et al., 2004).

Modern oceans
Planktonic foraminifera are most influential for the open marine calcite budget. Their annual flux rate of calcitic tests at 100 m water depth accounts for 1.3–3.2 Gt, equivalent to 23–56% of the global, open marine CaCO3 flux. During most of the year, dissolution takes place within the upper water masses (>700 m water depth), and only 1–3% of the initially exported CaCO3 reaches the seafloor. This biogeochemical pump is most influential for the alkalinity of surface and intermediate water masses. During short pulsed flux events, mass dumps of fast settling particles yield the major contribution to the formation of deep sea sediments. This pathway transfers 0.4–0.9 Gt CaCO3 annually, accounting for 32–80% of the total deep-marine calcite budget (Schiebel, 2002). Maximum carbonate production appears in open ocean realms where symbiotic and non-symbiotic planktonic foraminifera occur syntopic (Zaric et al., 2006). The contribution of coccolithophores (mean 12%) and calcareous dinoflagellates (0–3.5%) is comparably small (Schiebel, 2002; Sarmiento et al., 2002). Planktic gastropods (pteropods) may add another 10% only locally (Schiebel, 2002). Phototrophic endosymbionts play a major role in the carbonate production of planktic foraminifera. Such endosymbionts are the key factor for the foraminiferal successes in nutrient depleted environments, and for their enhanced turnover (Hemleben et al., 1989; Lee, 2006).

One sixth of the global carbonate production is shared by reef complexes, dominated by symbiotic-bearing corals, foraminifera, and coralline algae. With an annual production of 130 million tons of CaCO3, benthic foraminifera bearing phototrophic symbionts contribute nearly 5% of the annual present-day carbonate production in the world’s reef and upper shelf areas (Langer, 2008). Marine benthic foraminifera without phototrophic symbionts are much more diverse, inhabiting all ocean realms from estuaries to deep ocean trenches. But their share in marine biogenic carbonate production ranges only near 1% (Langer, 2008). This is due to the rapidly decreasing flux rate and nutritional value of organic matter reaching the deeper ocean floors (Altenbach and Struck, 2001). Deep-sea foraminifera have a metabolic turnover comparable to bacteria (Linke, 1992; Moodley et al., 2002), and their grazing rate exceeds that of common meiofaunal groups (Pascal et al., 2008). They have a considerable share in abyssal respiration rates (Witte et al., 2003), but this does not sum up to a significant impact in biogenic carbonate production.

Complete denitrification was observed as an endogenic metabolic pathway for benthic foraminifera in laboratory cultures (Risgaard-Petersen et al., 2006). The species facultatively switch to denitrification when high loads of organic matter are available in oxygen-depleted environments. Field observations in the upwelling region off Chile revealed that benthic denitrification is dominated by foraminifera, and not by bacteria (Høgslund et al., 2008). In view of the large benthic foraminiferal populations recovered from other oxygen minimum zones, and the large uncertainties in the global nitrogen budget (Gruber and Galloway, 2008), these findings may challenge our considerations on marine denitrification cycles (Høgslund et al., 2008). Several species related to denitrification acquire chloroplasts from settled algae in the deep ocean, which are kept functional in the foraminifers cytoplasm for at least one year (Grzymski et al., 2002). Such kleptoplasts produce hydroxyl radicals, which offer an alternative source of oxygen for the hosts metabolism by H2O2 breakdown (Bernhard and Bowser, 2008). Sulfur-oxidizing bacterial endosymbionts were considered for benthic foraminifera recovered from long-term sulfidic environments in the deep sea (Bernhard et al., 2006) and from temporal anoxic shelf areas (Bernhard, 2003). However, the definition of oxic or anoxic conditions is a problem by itself near the redox cline, as the interwoven, submillimetric structures of differing redox conditions build up a foamy, three dimensional space. Contrasting microbial turnover takes place in smallest pore water compartments. The intercalation may be so dense that foraminiferal inhabitants can not be attributed to either oxic or anoxic conditions (Bernhard et al., 2003).

Xenophyophores and Komokiaceans are very common deep-sea foraminifera with a comparatively large and loosely agglutinated test. They may cover up to 50% of hadal seafloors (Lemche et al., 1976). Early research on the role of Xenophyophores and Komokiaceans for biogenic metal dissolution and precipitation considered a significant impact for the formation of manganese nodules (Riemann, 1985). The microscopic barite crystals, commonly found in the endoplasm of Xenophyophores (Gooday and Nott, 1982), were considered a proof for their endogenic barite precipitation (Bertram and Cowen, 1997). However, these rhizarians ingest considerable amounts of highly refractory organic matter and produce large amounts of waste pellets (stercomata), offering an attractive food source for deep ocean microbial communities (Nozawa et al., 2006). Gardening of a specialized
bacterial flora growing on the stercomata of Xenophyophores was made plausible by Laureillard et al. (2004). Such affiliated microbial communities will move the redox state by degrading the stercomata, a factor that should interplay with the observed metal reactions. Gathering refractory material that is transferred to higher levels of the food chain by bacterial counterparts is highly effective. Parallel to the increasing number of reactions, a factor that should interplay with the observed metal movements is denitrification by bacteria, in most cases combined with the bacterial flora growing on the stercomata of Xenophyophores. We may presume that foraminiferal interactions seem to play a key role for anoxic and sulfidic microenvironments. The general outline of foraminiferal ecology and their overall impact on the marine food chains and calcite budgets is well documented and understood. Recently discovered, endogenic denitrification offers a facultative metabolic pathway for benthic foraminifera with potential impact for the marine nitrogen cycle. Additional complexity in synecology and ecophysiology is also indicated by observations on chemotrophic bacterial endosymbionts and bacterial gardening, not fully understood at present. Chemotrophic symbionts and functional kleptoplasts seem to play a key role for anoxic and sulfidic microenvironments. We may presume that foraminiferan interactions with bacteria play a more significant role in geobiology and biogeochemical cycles than has previously been suspected.

Conclusions

The general outline of foraminiferal ecology and their overall impact on the marine food chains and calcite budgets is well documented and understood. Recently discovered, endogenic denitrification offers a facultative metabolic pathway for benthic foraminifera with potential impact for the marine nitrogen cycle. Additional complexity in synecology and ecophysiology is also indicated by observations on chemotrophic bacterial endosymbionts and bacterial gardening, not fully understood at present. Chemotrophic symbionts and functional kleptoplasts seem to play a key role for anoxic and sulfidic microenvironments. We may presume that foraminiferan interactions with bacteria play a more significant role in geobiology and biogeochemical cycles than has previously been suspected.

Bibliography


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**Cross-references**

Algae (Eukaryotic)  
Bioerosion  
Carbonate Environments  
Chlorophyta  
Divalent Earth Alkaline Cations in Seawater  
Isotopes and Geobiology  
Protozoa (Heterotroph, Eukaryotic)  
Symbiosis

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**FRUTEXITES**

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**Synonyms**

Colloform limonitic crusts; Frutexites crusts; Frutexites-like forms; Frutexites microstromatolite; Frutexites tuffs; Haematitic/ferruginous/iron microstromatolites; Iron dendritic aggregates; Iron shrubs; Pillar-shaped microstromatolites

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**Frutexites, Figure 1** Original figures of genus of *Frutexites* described by Maslov (1960).
Definition

*Frutexites* is a problematic microfossil rich in iron. From a taxonomic point of view, only five species have been figured (*Frutexites arboriformis*, Maslov, 1960; *F. microstroma*, Walter and Awramik, 1979; *Frutexites* sp. 1, *Frutexites* sp. 2, and *New gen. 3*, Tsien, 1979), although the authors mostly use the term *Frutexites sensu lato*. The genus *Frutexites* was coined by Maslov (1960) in order to describe submillimeter-sized, iron-rich, and subordinate calcite microfossils (Figure 1). *Frutexites* have a dendritic shape formed by divergently branched microcolumns. The height and width of microcolumns as well as their composition and microstructure can vary (Table 1). The preservation of microstructure is strongly controlled by its dominant mono- or polymineral character. Microstructure is formed by convex-upward laminae, which sometimes show radially arranged fibers.

*Frutexites* can occur mainly as monomineral as well as polymineral structures with: iron-rich and/or manganese-rich (hematites, iron hydroxides, Fe-Mn oxyhydroxides), and/or carbonate-rich (calcite, ferroan calcite, dolomite), and/or siliciclastic-rich (argillite, microquartz), and/or phosphatic-rich zones.

Depositional environmental occurrences

*Frutexites* has been described in marine environments such as shallow and deep water stromatolites, microbial limestones, hardgrounds, and condensed pelagic limestones as well as in cavities, sheet-cracks, veins, and Neptunian dikes. However, comparisons with **Frutexites, Table 1**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Height</th>
<th>Width</th>
<th>Microstructure</th>
<th>Morphology</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maslov (1960)</td>
<td>Up to 400 µm</td>
<td>25–30 µm, more of 50 µm</td>
<td>Sheets with occasionally circular spaces</td>
<td>Radially diverging and branched sheets</td>
<td>Iron hydroxide and carbonate</td>
</tr>
<tr>
<td>Horodyski (1975)</td>
<td>20–500 µm</td>
<td>10–200 µm</td>
<td>2–10 µm convex-upward laminae</td>
<td>Pillar-shaped branched and not</td>
<td>Hematite, calcite, and argillite</td>
</tr>
<tr>
<td>Walter and Awramik (1979)</td>
<td>Up to 450 µm</td>
<td>5–120 µm</td>
<td>0.7–2.7 µm convex upward laminae and axial tube (trichome?)</td>
<td>Undulose layers, laminae with protruding pustules and erect branching microcolumns</td>
<td>Organic matter permineralized by silica</td>
</tr>
<tr>
<td>Myrow and Coniglio (1991)</td>
<td>250 µm–4 mm, most &lt;1 mm, average 250 µm–600 µm, 75–600 µm</td>
<td>Convex laminae with radially arranged fibers</td>
<td>Unbranching and branching columns</td>
<td></td>
<td>Calcite, ferroan calcite, hematite, and microquartz</td>
</tr>
<tr>
<td>Böhm and Brachert (1993)</td>
<td>Up to 5 mm, average 1–2 mm</td>
<td>Lighter and darker colored layers resulting chambered appearance</td>
<td></td>
<td></td>
<td>Fe-Mn oxides, calcite, and phosphates</td>
</tr>
<tr>
<td>Woods and Baud (2008)</td>
<td>Up to 800 µm</td>
<td>50–200 µm</td>
<td>Lighter and darker colored layers resulting chambered appearance</td>
<td></td>
<td>Hematite and/or Fe-Mn minerals, and calcite</td>
</tr>
</tbody>
</table>

*Frutexites, Figure 2* (a) Marine Upper Cenomanian/Lower Turonian *Frutexites* colonies from a deepwater hardground environment (Liencres coast, northern Spain; Reitner et al., 1995). The *Frutexites* facies is located on top of the hardground sequence and marks a fundamental change of the oceanographic conditions. (b) Deep subterranean *Frutexites* colonies growing on calcareous microstromatolites from tectonic fractures in the so-called “sole dolomite” at the base of the Naukluft Nappe Complex (NNC) in southern Namibia. The colonies are growing upside down from the fracture ceiling. The black ones are rich in manganese oxides, the ochre parts are enriched in iron oxides.
continental black shrubs are common, too. \textit{Frutexites} structures are also frequently found in veins and fractures of deep subterranean environments (Figures 2b, 3, and 4).

\textbf{Stromatolites}

The main record of \textit{Frutexites} has commonly been recognized from shallow to deep calcareous stromatolites from Proterozoic to recent examples (see Caldera lake stromatolites at Tonga, by Kazmierczak and Kempe, 2006). \textit{Frutexites} structures grow upward perpendicular to the stromatolite laminae; however, they can sometimes cross them (Horodyski, 1975) and even destroy them. For this reason, Böhm and Brachert (1993) interpreted that stromatolite accretion was independent of \textit{Frutexites}, which would act as dweller or secondary binder in the Jurassic deep water stromatolites from Germany and Austria. Descriptions about the associated macro- and microfauna in the \textit{Frutexites}-bearing stromatolites are not abundant. In the Canning Basin, the late Devonian \textit{Frutexites}-bearing stromatolites show abundant metazoans in life position as crinoids and coral holdfasts encrusting the successive laminae (Playford et al., 1976), which would indicate normal oxygenated water conditions (Nicoll and Playford, 1993). In Jurassic stromatolites, \textit{Frutexites} occur with shells of \textit{Bositra} and \textit{Lenticulina} foraminifers.

\textbf{Cavities, sheet cracks, veins, and Neptunian dikes}

The second most frequent occurrence of \textit{Frutexites} in the geologic record is related to cavity walls and fissures. On the horizontal surfaces \textit{Frutexites} could display a dominant upward growth, but, in general, their growth is normally perpendicular to the substrate where they were nucleated. \textit{Frutexites} occur interbedded with fibrous calcite cements as well as with internal sediment. The oldest described record of \textit{Frutexites} is in the Paleoproterozoic Gunflint chert (Walter and Awramik, 1979) and from sheet
cracks within stromatolites of the Upper Vendian to Lower Cambrian Chapel Island Formation, Canada (Myrow and Coniglio, 1991). Frutexites have been found in cavities of Devonian deep water mud mounds and grouped with *Renalcis-Epiphyton* calcimicrobes, and with deep water stromatolites assemblage (Tsien, 1979) as well as in stromatoloid cavities from Viséan microbial limestones where they occur interbedded with marine isopachous crusts of fibrous and botryoidal calcite cements (Gischler, 1996). The presence of *Frutexites* in Neptunian dikes has been described only in Devonian records from the Harz Mountains in Germany (Gischler, 1996) and mud mounds of the Hamar Laghdad Ridge in Morocco (Cavalazzi et al., 2007). Similar structures as *Frutexites* have also been described in voids from Toarcian Mn-rich layer at Tatra Mountains in Poland (Jach and Dudek, 2005) as well as in synsedimentary karstic cavities in Pleistocene travertines in Germany (Koban and Schweiger, 1993).

**Condensed pelagic limestones and hardgrounds**

The last most extended occurrence of *Frutexites* is associated with condensed hemipelagic and pelagic red to grey limestones like Griotte and Hallstatt Limestone as well as Rosso Ammonitico facies from Devonian up to Jurassic. These facies occur during very low sedimentation rates, from deep to relatively shallow water depths, during fast transgressive and/or drowning episodes. Nektonic organisms are the dominant fauna (goniatites, nautiloids, ammonoids) and *Frutexites* occur in (a) water-sediment interfaces colonizing sessile fauna, reworked bioclasts as well as ferromanganese hardgrounds (see below) and (b) within the micritic sediment. The first type of *Frutexites* growth in condensed pelagic limestones was described in Matagane Formation (Devonian of Belgium) by Tsien (1979) and in current-swept shallow pelagic ridge (Tafilalt Platform, Devonian of Morocco) by Wendt (1988). No less representative examples have been shown from the Triassic Hallstatt facies of Austria (Wendt, 1969; Rodriguez-Martinez et al., in press) and Oman Mountains (Woods and Baud, 2008). In the Oman Mountains, the sea-floor was directly colonized by *Frutexites*-bearing microbialites and synsedimentary cements. However, in the Northern Calcareous Alps, multiple ferromanganese crusts were colonized by ephibenthonic sessile agglutinated foraminifers which were successively encrusted by *Frutexites* forming pillar-like structures above the seawater-sediment interface.

A similar situation has been described from deep-water hardgrounds during Mid-Cretaceous times in Spain (Reitner et al., 1995) (Figure 2). In this case, a previous benthic community dominated by coralline sponges was replaced by thick limonitic stromatolites with encrusting foraminifera and colonies of *Frutexites*.

The growth of *Frutexites* within the sediment was firstly pointed out by Böhm and Brachert (1993). They described the changes in composition and shapes of *Frutexites* as a result of their growth in open spaces or in interstitial environments. Mamet and Prétat (2006) found *Frutexites* associated to other hematite microstructures in condensed Griotte facies (Coumiac Limestones, Montagne Noire, Baleas Limestone Spain) and Rosso Ammonitico Limestone (Subbetic Cordillera Spain).

**Continental environments**

Some authors (Myrow and Coniglio, 1991; Böhm and Brachert, 1993) have compared the marine records of *Frutexites* with similar arborescent, dendritic forms found in speleothems, desert varnish, and travertines. Also, continental manganese and iron-rich black shrubs have been compared with the marine *Frutexites* records (Koban and Schweiger, 1993; Chafetz et al., 1998). Shapes, sizes, and polymineral character are similar in both marine and continental records.

**Subterranean environments**

*Frutexites* structures are sometimes common in light-independent, deep continental caves, fractures, and veins forming small microbial crusts often associated with calcareous stromatolitic structures. A characteristic representative was found by J. Reitner in southern Namibia (Figure 2b) at the base of the Naukluft Nappe Complex (NCC), which is part of the early Cambrian Damara orogeny (Miller, 2008). The deep base of the NCC is formed by the so-called “Sole Dolomite” (Korn and Martin, 1959) which is often heavily fractured. Within these cryptic fractures *Frutexites* is very common and intergrows with calcareous stromatolitic structures. Thin-shelled freshwater type ostracodes are also abundant in this fracture system.

A modern example of *Frutexites* structures has been found in the deep tunnel of Äspö in southern Sweden (Heim et al., 2007), as part of a highly oxygenated system. Wherever groundwater drops from the tunnel ceiling, a net-like, mineralized micro-system is formed on any rock surface beneath (Figure 3). In general, the dropping system is associated to pending mineral cones which are dominated by the iron-oxidizing, chemolithophoretic bacterium *Gallionella ferruginea*.

The evolving net-like structures feature semi-solid ridges, harboring a particular, highly diverse microbial community. The cross-section of this mineralized biofilm shows laminae with *Frutexites*-like structures (Figure 4). These are mainly composed of iron hydroxides, iron oxides, and less abundant manganese oxides. Small amounts of siderite, calcite, and siliceous material occur side by side with iron and manganese oxides.

**Interpretation**

Holotypes of *Frutexites* spp. (*Frutexites arboriformis*, Maslov, 1960; *Frutexites microstroma*, Walter and Awramik, 1979) were originally described from stromatolites; thus, they were genetically linked to different types of cyanobacteria (Playford et al., 1976, 1984; Scytonematacean – Walter and Awramik, 1979;...
Rivulariacean – Hofmann and Grotzinger, 1985; Angulocellularia group – Riding, 1991). Hofmann and Grotzinger (1985) discussed the affinity of *Frutexites* to different cyanobacteria and proposed further alternatives (purely physicochemical accretion and/or iron bacteria due to the ferruginous character of stromatolite). The occurrence of *Frutexites* in cavities, fissures, and dykes was interpreted as a cryptobiont role (Myrow and Coniglio, 1991). However, Tsien (1979) was the first suggesting a non-phototrophic character, sometimes linked to chemoheterotrophic cyanobacteria (Gischler, 1996) or to chemohetero and autotrophic bacteria (Cavalazzi et al., 2007). Böhm and Brachert (1993) emphasized the roles of *Frutexites* as a cryptobiontic as well as a cryptoendopelagic organisms (living in interstitial habitats). Based on these aspects, the authors proposed the preference of *Frutexites* for oxygen-deficient environments (dyasaerobic to anaerobic conditions). Furthermore, they suggested a bacterial precursor for the formation of *Frutexites* but did not exclude a possible fungal and/or physicochemical origin. According to previous publications (Maslov, 1960; Myrow and Coniglio, 1991), Böhm and Brachert (1993) explained the ferromanganese or phosphate mineralogy of *Frutexites* as a replacement of primary carbonate mineralogy. However, a primary iron mineralization of *Frutexites* was postulated by Horodyski (1975) and Hurley and Van der Voo (1990). Others like Hofmann and Grotzinger (1985) believed that microbiota involved in *Frutexites* could regulate the local water chemistry and iron oxyhydroxides as well as aragonite could coprecipitate.

Finally, the occurrence of *Frutexites* in condensed pelagic limestones and hardgrounds has been linked to a physicochemical origin (Wendt, 1969) as well as to iron bacteria (Reitner et al., 1995; Mamet and Prétat, 2006). Reitner et al. (1995) interpreted the *Frutexites*–foraminifer assemblage as an R-strategic community which replaced the previous K-strategic community (sponges-microbes) due to fundamental changes in oceanographic conditions (from oligotrophic to more eutrophic conditions). Mamet and Prétat discussed the origin of red pigmentation in some Phanerozoic limestones, where some condensed pelagic limestones with *Frutexites* are included. They argued that under anoxic to dysoxic conditions, ferrous iron may be available for oxidation by microaerophilic iron microbes growing at the sediment-water interface. In contrast, the recent *Frutexites*-like structures found in the deep biosphere in fact grow in air-exposed environments under aerobic conditions. However, the presence of different mineral phases within these structures could be associated to changing redox conditions and/or to a different microbial community composition at micrometer-range.

**Conclusions**

The different modes of growth of *Frutexites* have been recognized within shallow to deep water stromatolites, on the seawater-sediment interface, marine micritic sediments, continental cavities, and fractures of deep subterranean environments. Such wider environmental distribution has been taken into consideration for paleoecological interpretations. According to the occurrence of *Frutexites*, some general aspects can be summarized: (a) its distribution does not show bathymetric control (although the dominant record is in deep waters facies), (b) it is formed in environments with very low sedimentation rate (in quiet as well as in agitated waters), (c) in marine environments, *Frutexites* mainly encrusts heterozoan assemblages (crinoids, sponges, solitary corals, and foraminifers), and finally, (d) there is no proof for an exclusive occurrence of *Frutexites* under anoxic and aphytic conditions.

Most of the authors considered a bacteriologically induced origin for *Frutexites*, but the final assessment strongly depends on which mineralogical composition they interpreted as primary (timing of iron mineralization) as well as its loci of growth.

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Cross-references

Bacteria
Calcified Cyanobacteria
Cyanobacteria
Fe(II)-Oxidizing Prokaryotes
Gallionella
Microbial Biominalization
Microbialites, Modern
Microbialites, Stromatolites, and Thrombolites
Stromatactis
Stromatolites

Fungi and Lichens

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Definition

Fungi. Saprophytic, parasitic, or symbiotic living organisms, mainly filamentous (hyphae), rarely unicellular. Cell walls are of chitin or other non-cellulose compounds.

Lichens. Symbiotic organisms composed of a fungal partner, the mycobiont, and one or more photosynthetic partners, the photobiont.

Characteristics

Fungi

Fungi are a group of eukaryotic, heterotrophic organisms with their thallus on or in the substratum. The thallus can be unicellular (rarely) or filamentous (mostly), the latter being either composed of septate or nonseptate hyphae and often forming a more or less defined web of filaments (mycelium). The cell wall is typically chitinized or composed of other non-cellulose compounds. The spore forming thallus (sporocarp) can be microscopic or macroscopic with limited tissue differentiation. Fungi are cosmopolitan and ubiquitous, saprotrophic, mutualistic (mycorrhizal, lichen-forming), or parasitic. A number of rock-inhabiting fungi belong to the Deuteromycetes, a heterogenic group of fungi that develop no or only asexual sporocarp types. The vast majority of rock fungi are dark brown to blackish pigmented and are filamentous or forming microcolonies (Büdel et al., 2000).

Lichens

Symbiotic mode of living

Lichens are mutualistic organisms consisting of a fungal partner, the mycobiont, and of one or more photosynthetic partners, the photobiont. Taxonomically speaking, they belong to the kingdom of fungi and represent here a special group, the lichenized fungi (Honegger, 1996). The lichen-forming fungi are polyphyletic, meaning that they have developed several times during the evolution of fungi. This brought about a taxonomically heterogeneous group with the large majority of them belonging to the Ascomycetes and only few Basidiomycetes and Deuteromycetes (= Fungi Imperfecti; Nash III, 1996a, Miadlikowska et al., 2006).

As photobiont of lichens, green algae with the most frequent genera Trebouxia and Trentepohlia make up about 90% of the photosynthetic partners, whereas cyanobacteria with the prevalent genus Nostoc account for the remaining 10%. The green algae, also referred to as “chlorobionts” (Lange and Wagenitz, 2003), belong to the eucaryotic algae which share many cytological features and their pigmentation (e.g., chlorophylls a and b) with land plants (Bold and Wynne, 1985; van den Hoek et al., 1993). Cyanobacteria (“cyanobionts”) are of
procaryotic nature, lacking a nucleus, mitochondria and chloroplasts, all of which are found in eucaryotic cells. The DNA, which in eucaryotic cells is stored within the nucleus, is found as circular DNA in the cytoplasm and also the thylacoids, which occur in the chloroplasts of eucaryots, are here embedded into the cytoplasm (Friedl and Büdel, 2008).

The interactions between the mycobiont and the photobiont are not completely balanced. There is metabolic transport from the autotrophic photobiont to the heterotrophic mycobiont in the form of carbohydrates, which passively leak from the photobiont cells. Metabolic transport processes from the mycobiont to the photobiont are not known (Schöller and Mollenhauer, 1997). However, the photobiont often also profits from growth within the mycelium of hyphae, since more balanced water and light conditions are granted within the thallus. Besides that, the mycobionts produce a variety of secondary substances that might be beneficial for the photobiont (Schöller and Mollenhauer, 1997). Therefore, the lichenization of the photobiont is probably most adequately described as a controlled parasitism by lichen-forming fungi (Ahmadjian, 1993).

The thallus of a lichen is formed by a more or less dense network of fungal hyphae, in which the photobionts are either evenly distributed (homoiomorous thalli), or are concentrated within a defined layer (heteromorous thalli). Homoiomorous thallus construction mainly occurs in gelatinous, crustose lichens (e.g., Collema, Pyreopsis) whereas heteromorous thallus construction is typically formed by an upper cortex followed by a photobiont layer, a medulla and a lower cortex (Büdel and Scheidegger, 2008). A surface tissue, like the epidermis with stomata and a protective cuticle as in higher plants, does not exist in lichens, neither do they have water and nutrient-transporting structures.

Lichen morphology
The appearance of a lichen thallus is determined primarily by the mycobiont and only in few cases the photobiont determines, to a certain extent, the form of a thallus (e.g., in the filamentous genera Coenogonium, Ephebe, Cystocoleus, and Rhacodium). However, only after the establishment of the symbiosis, under the influence of the photobiont, the characteristic thallus of a lichen is developed (Büdel and Scheidegger, 2008).

Based on their overall growth form, lichens are traditionally divided into three main morphological types: crustose, foliose, and fruticose. Crustose lichens are tightly attached to the substrate with their lower surface and cannot be removed without destruction of the thallus. Foliose lichens form leaf-like thalli that are partly attached to the substrate and have a dorsiventral organization (Büdel and Scheidegger, 2008). Thallus lobes of fruticose lichens are hair-like, strap-shaped, flat, or cylindrical, forming erect or hanging thalli. Some fruticose genera like Baemopyces and Cladonia develop a twofold thallus with a foliase horizontal and a fruticose vertical thallus part. Endolithic lichens represent a special form of crustose lichens, where the thallus partly or completely grows within the rock. According to Golubic et al. (1981), the endolithic growth form can be further separated into cryptoendolithic (= growing within the rock), chasmendolithic (= growing along cracks or fissures of the rock), and euendolithic (= actively boring into the rock) organisms.

Diversity
Fungi
Rock-inhabiting fungi belong to major groups of fungi: the hyphomycetes and the “black fungi,” the latter named because of the strong production of melanoid pigments that causes dark-brown or blackish pigmented hyphae. Formation of microcolonies is a universal feature of the latter group, and they are therefore named “microcolonial fungi” (= MCF; Figure 1f and i; Gorbushina, 2003). Hyphomycetes form a mycelium but lack a sporocarp and the conidia (asexual spores) are borne on hyphae. They occur on soil or epiphytic habitats and dominate on rock surfaces under milder climate regimes (e.g., high air humidity, abundant nutrients). The black MCF prevail under harsh climatic conditions and are slow-growing. They represent a diverse assemblage of non-lichenized ascomycetes. Because of a convergent microcolonial morphology and their morphological plasticity, they are extremely difficult to identify by classical methods. Identification procedures based on molecular methods are just in progress and have been applied to a few strains only. So far, identified samples and strains have been placed in the mostly anamorphic (asexual) genera Alternaria, Chaetomium, Cladosporium, Coniosporium, Cryomyces, Friedmanniomyces, Lichenothelia, Phaeococcomyces, Phaeosclera, Phoma, Sarcinomyces, and Scolecosbassidium (Sigler et al., 1981; Diakumakou et al., 1995; Wollenzen et al., 1997; Sterflinger et al., 1997; Büdel et al., 2000, Selbmann et al., 2005; Onofri et al., 2007). Most of them belong to the ascomycete order Pleosporales, Capnodiales, and Dothideales, summarized in the class Dothideomycetes (Schoch et al., 2006), few belong to the Chaetothyriales in the class Eurotiomycetes (Geiser et al., 2006).

Lichens
Lichens grow on a wide variety of substrates including rocks (= epi- or endolithic), soil (= epigaic), detritus, and the branches, stems (= epiphytic), and leaves of trees (= epiphylic). Most lichen species are specialized to one type of substrate comprising certain habitat characteristics. There are species with a wide ecological amplitude (euryoecious as compared to stenococious species), able to colonize different types of substrate, like e.g., Caloplaca citrina (Hoffm.) Th.Fr., Candelariella vitellina (Hoffm.) Müll.Arg., and Physcia adscendens (Fr.) Oliv.
and there are nutrient-tolerant species that occur epiphythically as well as epilithically (Figure 2b and c; Wirth, 1995).

On soils, lichens often represent part of a biological soil crust (BSC). BSC are formed by an association between soil particles and different proportions of cyanobacteria, algae, microfungi, bryophytes, and lichens (Belnap et al., 2003). In arid and semiarid regions, which make up more than 30% of the land surface of the earth, BSC cover up to 25% of the soil surface (Weber et al., 2008), but also in temperate regions like Central Europe, BSC occur in various habitats (Büdel, 2003).

Fungi and Lichens, Figure 1 (a) Granite inselberg at Soebatsfontein, Succulent Karoo, South Africa. (b) Close-up of the rock surface, showing dense colonization by the microlichens Peltula bolanderi (large olive-colored thalli), Peccania sp., Synalissa sp., cyanobacteria, and microcolonial fungi. (c) Detail with Peccania sp. (d) Detail with Synalissa sp. (e) Microscopical view on the lobes of Synalissa sp., showing the incorporation of the yellow pigment scytonemin (light and UV protectant) in the gelatinous sheath of the lichen. (f) Endolithic lecideoid lichen, thallus parts visible; where the rock surface was removed (arrows), the sporocarps (fruit bodies) are exposed on the rock surface (fb); MCF indicates the presence of numerous microcolonial fungi. (g) Microscopical view on a part of the algal layer of the endolithic lecideoid lichen showing the Trebouxia-like chlorobionts in an envelope of lichen hyphae. (h) Colonies of the red-sheathed cyanobacterium Gloeocapsa sp. growing on the rock surface between the lichens. (i) Microcolonial fungi on the rock surface.

Rock-inhabiting lichens come from most taxonomic groups of lichens as, for example, members of the class Lichinomycetes (e.g., Anema, Digitothyrea, Heppia, Lichinella, Peccania, Peltula, Phyllisciella, Synalissa, Thyrea; Figure 1b–e) that exclusively have cyanobacteria as photobionts. Although these lichens can be most abundant in arid and semiarid landscapes, the vast majority of lichens (more than 200 species) come from the largest class of lichenized fungi, the Lecanoromycetes. Within the Lecanoromycetes, green algal photobionts dominate (e.g., Acarospora, Buellia, Calopla, Dermatiscum, Dimelaena, Hymenelia,
Biogeography

Fungi

Since determination on the species level is a challenging task and not easily possible without culturing or before more molecular data are available, biogeographical information is very limited at present. For some species of the genus *Lichenothelia*, however, information on distribution patterns are available. While *L. intermedia* occurs on almost every granite rock surface throughout Africa, the species *L. gigantea* and *L. radiata* are restricted to Australia, and *L. globulifera* was only found on rock outcrops of the Seychelle island La Digue (Henssen 1987). On the basis of morphological and molecular data (SSU phylogeny and ITS sequence data), Selbmann et al. (2005) recently described two new genera of MCF, *Cryomyces*, and *Friedmanniomyces*, only known from Antarctic rocks so far. The authors provide good evidence that at least the new genus *Cryomyces* might be endemic to Antarctica. Since MCF diaspores are easily spread over large distances by the wind, many of them might have a cosmopolitan distribution (airborne fungi).

Lichens

Lichens have developed during evolutionary radiation of ascomycete fungi within the last 600–400 million years, possibly contemporaneous to the emergence of land plants (Galloway, 1996). Due to this long-lasting evolutionary process, their distribution patterns show similarities to other groups of organisms and can be explained utilizing the theory of plate tectonics (Galloway, 1996). Compared to vascular plants, lichens comprise a higher number of cosmopolitan species within different climate zones of the earth and endemism within smaller geographic regions is normally lower. Exemplarily, on the Kap Verde Islands 13.4% of vascular plants were endemics, whereas only 2.5% of the lichen species were found to be endemic (Schöller, 1997). Dispersal of both symbiotic partners is facilitated by the fact that fungal spores as well as algal cells are transported over vast distances within the air (Brown et al., 1964).

At least 16 major distribution patterns of lichens can be distinguished (Galloway, 1996). Besides lichen species that are constrained to one single continent, like austral and neotropical taxa, there are distribution patterns that comprise spatially contiguous regions, like the paleotropics that include most of Africa, the Arabian Peninsula, most of the Indian subcontinent, the Malaysian archipelago, and the Islands of the Pacific Ocean (Galloway, 1996). A third type of distribution pattern embraces disjunct distributions over continents, which have been close by or unified in history, but were separated by plate tectonic movements, like pantropical and American–Asian distribution patterns (Grotzinger et al., 2006). Taxa with bipolar distribution represent the last pattern, which results from climate change through earth history. These organisms occur at high latitudes of the
northern and southern hemisphere, but often also occur in alpine regions, forming relict habitats of the last ice age (Galloway, 1996).

**Physiology**

**Fungi**

There is not much known of the specific physiology of rock-inhabiting fungi except that they are able to tolerate extremely high (70–80°C; Palmer et al., 1987) and low temperatures (−40°C; Selbmann et al., 2005) due to their poikilohydric nature and related to that, their ability to withstand long periods (in the magnitude of tens of years) in the dry state. Many fungi are heavily melanized, most probably as a shelter against high radiation of both visible and UV light.

**Lichens**

Lichens are poikilohydric organisms, meaning that they cannot actively take up or lose water. Moistening of the thallus can be achieved by rain/snow precipitation, dew-fall, and equilibrium with high air humidity. This clearly distinguishes them from vascular plants that actively regulate water uptake and loss.

**Water**

Although water uptake, storage, and loss are modified by differences in thallus structure and morphology, these mechanisms are predominantly controlled by physical processes (Rundel, 1988). Therefore, the physiological activity of lichens is highly dependent on the prevailing water conditions. In general, the photosynthetic response of lichens to increasing water content can be described as an optimum curve, which, however, reveals large variability (Lange et al., 2001). Air-dried lichens with a water content below 15–30% show no photosynthetic response at all (Nash III, 1996b). With increasing water content, lichens first reveal net respiration rates before the compensation point is reached, above which positive photosynthetic activity is recorded. In general, cyanolichens commence positive net photosynthesis (NP) at higher water contents and are generally able to perform positive NP under higher water content than chlorolichens (especially the homoioemerous species; Lange et al., 2001).

Above an optimum water content, at which maximum NP rates are reached, free water is present and the lichens have a water potential close to zero (Green and Snelgar, 1982). These periods of water supra-saturation cause a depression in photosynthesis by increased thallus diffusive resistances; CO₂ pathways within the thallus are blocked by water (Lange et al., 1997). Water supra-saturation can occur in chloro- and cyanolichens of all growth forms (Lange et al., 1996, 2000, 2004; Lange and Green, 1997), and also endolithic lichens of various substrates have to cope with this problem (Tretiach and Pecchiari, 1995; Tretiach and Geletti, 1997; Winkler and Kappen, 1997). In a recent study, the photosynthetic performance of Hymenelia prevostii and H. coerulea, two endolithic green algal lichens growing on steep limestone outcrops in the eastern alpine mountains, have been investigated in detail (Weber et al., 2007). Both species were saturated at extremely low water content (H. prevostii: 0.26 mm, H. coerulea: 0.07 mm rainfall equivalent) and revealed very narrow ranges of positive NP values upon increasing thallus water content (0.2 mm vs. 0.1 mm). Their growth on steep rock surfaces therefore represents an adaptation to avoid excessively high water contents or the formation of CO₂ blocking water films.

A crucial difference in the physiology of cyano- and chlorolichens lies in the capability to use water vapor for photosynthetic activation. While air-dry chlorolichens gain positive NP in equilibrium with air of about 80% relative humidity and above, all cyanobacterial lichens investigated so far need liquid water for activation of NP (Büdel and Lange, 1991; Lange et al., 1993). This fundamental difference also affects the natural habitats of lichens with cyanolichens preferably occurring at moist habitats with frequent liquid water (e.g., Collema spp., Lichinella spp.), whereas green algal lichens often also occur in rain-shaded habitats of high air humidity (e.g., Lepraria spp., Usnea spp.). The endolithic Hymenelia species mentioned above were also shown to perform positive NP at relative air humidities of 90% and above (Weber et al., 2007).

**Light**

The capture of radiant energy is essential for photosynthesis. Only light of wavelengths between 400 and 780 nm, the photosynthetically active radiation (PAR), is utilized for photosynthesis (Nash III, 1996b). Under constant and favorable abiotic conditions, photosynthetic response of lichens to increasing light intensities exhibits a saturation curve with saturation for most species occurring between 100 and 400 µmol photons m⁻² s⁻¹ (Demmig-Adams et al., 1990). However, extremely shade-tolerant species have saturation values as low as 20 µmol photons m⁻² s⁻¹ (Green et al., 1991), whereas others, like Ramalina maciformis (Del.) Bory reveal values above 1,000 µmol photons m⁻² s⁻¹ (Demmig-Adams et al., 1990). At increasing temperatures, light saturation is achieved at higher levels (Kershaw, 1985) and also water content has an influence (Lange, 1969), probably due to alterations of light penetration within the thallus (Ertl, 1951; Dietz et al., 2000). Exposure of fully hydrated lichens to excess light can induce a depression of photosynthesis, both in chloro- and cyanolichens (Demmig-Adams et al., 1990).

As a protection against excessive light, lichens in general possess a cortex made of fungal tissue, and some species even form a so-called epinecral layer consisting of dead, collapsed, and often gelatinized hyphae and photobiont cells (Büdel and Scheidegger, 2008). Peltigera rufescens (Weiss) Humb., for example, has a cortical organization that causes a decrease of light transmittance to
40% at the upper boundary of the algal layer (Dietz et al., 2000).

The endolithic growth form (Figure 1f) can also be interpreted as a protection from excess light. Winkler and Kappen (1997) found that only 0.1–0.2% of the ambient light reaches the upper part of the algal layer in Sarcogyne cf. astrostrophiaca (Zahlbr.) H. Magn. Light reduction within the sandstone on top of the photosynthetically active layer is also reflected by light saturation values above 800 μmol photons m⁻² s⁻¹ measured for Sarcogyne cf. astrostrophiaca, Lecidea confluenta Müll. Arg. and Lithoglypha aggregata Brusse (Winkler and Kappen, 1997). In limestone, Hymenelia prevostii and H. coerulea were saturated at high light intensities (1,000 μmol photons m⁻² s⁻¹ vs. 800 μmol photons m⁻² s⁻¹; Weber et al., 2007).

Temperature
Lichens are generally known to survive a wide range of temperatures. Being dominant terrestrial organisms of Antarctica, they must be able to tolerate extremely low temperatures (Nash III, 1996b), whereas in sun-exposed microhabitats, lichens experience temperatures of up to 60°C (Hahn et al., 1989). However, lichens in general reveal a vast difference in temperature tolerance depending on whether the thalli are hydrated or dry. Whereas dry thalli of some lichen species are able to tolerate a stepwise cooling to temperatures as low as −196°C (Kappen, 1973) and heating up to 90 or 100°C (Lange, 1953), wet thalli are far more sensitive. Review of field measurements of lichen photosynthesis revealed that many lichens are primarily active at temperatures between 5°C and 20°C, although 0–10°C may better characterize some Antarctic species (Kappen, 1988; Kappen and Friedmann, 1983).

Photosynthetic capacity of some lichen species is known to reveal seasonal changes that involve an adjustment to higher summer temperatures (Kershaw, 1985). Recently, Lange and Green (2005) proved that dark respiration (DR) of lichens does exhibit seasonal changes of temperature sensitivity. At 5°C, for example, the respiration rates of Cladonia convoluta (Lam.) P. Cout., Lecanora muralis (Schreber) Rabenh., and Diploschistes muscorum (Scop.) R. Sant. were several times higher in winter than in summer. This acclimation of DR implies that maximal net carbon fixation rates remain similar throughout the year and are not depressed by increased carbon loss due to higher temperatures during the warmer season (Lange and Green, 2005).

CO₂
The photosynthetic CO₂-fixation rate of lichens at optimal water content generally increases with increasing CO₂-concentrations, typically reaching saturation around 600–1,300 ppm (Nash III, 1996b; Tretiach and Geletti, 1997; Lange et al., 1999; Lange, 2002). At natural CO₂ concentrations (around 360 ppm), many species only reach about 60–80% of maximal NP under optimal water conditions (Lange et al., 1999; Lange, 2002).

Under supra-saturated water conditions, NP of lichens is often depressed at natural CO₂ concentrations. Increased CO₂ values, however, in most cases result in attenuation of this depression (e.g., Lange, 2002). In some species, as for the epigaecic species Fulgensia fulgens (Sw.) Elekin, a severe depression in CO₂ uptake can even be completely counterbalanced by a CO₂ concentration of 4,500 ppm (Lange et al., 1999).

The CO₂ compensation point (CO₂ concentration, at which respiration equals photosynthesis) in many lichen species is very low as compared to vascular plants (Nash III, 1996b). This often results from a CO₂ concentrating mechanism (CCM), by which CO₂ is actively pumped into the photobiont cells (Badger et al., 1993). Such a CCM is also present within the photobiont Trebouxia irregularis Hildreth and Ahmadjian of the endolithically growing Hymenelia species, which makes them less susceptible to elevated CO₂ concentrations (Weber et al., 2007).

Interactions with the substrate
Bare natural rock surfaces are extremely rare on earth and might only be found under extreme climatic conditions of hot or cold deserts. Normally, rock surfaces are covered by a dense layer (film, crust; Figure 1a) composed of cyanobacteria (Figure 1h), algae, lichens (Figure 1b–f), and fungi (Figure 1f and i). Cyanobacteria, lichens, and fungi also can grow inside the rock itself, in the upper few millimetres (=endolithic, Figure 1f and g). On inselbergs of tropical savannas, these biofilms are even responsible for the characteristic black (cyanobacteria dominated) or brown color (lichen dominated) of the rock surface (Büdel et al., 2000).

Fungi
Fungi grow on rock surfaces (epilithic), inside the rock (endolithic) or are part of the desert varnish produced by microorganisms growing inside the up to 1 cm thick black layer (e.g., Krumbein and Jens, 1981). Also MCF have been shown to be involved in desert varnish formation in Arizona and Australia (Taylor-George et al., 1983). Microbial growth on rock surfaces follows the microscopic landscape formed by mineral grains, pores, and fissures of the substratum (Figure 1f and i). Characteristically, microorganisms of the resulting biofilms colonize in the way that fungal hyphae and some cyanobacteria penetrate the substrate, while algae, bacteria, and microcolonies of yeast-like fungi clump together (Gorbushina, 2007). They secrete extracellular polymeric substances (EPS), which envelope the microcolonies, keep them together and adhere them to the substratum.

Biogenic rock weathering is surely a multifactorial process. The role of fungi in the wear-down of rocks is difficult to separate from the activity of other microorganisms of biofilms. Generally, there are two main groups of weathering that can be distinguished. (1) Either the growth of cells forces separation of mineral grains (Dornieden
et al., 1997), or desiccation/hydration cycles of cell walls and EPS cause their separation (Friedmann and Weed, 1987; Warscheid et al., 1991). (2) Dissolution of rock minerals can be achieved by organic acids released from cells, chemical impact of respiratory CO$_2$, H$,^+$, and redox reactions with metal-ions (Gadd, 1999; Burford et al., 2003).

**Lichens**

During the 1980s and 1990s, a huge variety of literature on the patterns and effects of biogenic weathering by lichens has been published. However, a comprehensive understanding on the overall mechanisms, effects, and magnitude of effects could not yet be accomplished (Chen et al., 2000).

As for the fungi, the impact of lichens can be subdivided into physical and chemical effects. The physical effects comprise: (1) penetration of hyphae through intergranular cavities and along mineral cleavage planes; (2) thallus expansion and contraction due to varying water content; (3) freezing and thawing of the lichen thallus; (4) swelling of organic and inorganic salts originating from lichen activity; and (5) incorporation of mineral fragments into the thallus (Chen et al., 2000).

The chemical effects comprise the solubilization and the insolubilization of inorganic elements, resulting in the weathering of minerals (silicates, phosphates, carbonates, sulfides, oxides) and the formation of new deposits (carbonates, oxides, sulfides, phosphates; Benthelin, 1988). The solubilization processes, as induced by lichens include (1) the release of metal ions by acid and complexing compounds, and (2) the acidification or alkalization by uptake or release of alkaline compounds. Insolubilization processes are oxidation of Fe$^{2+}$ and Mn$^{2+}$, and uptake of elements by the organisms in different environments (Benthelin, 1988).

Besides the alkalization activity of endolithic cyanobacteria (Büdel et al., 2004), it is also expected that lichens perform a similar role on/in rocks. Wessels and Schoeman (1988) describe an exfoliation process initiated by the endolithic lichen *Lecidea aff. sarcogynoides* Koerb. In SEM imagery, they perceived a dissolution of the binding material along the growth zone of the hyphae and also pitting marks on the quartz crystals were observed, where hyphae had attached to the surface of the crystals (Wessels and Schoeman, 1988). Besides *L. aff. sarcogynoides*, also lichens of the genus *Caloplaca* are found to contribute to rock weathering in sandstones of South Africa (Figure 2d).

Friedmann (1982) and Friedmann and Weed (1987) report on cryptoendolithic microorganisms in the Antarctic cold desert (Ross Desert) being dominated by lichens that cause a exfoliative weathering pattern along the lichen growth zone very similar to the pattern described by Büdel et al. (2004).

**Conclusions**

Identification of lichens on the basis of their external and internal morphology, as well as chemical characteristics, is well-established and only in some cases, where lichens do not express a typical morphology or where they are often sterile, e.g., in the endolithic habitat, additional identification methods, using a molecular approach are necessary. Thus, lichen diversity of different rock types is quite well-known. Rock-inhabiting fungi, on the other hand, are much more difficult to identify. They often only express a reduced set of morphological features, insufficient for save identifications. Here, the use of molecular techniques is a must and already lead to a considerable increase in knowledge (e.g., Selbmann et al., 2005).

The interaction of fungi and lichens with their rock sub-stratum is manifold. Biofilms with fungi and lichens might either increase weathering rates of their substratum, or in some cases, protect it from weathering. However, these crusts and films on rocks are a complex mixture of bacteria, cyanobacteria, algae, fungi, and microlichens embedded in the common structure of an EPS. Macrolichens rising above the biofilm form a separate layer, comparable to that of emergent trees that rise above the canopy of rain forests.

Microbial biodiversity of rock surfaces and the complex interaction of these organisms among themselves and with the rock substratum, for example, their role in bioweathering, is still poorly understood at present and will surely be a field of intensive and fascinating research in future.

**Bibliography**


Cross-references

**Algae (Eukaryotic)**

**Biodeterioration (of Stone)**

**Bioerosion**

**Biofilms**

**Chroococcidiopsis**

**Cyanobacteria**

**Endoliths**

**Geomycology**

**Microbial Communities, Structure, and Function**

**Microbiocorrosion**
GALLIONELLA
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Definition
Gram-negative, microaerophilic, and chemolithotrophic bean-shaped cells that secrete an extracellular twisted stalk and can grow with ferrous iron as source of energy and carbon dioxide as the sole source of carbon.

The genus Gallionella is characterized by its chemolithotrophic growth with ferrous iron as source of electrons and energy and carbon dioxide as source of carbon. Mixotrophic metabolism has been demonstrated with glucose, fructose, and sucrose (Hallbeck and Pedersen, 1991). The family Gallionellaceae has one known species, Gallionella ferruginea (Hallbeck et al., 1993; Hallbeck and Pedersen, 2005). The organism produces a twisted stalk that consists of a bundle of numerous organic fibers, which makes Gallionella very easy to identify (Figure 1). The potential functions of the stalk is to act as a holdfast (Figure 2) and possibly also to act as a sorbent for the iron oxides produced by the organism during its oxidation of ferrous iron to ferric iron. This stalk allows easy identification not only in modern systems but it also allows use as fossil to trace the activity of Gallionellaceae in the past. The stalk production appears to be stimulated by pH values above 6 (Hallbeck and Pedersen, 1990).

Optimal growth and stalk formation under in situ conditions occur under microaerophilic conditions (0.4–1.5 mg oxygen L⁻¹) in a an Eₘₚ range between +150 and +250 mV (Hallbeck and Pedersen, 1995; Anderson and Pedersen, 2003). The environment where stalk-forming Gallionella can be observed, commonly attached to surfaces, is slowly flowing groundwater that is rich in ferrous iron but has low organic carbon and oxygen concentrations. Typical places to find Gallionella are in drain pipes, storage basins for groundwater from deep wells, in tunnels, and on rock walls with seeping groundwater. Colonies of Gallionella rapidly develop to visible structures in

Gallionella, Figure 1 Gallionella ferruginea produces a twisted stalk that consists of numerous thin organic fibers. As the stalks age, iron oxides precipitate on the stalks and many trace elements co-precipitate (Anderson and Pedersen, 2003). The attachment of the cell to the stalk is very brittle and they are, therefore, commonly detached during the preparation of specimens for the microscope. Diameter of the stalks is typically 0.3–0.5 μm in width and up to 400 μm or more in length. Photo: Karsten Pedersen.
water (Figure 2). Growth of Gallionella in groundwater seeping out from fractures in rock walls develops to compact mats of cells, stalks, and iron oxides. Such mats can reach a thickness of several centimeters in less than a year and they may enrich trace elements up to 10^6 times their respective concentrations in the seeping groundwater (Anderson and Pedersen, 2003).

**Bibliography**


**Cross-references**

Banded Iron Formations
Chemoolithotrophy
Fe(II)-Oxidizing Prokaryotes

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**GEOBACTER**

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**Definition**

*Geobacter* is the name of a genus that is located within the *Deltaproteobacteria* comprising several species of Fe(III)-reducing bacteria (Lovley et al., 1993). The genus *Geobacter* represents the type genus of the family Geobacteraceae which includes also the genera *Desulfuromonas*, *Desulfuromusa*, *Geoalkalibacter*, *Geopsychrobacter*, *Geothermobacter*, *Malonomonas*, and *Pelobacter*.

The type species of the genus *Geobacter* is *Geobacter metallireducens* strain GS-15, which was enriched and isolated from pristine freshwater sediments of the Potomac River with acetate as the electron donor and poorly crystalline ferric iron as the electron acceptor. In addition to pristine aquatic habitats, *Geobacter* species were isolated from deep aquifer sediments (e.g., *G. chapellei*), subsurface sediments (e.g., *G. bemidiensis*), or various contaminated sites (e.g., *G. sulfurreducens*). Cells of *Geobacter* species stain Gram-negative and are non-spore-forming. All *Geobacter* species have midrange temperature optima (25–35°C) and grow only under strictly anoxic conditions. Alternative electron acceptors for *Geobacter* species include nitrate, elemental sulfur, fumarate, malate, Mn (IV), U(VI), Co(III), anthraquinone-2,6-disulfonic acid,
and humics. Furthermore, *G. metallireducens*, *G. psychrophilus*, and *G. sulfurreducens* were shown to transfer electrons from the oxidation of organic compounds onto the surface of electrodes. All *Geobacter* species utilize acetate as electron donor and carbon source; other electron donors that are utilized by the majority of *Geobacter* species include ethanol, pyruvate, lactate, hydrogen, and formate. *Geobacter* species are unable to grow by fermentation. Ecological studies indicate that *Geobacter* species predominate under iron-reducing conditions. Furthermore, the combination of ecological and physiological studies implies that *Geobacter* species might play important roles in bioremediation processes such as the oxidation of aromatic compounds, the reduction of tetrachloroethene, or the immobilization of U(VI).


Among the validly described *Geobacter* species, *G. metallireducens* strain GS-15 and *G. sulfurreducens* strain PCA are the most intensively studied in terms of physiology, biochemistry, and genetics. The genomes of both species were sequenced and a genetic system was established for *G. sulfurreducens* (Methé et al., 2003).

### Bibliography


### Cross-references

Fe(II)-Oxidizing Prokaryotes
Fe(II)-Reducing Prokaryotes
Gallionella

### GEOCHRONOLOGY

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### Synonyms

Age determination; Dating

## Definition

**Geochronology.** Determination of ages and time intervals for geologic materials and processes on geologic, archeologic, and historic time scales. The science of investigating and reflecting the chronology of the earth constituents as induced from geologic data, based on absolute and relative dating methods.

**Age, absolute.** Age determination based on radioactive elements, their rates of decay and physical measurements, resulting in an actual age given in years for the analyzed geologic material (e.g., mineral, rock, fossil, fluid).

**Age, relative.** Age information based on stratigraphic anomalies (e.g., lithostratigraphic and biostratigraphic principles, field observations) or utilizing chemical reaction rates (e.g., diffusion, exchange, oxidation, hydration) as a measure of time.

**Units.** Geochronologic data are presented as yr BP (years before present), as ka (age date in thousands of years), as kyr (time interval in thousands of years, e.g., between distinct age data), following the same principle as Ma and Myr (millions of years, respectively). Alternatively, the mathematical expression in years, times order of magnitude is used and favored for age data and time intervals in the range of billions of years and the description of half-lives (e.g., $4.47 \times 10^9$ years, half-life of the $^{238}$U isotope).

### Introduction

The term “geochronology” may reasonably be used in reference to all principles and methods employed in geology to determine the relative and numerical ages of all kinds of rocks (Walsh, 2001). The immense age of the Earth and the long-term duration of processes changing the morphology and composition of the Earth’s crust and interior were progressively recognized throughout the last centuries. Geoscientists developed and established a relative chronological systematic based on lithostratigraphic and biostratigraphic principles, including global correlations and ongoing refinement. The actual state of the art is presented by the International Commission on Stratigraphy (ICS, 2009; [http://www.stratigraphy.org/upload/ISChart2009.pdf](http://www.stratigraphy.org/upload/ISChart2009.pdf)).

In contrast, the direct determination of absolute ages required fundamental new physical approaches and analytical methods in the field of geochemistry. The basis for “geochronology” as a new geoscientific discipline was provided by the early description of radioactive transformations more than 100 years ago (Rutherford, 1906). An actual status in combining detailed knowledge of relative age successions with precise absolute ages and the impact of resulting chronostratigraphy on the understanding of the Earth’s geologic evolution is presented in concise manner by Ogg et al. (2008). A still valid and systematic overview about the broad variety, principles, scopes, potentials, and limitations of physical and chemical dating methods is given in a comprehensive way by Geyh and Schleicher (1990). This work is especially
useful for non-geochronologists on search for analytical methods most suitable for their specific demand in geologic time series data, providing fundamental knowledge for collaborative approaches.

**Geochronology in geobiology**

The science of geochronology provides the fourth dimension of geological and geobiological processes as a crucial requirement for their reconstruction in space and time, e.g., the determination of changes in biochemical fluxes, habitat conditions, and their comparison through Earth’s geological and biological evolution.

Due to the fast developing field of geobiology, the increase in recognition and understanding of biological mediated formation and destruction of minerals, stones, and rock sequences the demand for high resolution, and absolute age data is continuously evolving.

In the following some essential, very different, and often in geobiologic approaches preferred concepts of age determination are explained.

**Some essential concepts**

**Counting of structural and chemical anomalies**

(High resolution, high precision, robust, low analytical effort, short time scales, strict prerequisites on growth systematic for high reliability):

Well known is the counting of growth, sedimentation, and precipitation cycles in correlation to short time intervals of orbital cycles in a broad variety of archives. Typical examples are changes of tree-ring structures (seasonal density, dendrochronology), layering of varves in sediments (seasonal change in detrital and organic input), sclerochronology of corals (annual growth bands), chemical lamination within sclerosponges, and fast growing Fe-Mn precipitates (reflecting seasonal changes in temperature and oxygenation, respectively).

Assuming the youngest part of a succession as recent reference point, this concept allows very precise and reliable age data, as long as continuous growth is ensured and no hiatus is observed. The latter would interrupt and extend the age record to unknown length, until independent radiometric age data provide new reference points. Alternatively, the identification of characteristic markers for over-regional to global events allows the combination of such archives from different locations and their sequential connection to longer time series (e.g., regionally resolved reconstruction of the Little Ice Age; Mann, 2001).

**Radiometric age determination/radiochemistry**

(Precise absolute ages, independent from growth structure and stratigraphy, decadal to billions of years time scales, robustness, and uncertainties depending on method and age, high analytical effort, on highest level of precision often depending on physico-chemical models, and correction assumptions):

Radiochemistry and related methods for radiometric age determination are the most powerful tools in geochronology, providing independent absolute ages for samples throughout the Earth’s evolution. Their principles are based on changes of the isotopic composition of an element in the sample, due to the decrease of radioactive parent isotopes by decay and/or the increase of radiogenic isotopes as product of decay. Both processes are depending on isotope-specific half-lives, therefore, providing directly chronologic information. Radiometric methods belong to the broad field of Isotope Geochemistry. Detailed information about isotope systematic, analytical procedures, data interpretation, and geologic and geochemical background is presented by Dickin (2005), Faure and Mensing (2005), and Allegre (2008).

Two major geochronological principles, **deficiency** and **in-growth**, are dominating these methods and are explained in examples given below. In general, the deficiency principle can be described as determining the age by calculating the time required for the decay of a radioactive isotope from an assumed starting concentration or ratio down to the actually measured value. The starting value is equivalent to an excess concentration of a radioactive isotope above its equilibrium activity within its decay chain. The initial degree of excess depends on the investigated environment and the isotope system of interest. Required assumptions on the initial values are generally based on the measurements of recent analogues. Deduced ages are often presented by adding “excess” or the abbreviation (exc.) to the related isotope for method identification, especially if this isotope or isotope ratio is relevant for different geochronological methods.

In contrast, if a sample is strongly depleted in a decay product down to negligible or well-known initial concentration, e.g., by element fractionation during sample growth or crystallization, the in-growth principle can be used for direct age determination.

The activity ratio between decay product and parent isotope is increasing with time until equilibrium is reached. The time span required to reach the actually measured concentration or ratio is directly related to the half-life.

The maximum limit of age determination is reached for both principles in samples reflecting equilibrium activity within the analytical uncertainty, just reached from different directions (decay/in-growth). As a rule of thumb, for the robust estimation of the dynamic range of a geochronological approach, a factor of 4–8 (depending on the analytical technique and its precision) could be applied on the half-life of the relevant isotope. Best control on age reliability and accuracy is given where both principles could be applied on the absolute determination of a sample age or duration of a process.

It should be considered that radiometric methods can be easily overestimated and misinterpreted due to manifold requirements in sample quality, preparation, and analytical
effort. As more precise modern analytical techniques allow the determination of isotope ratios and concentrations, more attention should be given to the clear distinction between precision of a measurement and the accuracy of the deduced age. Especially in terms of realistic age uncertainties, the range of whole procedure blanks, reproducibility, and applied correction factors must be well known.

A general requirement of radiometric dating methods is a closed-system behavior of the sample material after formation until preparation for measurement. Differential analyses combining relative age successions with subsequent absolute age determinations are fundamental to achieve the highest level of accuracy, as, e.g., required for international chronostratigraphic calibration approaches. Relative age determination is generally based on the field and the microscopic observation and biostratigraphic classification. Absolute age determination is most reliable if independent measurements could be applied. In any case, the expensive isotope analyses should be combined with mineralogical and/or chemical pre-investigations for verification or estimation of potential secondary perturbations. Main issues are mineral composition and purity, the detection of secondary recrystallization processes, and chemical exchange reactions. For robust results of isotope approaches, a useful guiding principle includes to (1) look carefully, then to (2) select well-documented samples, before (3) crushing and homogenizing these materials for lab procedures.

Radiometric example A: cosmogenic
\[ ^{14}\text{C}/\text{radiocarbon method/deficiency principle} \]

Substrates for geochronological approaches in geobiology often consist of carbonic components, therefore the radiocarbon method, based on the cosmogenic nuclide \(^{14}\text{C}\), is especially advantageous and often suitable. \(^{14}\text{C}\) is the least abundant of the three natural carbon isotopes \((^{12}\text{C}, \text{98.98\%}; ^{13}\text{C}, \text{1.11\%; } ^{14}\text{C}, \text{1.176 \times 10^{-12} \text{ atoms per } ^{12}\text{C atom}}} \) and decays with a half-life of 5,730 years. Due to its general dependence on cosmic activity and processes, it belongs to a group of cosmogenic and geochronologic relevant nuclides like \(^{10}\text{Be} \text{(Beryllium), } ^{36}\text{Cl} \text{(Chlorine), and } ^{129}\text{I} \text{(Iodine) of very different chemical affinity and dating potential.} \)

\(^{14}\text{C}\) is mainly produced by the collision of low-energy cosmic ray neutrons with nitrogen atoms \((^{14}\text{N})\) in the upper atmosphere, rapidly oxidized to \(\text{CO}_2\) and available for metabolic processes and mineral precipitation. A complex system of various exchange processes between the atmosphere, the biosphere, and the hydrosphere results in a kind of dynamic equilibrium resulting in rather constant \(^{14}\text{C}/^{12}\text{C}\) ratios.

As soon as organic matter (e.g., by death of an organism) or a mineral (e.g., by biomineralization) is excluded from equilibration processes with the ambient carbon, the carbon isotopic composition changes systematically by decay of \(^{14}\text{C}\). The decreasing \(^{14}\text{C}\) concentration is ideally transferable to direct age information, i.e., about the date since the system was closed.

The rather short half-life of \(^{14}\text{C}\) limits this method in general to samples younger than 50 ka. Extremely challenging is the application on samples of heterogeneous carbon supply, like marine cold seep and AOM (anaerobic oxidation of methane)-related carbonates, which tend to be influenced by varying proportions of buried methane-driven \(^{14}\text{C}\) from older sources. Due to the low abundance, precise, and high resolution, \(^{14}\text{C}\) measurements on small samples or growth increments (e.g., 0.002 g of a coral fragment) are restricted on very expensive and rare accelerator mass spectrometry (AMS) facilities. High-resolution analyses and chronological profiles are restricted to mechanical preparation methods using handheld mini- and computer-controlled micro-drilling devices.

Radiometric example B: Uranium decay series/\( (^{230}\text{Th}/^{234}\text{U})/\text{in-growth principle} \)

The U decay series offers a wide range of established geochronological methods, suitable from marine mineral precipitation processes of the last decade \((^{230}\text{Th}/^{234}\text{U})\) back to the oldest rocks and minerals \((^{238}\text{U}/^{206}\text{Pb})\) on Earth, billions of years in age. Bourdon et al. (2003) presented an outstanding selection of papers covering the broad scope of Uranium series geochemistry.

Considering geobiological approaches, the affinity of many organisms to build directly calcium carbonate skeletons and indirectly remnants of biologically mediated precipitation processes (chemoherms) favors the formation of high-resolution archives, enriched in U and poor in Thorium.

An important and often applied approach for carbonate samples of the last 350 kyr is the in-growth of \(^{230}\text{Th}\) (half-life of \(7.5 \times 10^4\) years) by the decay of primary incorporated \(^{234}\text{U}\) (half-life of \(2.45 \times 10^5\) years). The change of their isotopic ratio with time during closed-system conditions provides direct geochronologic information. In regard to precision, reliability, and robustness it is crucial to select and prepare pristine sample parts without secondary overprint and with lowest \(^{233}\text{Th}\)-content. The latter reflects the degree of initial Th incorporation which controls the influence of correction factors for initial element and isotope ratios on the final age calculation.

The state-of-the-art analytical procedure is based on multicolonator – inductively coupled plasma – mass spectrometry (MC-ICP-MS) combined with low-blank cleanlab preparation procedures. A direct comparison of sclerochronology and U-series analyses on smallest sample amounts of a continuous drill core from a tropical reef demonstrated the analytical potential of extending this technique to multi-static multiple ion counting (MIC)-ICP-MS (Fietzke et al., 2005). Another promising step toward high-resolution U-Th age data was presented by Potter et al. (2005) who used laser-ablation-MC-
ICP-MS. Unfortunately, this in situ approach is still hampered by the high uncertainties of low-counting statistics on samples younger than 10 ka. Meanwhile, recent efforts in conventional data precision and sample selection pushed the maximum limit of age determination with the U-Th method to the successful dating of extraordinarily well preserved 600-ka-old corals (Andersen et al., 2008).

Summary

Geochronology in geobiologic approaches offers detailed insights into the time scales of biological processes, changes in biochemical fluxes, and the identification of driving mechanisms. Still open challenges are the rather low sample throughput of conventional clean-lab preparation procedures, the necessary achievements in precision for in situ approaches, and the methodical extension toward reliable dating tools covering the last millions of years.

Bibliography


Cross-references

Anaerobic Oxidation of Methane with Sulfate
Chemolithotrophy
Cold Seep
Reefs

Introduction

Fungi are chemoorganotrophic (heterotrophic) organisms relying on organic carbon sources for energy and metabolism, and ubiquitous in aquatic and terrestrial environments. They are important as decomposers, animal and plant mutualistic symbionts and pathogens, and spoilage organisms of natural and manufactured materials (Gadd, 1993a, 1999, 2006; Burford et al., 2003a). They also have a role in the maintenance of soil structure due to their predominantly filamentous branching growth habit (each filament is called a hypha; plural = hyphae; all the hyphae constitute the mycelium) and frequent exopolymer production. Fungi have important roles in the biogeochemical cycling of the elements (e.g., C, N, P, S, metals), and this is linked with their ability to adopt a variety of growth, metabolic, and morphological strategies, their adaptive capabilities to environmental extremes and, their mutualistic associations with animals, plants, algae, and cyanobacteria (Gadd and Griffiths, 1980; Burford et al., 2003a; Gadd, 2004a, 2007a, b; Braissant et al., 2004; Gadd et al., 1984, 2005; Fomina et al., 2005a, 2006a, b) (Table 1). Fungal polymorphism and reproduction by huge numbers of spores underpins successful colonization of many different environments. Most fungi exhibit a filamentous growth habit which enables explorative or exploitative growth strategies, and many form linear organs of aggregated hyphae for protected translocation of nutrients (Jacobs et al., 2002, 2004; Boswell et al., 2002, 2003, 2006; Fomina et al., 2005a, b). Some fungi are polymorphic, occurring as both filamentous mycelium and unicellular yeasts or yeast-like cells, as in the black microcolonial fungi colonizing rocks (Gadd and Griffiths, 1980; Sterflinger, 2000; Gorbushina et al., 2002a, b, 2003). Some fungi are predominantly unicellular such as the yeasts, exemplified by Saccharomyces cerevisiae.

A broad appreciation of fungi as agents of biogeochemical change is lacking within geobiology, and apart from obvious connections with the carbon cycle, they are frequently neglected within broader geomicrobiological and geochemical contexts. While the geochemical activities
**Geomycology, Table 1** Summary of important roles and activities of fungi in geomycological processes. These may take place in aquatic and terrestrial ecosystems, as well as in artificial and man-made systems, their relative importance depending on the species and active biomass present and physicochemical factors. The terrestrial environment is the main site of fungal-mediated biogeochemical changes, especially in mineral soils and the plant root zone, decomposing vegetation, and on exposed rocks and mineral surfaces. There is rather a limited amount of knowledge on fungal geobiology in freshwater and marine systems, sediments, and the deep subsurface. In this table, fungal roles have been arbitrarily split into categories based on growth, organic and inorganic metabolism, physicochemical attributes, and symbiotic relationships. It should be noted that many if not all of these are linked, and almost all directly or indirectly depend on the mode of fungal growth (including symbiotic relationships) and accompanying chemoorganotrophic metabolism, in turn dependent on a utilisable C source for biosynthesis and energy, and other essential elements, such as N, O, P, S, and many metals, for structural and cellular components. Mineral dissolution and formation are detailed separately although these processes clearly depend on metabolic activity and growth form (adapted from Gadd, 2007a)

<table>
<thead>
<tr>
<th>Fungal attribute or activity</th>
<th>Geomycological consequences</th>
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<tr>
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<tr>
<td>Growth and mycelium development; hyphal differentiation; melanization</td>
<td>Stabilization of soil structure</td>
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<td>Penetration of rocks and minerals</td>
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<td>Biomechanical disruption of solid substrates, building stone, cement, plaster, concrete, etc.</td>
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<td></td>
<td>Plant, animal and microbial colonization, symbiosis and/or infection; mycorrhizas, lichens, pathogens</td>
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<td>Nutrient and water translocation</td>
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<td>Surfaces for bacterial growth, transport, and migration</td>
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<td>Mycelium acting as a reservoir of N and/or other elements</td>
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<td><strong>Metabolism</strong></td>
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<tr>
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<td>Organic matter decomposition and cycling of component elements, e.g., C, H, O, N, P, S, metals, metalloids, radionuclides</td>
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<td>Altered geochemistry of local environment, e.g., changes in redox, O₂, pH</td>
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<td>Production of inorganic and organic metabolites, e.g., H⁺, respiratory CO₂, organic acids, siderophores</td>
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<td>Transport, accumulation, incorporation of elements into macromolecules</td>
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<td>Translocation of water, N, P, Ca, Mg, K, etc. through mycelium and/or to plant hosts</td>
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<td>Biodeterioration of building stone, cement, plaster, concrete etc.</td>
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of bacteria and archaea receive considerable attention, especially in relation to carbon-limited and/or anaerobic environments, in aerobic terrestrial environments fungi are of great importance, especially when considering rock surfaces, soil, and the plant root–soil interface (Table 1). For example, symbiotic mycorrhizal fungi are associated with most plant species and are involved in major mineral transformations and redistributions of inorganic nutrients, e.g., essential metals and phosphate, as well as carbon flow (Fomina and Gadd, 2007) (Figure 1). Free-living fungi have major roles in the decomposition of plant and other organic materials, including xenobiotics, as well as in mineral solubilization. Lichens (a fungal growth form comprising a mutualistic symbiosis between an alga and/or cyanobacterium and a fungus) commonly inhabit exposed rock (and other) substrates, and play fundamental roles in early stages of rock colonization and mineral soil formation (Haas and Purvis, 2006). Fungi are also major biodeterioration agents of stone, wood, plaster, cement, and other building materials, and they are important components of the rock-inhabiting microbial communities with involvement in mineral dissolution and secondary mineral formation (Burford et al., 2003a, b; 2006; Fomina et al., 2005a, b; Gadd, 2007a; Gadd et al., 2005, 2007).

The earliest fossil filamentous fungal remains appear to be from the mid- to late Precambrian (1430–1542 Myr ago), and they were extremely diverse by Devonian times, when forms belonging to major groups and even some

<table>
<thead>
<tr>
<th>Fungal attribute or activity</th>
<th>Geomycological consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exopolymer production</td>
<td>Complexation of cations</td>
</tr>
<tr>
<td></td>
<td>Provision of hydrated matrix for mineral formation</td>
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<td></td>
<td>Enhanced adherence to substrate</td>
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<td>Clay mineral binding</td>
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<td>Stabilization of soil aggregates</td>
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<td>Matrix for bacterial growth</td>
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<td>Chemical interactions of exopolymers with mineral substrates</td>
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<tr>
<td>Symbiotic associations</td>
<td>Altered mobility and bioavailability of nutrient and inessential metals, N, P, S, etc.</td>
</tr>
<tr>
<td>Mycorrhizas</td>
<td>Altered C flow and transfer between plant, fungus, and rhizosphere organisms</td>
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<td></td>
<td>Altered plant productivity</td>
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<td></td>
<td>Mineral dissolution and metal and nutrient release from bound and mineral sources</td>
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<td></td>
<td>Altered biogeochemistry in soil-plant root region</td>
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<td>Altered microbial activity in plant root region</td>
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<td>Altered metal distributions between plant and fungus</td>
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<td></td>
<td>Water transport to and from the plant</td>
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<td>Lichens</td>
<td>Pioneer colonizers of rocks and minerals, and other surfaces</td>
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<td></td>
<td>Bioweathering</td>
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<td></td>
<td>Mineral dissolution and/or formation</td>
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<td></td>
<td>Metal accumulation by dry or wet deposition, particulate entrapment, metal sorption, transport, etc.</td>
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<tr>
<td></td>
<td>Enrichment of C, N, P, etc. in thallus and alteration of elemental concentrations and distribution in local microenvironment</td>
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<td></td>
<td>Early stages of mineral soil formation</td>
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<td></td>
<td>Development and stimulation of geochemically active microbial populations</td>
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<td></td>
<td>Mineral dissolution by metabolites including “lichen acids” Biomechanical disruption of substrate</td>
</tr>
<tr>
<td>Insects and invertebrates</td>
<td>Fungal populations in gut aid degradation of plant material</td>
</tr>
<tr>
<td></td>
<td>Invertebrates mechanically render plant residues more amenable for decomposition</td>
</tr>
<tr>
<td></td>
<td>Cultivation of fungal gardens by certain insects (organic matter decomposition and recycling)</td>
</tr>
<tr>
<td></td>
<td>Transfer of fungi between plant hosts by insect vectors (aiding infection and disease)</td>
</tr>
<tr>
<td>Pathogenic effects</td>
<td>Plant infection and colonization</td>
</tr>
<tr>
<td>Plant and animal pathogenicity</td>
<td>Animal predation (e.g., nematodes) and infection (e.g., insects, etc.)</td>
</tr>
<tr>
<td></td>
<td>Redistribution of elements and nutrients</td>
</tr>
<tr>
<td></td>
<td>Increased supply of organic material for decomposition</td>
</tr>
<tr>
<td></td>
<td>Stimulation of other geochemically active microbial populations</td>
</tr>
</tbody>
</table>
Geomycology, Figure 1 Simple model of fungal action on naturally occurring and/or anthropogenically derived organic and inorganic substrates. (1) organic and inorganic transformations mediated by enzymes and metabolites, e.g., H⁺, CO₂, and organic acids, and physicochemical changes occurring as a result of metabolism; (2) uptake, metabolism, or degradation of organic substrates; (3) uptake, accumulation, sorption, metabolism, or degradation of inorganic substrates; (4) biosynthesis and production of organic metabolites, exopolymers, macromolecules, and biomass; (5) production of inorganic metabolites, secondary minerals, and transformed (organo)metalloid; and (6) chemical interactions between organic and inorganic substances, e.g., complexation and chelation. (Adapted from Gadd, 2004a, 2007a.)

genera present today were found (Taylor and Osborn, 1996; Taylor et al., 1994, 1997, 2005; Heckman et al., 2001; Butterfield, 2005). Since that time fungi have been ubiquitous components of the microbial communities of any terrestrial environment (Hawksworth, 2001), including such hostile locations as the arctic and Antarctic, hot deserts, and metal-rich and hypersaline soils (Burford et al., 2003a). One of the most successful mechanisms that enables fungi to survive in extreme subaerial environments is by the formation of mutualistic symbioses with algae and/or cyanobacteria as lichens, where the phototrophs provide a source of carbon and are protected to some degree from light and other external factors (Gorbushina et al., 1993; Sterflinger, 2000). The majority of terrestrial fungi inhabit soil environments which are perhaps more hospitable than bare rock surfaces. Fungal communities in soil include free-living and symbiotic fungi, as well as plant and animal pathogens, and unicellular yeasts.

Fungi encounter metals as components of the natural environment as well as those introduced or redistributed by human activities. Like other organisms, fungi possess a variety of properties that can influence interactions with metals, while “normal” growth and metabolism is dependent on metal and metal–mineral interactions to satisfy essential metal and associated nutrient requirements (Gadd and Griffiths, 1978). At potentially toxic metal concentrations, a variety of resistance mechanisms may be expressed: sensitive organisms may be vulnerable and population changes can result. Metal toxicity can be influenced by the physicochemical attributes of the environment, while fungi possess a variety of properties that can ensure survival. It seems fungi can be isolated from any habitats polluted by toxic metals.

This article will outline important fungal roles and functions in rock, mineral, metal, and soil transformations, and will emphasize the importance of fungi as agents of geochemical change. It will also include fungal-mediated metal transformations, the role of fungi in the geochemistry of metal cycling, and the applied significance of these processes in environmental biotechnology. Such roles can all be included under the term “geomycology,” which can be simply defined as “the scientific study of the role of fungi in processes of fundamental importance to geology.” This includes past, current, and potential future fungal activities and such roles include the alteration and weathering of rocks and minerals, soil formation, the transformation and accumulation of metals, decomposition, and nutrient cycling. In this article, the decomposition of organic substances is included under the heading of “geomycology” since this process results in major geochemical cycling of elements in the biosphere, and, as carbon and energy sources, the metabolism of organic compounds underpins all fungal activities and interactions with environmental components. “Geomycology” can be considered to be a subset of “geomicrobiology,” namely the role of microorganisms and microbial processes in geological and geochemical processes, and is itself a subset of “microbiology,” the scientific study of microorganisms. “Geomycology” can also be considered to be a subset of “mycology” which is the scientific study of fungi. Such definitions are clearly imprecise and important topics dealt with under “geomycology” and “geomicrobiology” headings are also relevant to and also important to such research areas as geochemistry, mineralogy, hydrometallurgy, and biogeochemistry, for example. Some geomycological activity involves enzymatic oxidation or reduction of inorganic substances, although prokaryotes are more usually associated with such reactions, and these may contribute to mineral formation, transformation, and degradation. Other activities involve the biosynthesis of inorganic and organic compounds and metabolites, and the degradation of natural and anthropogenically produced carbon compounds in which both prokaryotes and eukaryotes participate. Some geomicrobial activity involves reactions where inorganic or organic products of metabolism serve as chemical reagents in reactions such as metal precipitation, metal solubilization, and mineral weathering and dissolution. Some geomicrobial activity depends on physical effects exerted on the environment by growing microorganisms which can depend on growth form and metabolism, e.g., biomechanical pressure exerted by growing fungi in rock fissures, and pH reduction due to acid excretion. Some processes may be the result of a combination of several of these general activities. While the majority of processes discussed pertain to the terrestrial environment, it should be noted that the same processes may also occur in aquatic...
environments and sediments, though their significance may be different as well as influenced strongly by spatial and environmental factors. It should also be noted that detailed discussion of fungal roles and the mechanisms involved in the cycling and transformations of elements like N, P, and S is precluded here because of space limitations.

**Organic matter degradation and biogeochemical cycling**

The ability of fungi to utilize a wide spectrum of organic compounds is well-known. These range from simple compounds such as sugars, organic acids, and amino acids to more complex molecules which are broken down by extracellular enzymes before cellular entry. These latter compounds include natural substances like cellulose, pectin, lignin, lignocellulose, chitin and starch, but also anthropogenic products such as hydrocarbons, pesticides, and other xenobiotics. Utilization of these substances results in redistribution of component elements, primarily C, H, and O, but also N, P, S, and others (see later).

Some fungi have remarkable degradative properties, and lignin-degrading white rot fungi like *Phanerochaete chrysosporium* can degrade aromatic hydrocarbons, chlorinated organics, polychlorinated biphenyls, nitrogen-containing aromatics, and many other pesticides, dyes, and xenobiotics. Such activities are relevant to bioremediation where ligninolytic fungi have been used to treat soil contaminated with, e.g., pentachlorophenol (PCP) and polycyclic aromatic hydrocarbons (PAHs), the latter being constituents of creosote. Treatment generally involves inoculation of contaminated soil, nutrient addition, irradiation and aeration, and maintenance by land-farming procedures (Singleton, 2001). Xenobiotic-transforming fungi may need additional utilizable carbon sources because they cannot utilize these complex substrates. Inexpensive utilizable lignocellulosic wastes, such as corn cobs, straw, and sawdust are therefore supplied as nutrients. Wood-rotting and other fungi are also receiving attention for the bleaching of dyes and industrial effluents, and the biotreatment of agricultural wastes, such as forestry, pulp and paper by-products, sugarcane bagasse, coffee pulp, sugar beet pulp, apple and tomato pulp, and cyanide.

Polycyclic aromatic hydrocarbons (PAHs) enter the environment via fossil–fuel combustion, vehicle exhaust emissions, gas and coal tar manufacture, wood-preservation processes, and waste incineration (Harvey, 1997; Cerniglia and Sutherland, 2001, 2006). Aerobic biodegradation of PAHs by soil microorganisms depends on monoxygenase, peroxidase, and dioxygenase pathways; the first and third of these pathways are employed by bacteria while the first and second are found in fungi. Many fungi can metabolize PAHs but since fungi cannot generally use PAHs as their sole carbon and energy source, they must be supplemented with other carbon sources to allow co-metabolism (Cerniglia and Sutherland, 2001, 2006; Sutherland, 2004). The transformation of PAHs by ligninolytic wood-decaying fungi involves several enzymes. Those produced by white-rot fungi include lignin peroxidase, manganese peroxidase, laccase, cytochrome P450, and epoxide hydrolase. Ligninolytic fungi metabolize PAHs via reactions involving reactive oxygen species to phenols and quinones and these may be further degraded by ring-fission enzymes. Several wood-decaying fungi (e.g., *Bjerkandera*, *Coriolopsis*, *Irpex*, *Phanerochaete*, *Pleurotus*, and *Trametes* spp.), have been investigated for bioremediation of PAH-contaminated soils. Non-ligninolytic fungi, including *Cunninghamella*, *Mucor*, *Fusarium*, and *Penicillium* spp., have also been considered for PAH bioremediation. Complete biodegradation may require the presence of mixed bacterial and fungal communities.

Fungi are also important in the degradation of naturally occurring complex molecules in the soil, an environment where the filamentous hyphal mode of growth provides several advantages, and also in aquatic habitats. Since 95% of plant tissue is composed of carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulphur, the decomposition activities of fungi are important in redistribution of these elements between organisms and environmental compartments (Table 2). As well as C, H, O, N, P, and S, another 15 elements are typically found in living plant tissues — K, Ca, Mg, B, Cl, Fe, Mn, Zn, Cu, Mo, Ni, Co, Se, Na, Si. However, all 90 or so naturally occurring elements may be found in plants, mostly at low concentrations, although this may be highly dependent on environmental conditions. These include Au, As, Hg, Pb, and U, and there are some plants that accumulate relatively high concentrations of metals Ni and Cd. Animals likewise contain a spectrum of elements in varying amounts. For example, the human body is mostly water, and 99% of the mass comprises oxygen, carbon, hydrogen, nitrogen, calcium, and phosphorus. However, many other elements are present in lower amounts including those in substances taken up as contaminants in food, air, and water. A similar situation occurs throughout the plant, animal, and microbial worlds. Therefore, any decomposition, degradative and pathogenic activities of fungi are linked to the cycling of all these constituent elements, some of which may be radionuclides accumulated from environmental sources (Table 2). This simple perspective on organic matter decomposition illustrates the global significance of fungi in geochemical element cycling and their involvement in almost all elemental cycles. Space considerations preclude detailed discussion of the mechanisms involved in fungal transformations and cycling of elements like N, P, and S and their compounds.

Organometals (compounds with at least one metal–carbon bond) can be attacked by fungi. Degradation of organometallic (and organometalloid) compounds, which are still widely used in agriculture and industry, can be carried out by fungi, either by direct biotic action (enzymes), or by the fungi facilitating abiotic degradation, for instance by alteration of pH and excretion of metabolites.
Geomycology, Table 2 Fungal roles in key biogeochemical cycles of the elements. Some of the major or representative roles of fungi in elemental cycles are indicated without reference to their global significance. Major elemental movements relate to decomposition activities reliant on metabolism and the hyphal mode of growth. Note that only representative elements are shown here: virtually all elements in the Periodic Table (including actinides, lanthanides, and radionuclides) can be accumulated within or associated with fungal biomass depending on the environment. Fungi possess transport systems for essential metals; inessential metal species can also be taken up. Fungi are also capable of mediating metal bioprecipitation by, e.g., metabolite production, changing the physicochemical microenvironmental conditions around the biomass, and indirect release of metal-precipitating substances from other activities, e.g., phosphate. Fungal walls and exopolymers can sorb, bind, or entrap many substances. Redox transformations are also widespread in fungal metabolism. While most roles occur in the terrestrial aerobic environment, similar transformations may occur in aquatic environments and in sediments where fungal populations occur.

<table>
<thead>
<tr>
<th>Element(s)</th>
<th>Fungal roles in elemental cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Decomposition of organic substances; respiration (CO₂ production); synthesis of polymers, organic metabolites, etc.; humus formation; CN⁻ production; carbonate formation; oxalate formation; oxalate-carbonate cycle; dissolution of carbonates</td>
</tr>
<tr>
<td>H, O</td>
<td>Uptake, assimilation, degradation, and mobilization of organic and inorganic compounds; water uptake, transport, translocation, and conduction; respiration (CO₂); organic and inorganic metabolite excretion</td>
</tr>
<tr>
<td>N</td>
<td>Decomposition of nitrogenous compounds; assimilation and transformations of organic and inorganic N compounds; fungal nitrification and denitrification; biosynthesis of N-containing biopolymers, e.g., chitin; production of N-containing metabolites and gases, e.g., N₂O; ammonia fermentation under anaerobic conditions; mycorrhizal N transfer to plants</td>
</tr>
<tr>
<td>P</td>
<td>Dissolution of inorganic phosphates and P-containing minerals in soils and rocks; decomposition of organic P-containing compounds; formation of insoluble P, e.g., polyphosphate, secondary phosphate minerals; release of organically bound P by phosphatases; assimilation and transformation of inorganic P species; oxidation of reduced forms of phosphate, e.g., phosphate; transformations of soil organic P; production of diphosphates and phosphonates; mycorrhizal P transfer to plants</td>
</tr>
<tr>
<td>S</td>
<td>Degradation of organic S-containing compounds; organic-inorganic S transformations; uptake and assimilation of organic and inorganic S compounds; SO₄²⁻ reduction and assimilation; oxidation of reduced S compounds, e.g., S(0), thiosulfate, tetrathionate; sulfide production; oxidation of H₂S to S(0); reduction of S(0) to H₂S; dissolution of S-containing minerals in soils and rocks, e.g., sulfides, sulfates</td>
</tr>
<tr>
<td>Fe</td>
<td>Bioweathering of iron-containing minerals in rocks and soils; iron solubilization by siderophores, organic acids, metabolites, etc.; Fe(III) reduction to Fe(II)</td>
</tr>
<tr>
<td>Mn</td>
<td>Mn(II) oxidation and immobilization as Mn oxides; Mn(IV) reduction; indirect Mn(IV)O₂ reduction by metabolites, e.g., oxalate; bioaccumulation of Mn oxides to surfaces, exopolymers; contribution to desert varnish formation; biosorption; accumulation; intracellular precipitation</td>
</tr>
<tr>
<td>Cr</td>
<td>Cr(III) reduction to Cr(VI); accumulation of Cr oxyanions</td>
</tr>
<tr>
<td>Mg, Ca, Co, Ni, Zn, Cd, Sr</td>
<td>Bioweathering of minerals in rocks and soil; biosorption; uptake and accumulation; bioprecipitation, e.g., oxalates, sulfides, phosphates, carbonate</td>
</tr>
<tr>
<td>Ag</td>
<td>Reduction of Ag(I) to Ag(0); biosorption; accumulation</td>
</tr>
<tr>
<td>K, Na, Cs</td>
<td>Uptake and accumulation; translocation through mycelium; concentration in fruit bodies; mobilization from minerals in soil</td>
</tr>
<tr>
<td>Cu</td>
<td>Mobilization from Cu-containing minerals in rocks and soils; CuS formation; biosorption; uptake and accumulation; bioprecipitation, e.g., oxalates</td>
</tr>
<tr>
<td>Se</td>
<td>Reductive transformation of Se oxyanions, e.g., Se(VI) to Se(IV) to Se(0); Biomethylation of Se compounds; assimilation of organic and inorganic Se compounds</td>
</tr>
<tr>
<td>Te</td>
<td>Reductive transformation of Te oxanions, e.g., Te(VI) to Te(IV) to Te(0); Biomethylation of Te compounds; assimilation of organic and inorganic Te compounds</td>
</tr>
<tr>
<td>Pb</td>
<td>Biosorption; lead oxalate formation</td>
</tr>
<tr>
<td>Cl, Br, I</td>
<td>Methylation</td>
</tr>
<tr>
<td>Sn</td>
<td>Organotin degradation; sorption and accumulation of soluble Sn species</td>
</tr>
<tr>
<td>Au</td>
<td>Reduction of soluble Au species to Au(0)</td>
</tr>
<tr>
<td>As</td>
<td>Methylation of arsenic species, e.g., arsenite to trimethylarsonic; reduction of As oxanions, e.g., arenate to arsenite; oxidation of As oxanions, e.g., arsenite to arsenate</td>
</tr>
<tr>
<td>Hg</td>
<td>Hg methylation; reduction of Hg(II) to Hg(0); Hg volatilization as Hg(0); degradation of organomercurials; biosorption; accumulation</td>
</tr>
<tr>
<td>Al</td>
<td>Al mobilization from Al-containing minerals in soils and rocks; aluminosilicate dissolution; Al precipitation as oxides (early stage of bauxitization); biosorption</td>
</tr>
<tr>
<td>Si</td>
<td>Uptake of soluble Si species; organic Si complex formation from inorganic silicates; organic siloxane formation; silica, silicate, and aluminosilicate degradation; Si mobilization through production of chelators, acids, bases, exopolymers</td>
</tr>
<tr>
<td>U, Th</td>
<td>Biosorption; deposition of hydrolysis products; intracellular precipitation</td>
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</table>
Biomass is strongly influenced by fungal activity (Gadd, 1993b). Organotin compounds, such as tributyltin oxide and tributyltin naphthenate, may be degraded to mono- and dibutyltin by fungal action, inorganic Sn(II) being the ultimate product (Gadd, 2000a). Organomercury compounds may be detoxified by fungal organomercury lyase, the resultant Hg(II) being subsequently reduced to less toxic, diffusible, and volatile Hg(0) by mercuric reductase (Gadd, 1993b). Degradation of persistent carbon sources, such as charcoal and black shale, can be accelerated by fungal activity, which in turn may accelerate the release of toxic metals as organic metal complexes (Wengel et al., 2006).

Transformations of rocks and minerals
Minerals are naturally occurring inorganic solids of definite chemical composition with an ordered internal structure; rocks can be considered to be any solid mass of mineral or mineral-like material. Silicates are the most common minerals with non-silicates constituting <10% of the Earth’s crust, the most common being carbonates, oxides, sulfides, and phosphates. Rocks and minerals are a vast reservoir of elements, many essential for life, and such elements must be released in forms that may be assimilated by the biota. These include essential metals as well as nutrient elements like S and P (Gadd, 2007a; Gadd et al., 2005, 2007).

Microbial processes influenced by minerals
Many important microbial processes can be influenced by minerals including energy generation, nutrient acquisition, cell adhesion, and biofilm formation (Hochella, 2002), and such effects are also likely to be found within fungal populations. Essential nutrients may be acquired from mineral surfaces and this concentrates these substances above surrounding environmental levels (e.g., C, N, P, Fe, essential metals, various organic compounds) (Vaughan et al., 2002). Environmental contaminants may also be sorbed to mineral surfaces and these can be displaced by microbial activity (Kraemer et al., 1999). Potentially toxic metals released from minerals as a result of physicochemical and biological processes, may also affect microbial communities (Gadd, 2005). Such properties of mineral surfaces as microtopography, surface composition, surface charge and hydrophobicity play an important role in microbial attachment and detachment, and are therefore critical for colonization and biofilm formation, and the ecology of microbial populations, including fungi, associated with mineral substrates.

Bioweathering by fungi
Bioweathering can be defined as the erosion, decay, and decomposition of rocks and minerals mediated by living organisms. Fungi are well-suited as biological weathering agents since they can be highly resistant to extreme environmental conditions such as metal toxicity, UV radiation, and desiccation; they can adopt a variety of growth, metabolic, and morphological strategies; they can exude protons and metal-complexing metabolites, and form mutualistic associations with animals, plants, algae, and cyanobacteria (Sterflinger, 2000; Burford et al., 2003a, b; Gadd, 2007a). Most fungi exhibit a filamentous growth habit, while some are polymorphic occurring as filamentous mycelium and unicellular yeasts or yeast-like cells, e.g., black microcolonial rock-dwelling fungi (Gorbushina, 2007). The ability of fungi to translocate water, nutrients, and organelles within the mycelial network is another important feature for exploiting heterogeneous environments (Boswell et al., 2002, 2003, 2006).

Rock surfaces may be an inhospitable habitat due to moisture deficit and nutrient limitation although many fungi can tolerate extremes in such factors as light, salinity, pH, and water potential (Gorbushina et al., 1993; Sterflinger, 2000; Verrecchia, 2000; Burford et al., 2003a, b). Many fungi can scavenge nutrients from the atmosphere and rainwater. In the subaerial rock environment, fungi can also use organic and inorganic residues on surfaces or within cracks and fissures, waste products of other microorganisms, decaying plants and insects, dust particles, aerosols, and animal feces as nutrient sources (Sterflinger, 2000). Fungi may receive protection from the presence of melanin pigments and mycosporines in their cell walls, and by the production of mucilaginous exopolymeric slime that may entrap clay particles providing extra protection (Gadd, 1993a; Gorbushina, 2007). Fungi appear to be ubiquitous components of the microbiota of all rocks, building stone, and concrete, and have been reported from a wide range of rock types, e.g., limestone, marble, granite, sandstone, basalt, gneiss, dolerite and quartz, even from the most extreme environments (Burford et al., 2003a, b; Gorbushina, 2007).

Elements found in soil largely reflect the composition of the Earth’s crust, though some modification occurs by weathering, biogenic and anthropogenic activities: chemical changes include mineral dissolution while biological activity causes enrichment of C, N, and S. Elements and minerals that remain can reform as secondary minerals. In soil, fungus–mineral interactions are an integral component of environmental cycling (Figure 2). Mycorrhizal fungi are particularly important in mineral weathering and dissolution of insoluble metal compounds. Fungi are also important components of lithobiotic biofilm communities at mineral–microbe interfaces where they interact with the substrate both geophysically and geochemically: this can result in the formation of patinas, films, varnishes, crusts, and stromatolites (Gadd, 2007a; Gorbushina, 2007).

Biomechanical deterioration of rocks can occur through hyphal penetration and burrowing into decaying material and along crystal planes in, e.g., calcite and dolomitic rocks (Sterflinger, 2000; Gadd, 2007a). Cleavage penetration can also occur with lichens (Banfield et al., 1999). Spatial exploration of the environment to locate and exploit new substrates is facilitated by a range of sensory responses that determine the direction of hyphal growth. For example, thigmotropism (or contact guidance) is a well-known property of fungi that grow on and within solid substrates with the direction of fungal growth being influenced by grooves,
ridges, and pores (Bowen et al., 2007a, b). However, biochemical actions are believed to be more important processes than mechanical degradation. Biochemical weathering of rocks and minerals can occur through excretion of, e.g., H+, organic acids and other metabolites. This can result in changes in the mineral microtopography through pitting and etching of surfaces to complete dissolution of mineral grains (Ehrlich, 1998; Gharieb et al., 1998; Kumar and Kumar, 1999; Adeyemi and Gadd, 2005). Fungi generally acidify their microenvironment via a number of mechanisms, which include the excretion of protons and organic acids, while respiratory CO₂ can result in carbonic acid formation. In addition, fungi excrete a variety of other metal-complexing metabolites (e.g., siderophores, carboxylic acids, amino acids, and phenolic compounds) (Burgstaller and Schinner, 1993). The weathering of sandstone monuments by fungi has been attributed to the production of, e.g., acetic, oxalic, citric, formic, fumaric, glyoxylic, gluconic, succinic, and tartaric acids.

Concrete biodeterioration in radioactive waste disposal

Cement and concrete are used as barriers in all kinds of nuclear waste repositories. Despite the theoretical service life of concrete reaching up to one million years, biocorrosion is an important factor to take into account. All types of building and ceramic materials, concrete, and cement can be deteriorated by microorganisms and in some environments, fungi dominate the microbiota and play an important role in biodeterioration of concrete (Gu et al., 1998; Nica et al., 2000; Zhdanova et al., 2000). Fungal attack on concrete can be strongly and mildly aggressive caused by protons and organic acids and production of hydrophilic slimes leading to biochemical and biophysical/biomechanical deterioration (Fomina et al., 2007a). Fungal degradation may proceed more rapidly than bacterial degradation with complexolysis suggested as the main mechanism of calcium mobilization. Microfungi from the genera Aspergillus, Alternaria, and Cladosporium were able to colonize samples of the concrete used as the radioactive waste barrier in the Chernobyl reactor and leached iron, aluminium, silicon, and calcium, and re-precipitated silicon and calcium oxalate in their microenvironment (Fomina et al., 2007a). Fungi are also important members of the microbial communities (including lichens) that colonize and deteriorate “normal” concrete and cement used in buildings and other structures.
**Geomycology, Table 3** Some examples of biomineralization of fungal hyphae and lichen thalli with secondary minerals. The hydration state of some minerals is unclear and only stated when this is specifically identified. The list is not exhaustive and many other mycogenic minerals are possible, as well as many other fungal species capable of mediating their formation (taken from Gadd, 2007a; Burford et al., 2003a, b, and also adapted from a number of sources including Grote and Krumbein, 1992; de la Torre and Gomez-Alarcon, 1994; Easton, 1997; Verrecchia, 2000; Haas and Purvis, 2006; Burford et al., 2006)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Fungal hyphae</th>
<th>Lichen thalli</th>
<th>Organism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birnessite ((Na, Ca, K) Mn₇O₁₄·3H₂O)</td>
<td>Fungi on siderite boulder and Natraqualf soil</td>
<td>Alternaria spp., Cladosporium spp., Beauveria caledonica</td>
<td></td>
</tr>
<tr>
<td>Cadmium oxide (CdC₂O₄)</td>
<td>Fungi cultured with, e.g., cadmium phosphate, or other cadmium compounds and minerals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcite (CaCO₃)</td>
<td>Fungi on stalactites, Quaternary eolianites and calcrites; fungi grown in limestone cement microcosms and laboratory media containing insoluble calcium compounds, e.g., calcite, or other Ca-containing compounds and minerals</td>
<td>Caloplaca auranti, Doratomyces sp., Penicillium coryliphilum, Penicillium simplicissimum, Verrucaria spp.</td>
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<tr>
<td>Cobalt oxide (CoC₂O₄)</td>
<td>Fungi cultured with Co compounds</td>
<td></td>
<td>A. niger, Alternaria alternata, Cladosporium cladosporioides, Lichenothelia spp., Penicillium frequentans, Penicillium steckii, Phoma glomerata, Pertusaria corallina, Stereocaulon vulcani</td>
</tr>
<tr>
<td>Desert Varnish (MnO and FeO)</td>
<td>Fungal action on siderite and rhodochrosite in desert regions and sandstone limestone and granite monuments</td>
<td></td>
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<tr>
<td>Ferricydrite (Fe₂H₂O₅ · H₂O or 5Fe₂O₃ · 9H₂O)</td>
<td>Fungi cultured with hydromagnesite</td>
<td>Lichen on recent lava flow, on olovine of basalt, gabbro and augite</td>
<td>Lecanoraatra, Penicillium simplicissimum, Parmelia conspersa, Parmelia tilacea</td>
</tr>
</tbody>
</table>
| Glushinskite (MgC₂O₄ · 2H₂O) | Fungi cultured with hydromagnesite | Lichen on metamorphic rocks, feldspars, granite, and gneiss | Lasallia spp., Mucor spp., Parmelia spp., Penicillium spp., Rhizocarpon spp., Rhizopus spp., Acaeospora smargdula, 
Aspicilia alpina, Lecidea lactea, Stereocaulon vesuvianum | |
| Goethite (FeO(OH)) | | | |
| Halloysite (Al₂Si₂O₅(OH)₄ · 2H₂O) | Fungi cultured in laboratory media containing Mn compounds | Lichen on Mn ore | Aspergillus niger, Pertusaria corallina, Lasallia spp., Mucor spp., Parmelia spp., Penicillium spp., Rhizocarpon spp., Rhizopus spp., Acaeospora smargdula, Aspicilia alpina, Lecidea lactea, Stereocaulon vesuvianum | |
| Humboldtine (FeC₂O₄ · 2H₂O) | | | |
| Hydrocerussite (Pb₂(CO₃)₂(OH)₂) | Fungi cultured with hydromagnesite | | Penicillium simplicissimum | |
| Hydromagnesite (Mg₅(CO₃)₄(OH)₂ · 4H₂O) | Fungi cultured with pyromorphite, or in laboratory media containing Pb compounds | Lichen on Mn ore | Aspergillus niger, Beauveria caledonica | |
| Lead oxalate, lead oxalate dehydrate (PbC₂O₄, PbC₂O₄·2H₂O) | Fungi cultured in laboratory media containing Mn compounds | Lichen on Mn ore | Aspergillus niger, Pertusaria corallina, Lasallia spp., Mucor spp., Parmelia spp., Penicillium spp., Rhizocarpon spp., Rhizopus spp., Acaeospora smargdula, Aspicilia alpina, Lecidea lactea, Stereocaulon vesuvianum | |
| Mno–oxalate (MnC₂O₄ · 2H₂O) | | | |
| Montmorillonite (X₀.₃₃Al₂Si₂O₁₀(OH)₂ · nH₂O) | Fungi cultured in laboratory media containing Mn compounds | Action of lichens on cave deposits and waters | Lasallia spp., Mucor spp., Parmelia spp., Penicillium spp., Rhizocarpon spp., Rhizopus spp., Acaeospora smargdula, Aspicilia alpina, Lecidea lactea, Stereocaulon vesuvianum | |
| Moolooite (CuC₂O₄ · nH₂O (n < 1)) | Fungi cultured with, e.g., copper phosphate, or other Cu-containing compounds and minerals | Lichens on cupriferous rocks | A. niger, Beauveria caledonica, Lecidea inops, Lecidea lactea, Rhizopogon rubescens, Serpula himantioides | |
Formation of secondary mycogenic minerals

Formation of secondary minerals by fungi can occur through metabolism-independent and -dependent processes (Gadd, 2007a) (Table 3). Precipitation, nucleation, and deposition of crystalline material on and within cell walls are influenced by factors such as pH and wall composition. This process may be important in soil as the precipitation of carbonates, phosphates, and hydroxides increases soil aggregation. Cationic species of Si(IV), Fe(III), Al(III), and Ca(II) that may be released through dissolution mechanisms, stimulate precipitation of compounds that may bond soil particles. Hyphae and any

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Fungal hyphae</th>
<th>Lichen thalli</th>
<th>Organism(s)</th>
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<tbody>
<tr>
<td>Strontium oxalate hydrate</td>
<td>Fungi cultured with strontianite (SrCO₃), or other</td>
<td></td>
<td>Penicillium simplicissimum</td>
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<tr>
<td>(SrC₂O₄ · H₂O)</td>
<td>Sr-containing compounds and minerals</td>
<td></td>
<td>Pseudallescheria boydii</td>
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<tr>
<td>Todorokite (Mn,Ca,Mg) Mn₃O₇·H₂O</td>
<td>Fungi in cave deposits and waters</td>
<td></td>
<td>Serpula himantioides</td>
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<tr>
<td>Urampithle (NH₄(UO₂)</td>
<td>Fungi cultured with uranium oxides,</td>
<td></td>
<td>Macer spp.</td>
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<tr>
<td>(PO₄)·3H₂O and Chernikovite</td>
<td>metallic depleted uranium, or other U-containing</td>
<td></td>
<td>Penicillium ssp.</td>
</tr>
<tr>
<td>Weddellite (CaC₂O₄·2H₂O)</td>
<td>compounds and minerals</td>
<td></td>
<td>Rhizopus ssp.</td>
</tr>
<tr>
<td>Whewellite (CaC₂O₄·2H₂O)</td>
<td>In leaf litter and soils; fungi grown on</td>
<td>On serpentinite, cupriferous rocks, andesite and</td>
<td>Acarospora rugulosa</td>
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<td></td>
<td>limestone cement microcosms, and laboratory media</td>
<td>volcaniclastite</td>
<td>Hysterangium crassum</td>
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<td></td>
<td>containing insoluble calcium compounds, e.g.,</td>
<td></td>
<td>Lecanora atra</td>
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<tr>
<td></td>
<td>calcite, or other Ca-containing compounds and</td>
<td></td>
<td>Lecanora rupicola</td>
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<tr>
<td></td>
<td>minerals</td>
<td></td>
<td>Lecidea inops</td>
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<td>Lecidea lactea</td>
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<td></td>
<td>Ochrolechia parella</td>
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<td>Penicillium corylphilum</td>
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<td>Penicillium simplicissimum</td>
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<td>Pseudallescheria boydii</td>
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<td>Serpula himantioides</td>
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<td>In Nari limecrusts, Quaternary calcretes,</td>
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<td>Acarospora rugulosa</td>
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<td>forest leaf litter and soils; fungi grown on</td>
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<td>Acarospora smargdula</td>
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<td>limestone cement microcosms, and laboratory media</td>
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<td>Aspicilia alpina</td>
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<td>containing insoluble calcium compounds, e.g.,</td>
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<td>Aspicilia calcarea</td>
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<td>calcite, or other Ca-containing compounds and</td>
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<td>Aspicilia radiosia</td>
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<td>minerals</td>
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<td>Caloplaca flavescens</td>
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<td>Doratomyces sp.</td>
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<td>Hypogymnia physodes</td>
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<td>Lecanora atra</td>
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<td>Lecanora rupicola</td>
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<td>Lecidea inops</td>
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<td>Ochrolechia parella</td>
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<td>Parmelia conspersa</td>
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<td>Parmelia subrubdata</td>
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<td>Penicillium corylphilum</td>
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<td>Penicillium simplicissimum</td>
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<td>Persatia carallina</td>
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<td>Pseudallescheria boydii</td>
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<td>Serpula himantioides</td>
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<td>Xanthoria octaneoid</td>
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<td>Aspergillus niger</td>
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<td>Beauveria caledonica</td>
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<td>Rhizopogon rubescens</td>
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<td></td>
<td>Fungi exposed to, e.g., zinc oxide, zinc</td>
<td></td>
<td>Suillus collimitus</td>
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<td>phosphate</td>
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associated exopolymeric material can also enmesh soil particles, and also release organic metabolites that enhance aggregate stability (Bronick and Lal, 2005).

**Carbonates**

Microbial carbonate precipitation coupled with silicate weathering could provide an important sink for CO$_2$ in terrestrial environments. In limestone, fungi and lichens are considered to be important agents of mineral deterioration. In contrast, many near-surface limestones (calcretes), calcite, and petrocalcic horizons in soils are secondarily cemented with calcite (CaCO$_3$) and whewellite (calcium oxalate monohydrate, CaC$_2$O$_4$·H$_2$O). The presence of fungal filaments mineralized with calcite (CaCO$_3$), together with whewellite (calcium oxalate monohydrate, CaC$_2$O$_4$·H$_2$O), has been reported in limestone and calcareous soils from a range of localities (Verrecchia, 2000). Calcium oxalate can also be degraded to calcium carbonate, and this may again cement preexisting limestones (Verrecchia et al., 2006). During the decomposition of fungal hyphae, calcite crystals can act as sites of further secondary calcite precipitation. Calcite will also readily nucleate on chitin, the major component of fungal cell walls. Other experimental work has demonstrated fungal precipitation of secondary calcite, whewellite, and glushkinskite (MgC$_2$O$_4$·2H$_2$O) (Figure 3) (Burford et al., 2003b, 2006; Gadd, 2007a).

**Oxalates**

Many fungi can produce metal oxalates by interaction with a variety of different metals and metal-bearing minerals (Ca, Cd, Co, Cu, Mg, Mn, Sr, Zn, Ni, and Pb) (Figure 4) (Sayer and Gadd, 1997; Gadd, 2007a) (Table 3). Calcium oxalate dihydrate (weddelite) and the more stable calcium oxalate monohydrate (whewellite) are the most common forms of oxalate associated with fungi: biotic fungal calcium oxalate can exhibit a variety of crystalline forms (tetragonal, bipyramidal, plate-like, rhombohedral or needles) (Arnott, 1995). Precipitation of calcium oxalate can act as a reservoir for calcium and also influences phosphate availability. The formation of toxic metal oxalates may also provide a mechanism enabling fungi to tolerate high concentrations of toxic metals (Gadd, 1993a; Jarosz-Wilkolazka and Gadd, 2003).

**Reductive and oxidative precipitation**

Reduced forms of metals and metalloids (e.g., elemental silver, gold, selenium, tellurium) can be precipitated by many fungi (Kierans et al., 1991; Gharieb et al., 1995, 1999; Gadd, 1993b). The reductive ability of fungi is manifest by black coloration of fungal colonies precipitating elemental Ag or Te, or a red coloration for those precipitating elemental Se. An oxidized metal layer (patina) a few millimetres thick found on rocks and in soils of arid and semi-arid regions, called desert varnish, is also believed to be of microbial origin with some proposed fungal involvement. Fungi can oxidize Mn(II) and Fe(II) in metal-bearing minerals such as siderite (FeCO$_3$) and rhodochrosite (MnCO$_3$) and precipitate them as oxides (Grote and Krumbein, 1992).

**Other mycogenic minerals**

Combinations of biotic and abiotic factors can lead to deposition of a variety of other secondary minerals associated with fungal biomass, e.g., birnessite, MnO and FeO, ferrhydrite, iron gluconate, calcium formate, forsterite, goethite, halloysite, hydrocerussite, todorokite, moolooite, montmorillonite, and uranium phosphates (Figure 5) (Gadd, 2007a) (Table 3). Such biomineralization immobilizes metals and therefore limits metal bioavailability.

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**Geomycology, Figure 3** Glushinskite (MgC$_2$O$_4$·2H$_2$O) and hydromagnesite (Mg$_5$(CO$_3$)$_4$(OH)$_2$·4H$_2$O) deposition around hyphae of Penicillium simplicissimum cultured on media containing hydromagnesite. (Burford E. P., and Gadd G. M., 2004 unpublished data.)

**Geomycology, Figure 4** Moolooite (copper oxalate, CuC$_2$O$_4$nH$_2$O (n < 1)) formation around hyphae of Beauveria caledonica grown on media containing copper phosphate. The moolooite has been fractured showing the extensive precipitation around hyphae, and the resulting holes or tunnels. (Fomina M., and Gadd G. M., 2005 unpublished data; see also Fomina et al., 2005a.)
Fungal–clay mineral interactions

Clay mineral formation and impact on soil properties

Silicon dioxide, when combined with oxides of Mg, Al, Ca, and Fe, forms the silicate minerals in rocks and soil. Silicates are the largest class of minerals comprising 30% of all minerals and making up 90% of the Earth’s crust (Ehrlich, 2002). These minerals are unstable in the biosphere and break down to form clays. Microorganisms, including fungi, play a fundamental role in the dissolution of silicates in rock weathering, and therefore in the formation of clay minerals, and soil and sediment formation (Banfield et al., 1999). The presence of clay minerals can be a typical symptom of biogeochemically weathered rocks, and this has been observed for symbiotic fungal associations (lichens and ectomycorrhizas) (Barker and Banfield, 1998; Arocena et al., 1999). Some studies have shown that the transformation rate of mica and chlorite to 2:1 expandable clays was predominant in the ectomycorrhizosphere compared to non-ectomycorrhizosphere soils, likely to be a result of the high production of organic acids in the rhizosphere compared to non-ectomycorrhizosphere soils.

Clay minerals are generally present in soil in larger amounts than organic matter and because of their adsorptive and ion-exchange capacity, they perform a significant buffering function in mineral soils and are important reservoirs of cations and organic molecules.

Biological effects of clay minerals

Fungi are generally in close proximity to clay minerals in soils and sediments. Several studies have shown that interactions of microorganisms with solid adsorbents lead to an increase in biomass, growth rate, and production of various enzymes and metabolites. Stimulatory effects may arise from the abilities of different clays to serve as pH buffers, as a source of metal cationic nutrients, as specific adsorbents of metabolic inhibitors, other nutrients and growth stimulators; and modifiers of the microbial microenvironment because of their adsorptive capacity. However, some clays may inhibit fungal growth and metabolism (Stotzky, 1966).

Fungal–clay mineral interactions in soil aggregation

Fungal–clay mineral interactions play an important role in soil development, aggregation and stabilization (Burford et al., 2003a). Fungi entangle soil particles in their hyphae forming stable microaggregates and also take part in exopolysaccharide-mediated aggregation. Interactions between hyphae and solid particles are subject to complex forces of both a physicochemical (electrostatic, ionic, hydrophobic effects, etc.) and biological nature (chemothropism, production of specific enzymes, polysaccharides, lectins and other adhesins, etc.) (Ritz and Young, 2004). Interactions between clay minerals and fungi alter the adsorptive properties of both clays and hyphae (Morley and Gadd, 1995; Fomina and Gadd, 2002).

Clay and silicate weathering by fungi

Fungi and bacteria play an important role in the mobilization of silica and silicates. Their action is mainly indirect, either through the production of chelates or the production of acids, mineral or organic (including oxalic), or, as for certain bacteria, the production of ammonia or amines (Ehrlich, 2002).

Metal, metalloid, radionuclide, and organometal transformations

Metals and their compounds interact with fungi in various ways depending on the metal species, organism, and environment, while fungal metabolism also influences metal speciation and mobility (Gadd and Griffiths, 1978; Gadd, 1992, 1993a, 2004a, b, 2005, 2007a). Many metals are essential for life, e.g., Na, K, Cu, Zn, Co, Ca, Mg, Mn, and Fe, but all can exert toxicity when present above certain threshold concentrations. Other metals, e.g., Cs, Al, Cd, Hg, and Pb, have no known metabolic function in fungi but all can be accumulated. Metal toxicity is affected by environmental conditions and the chemical behavior of the particular metal species. Despite potential toxicity, many fungi survive and grow in metal-rich locations and a variety of mechanisms, both active and incidental, contribute to tolerance. Fungi have many properties which influence metal mobility and toxicity including the production of metal-binding proteins, organic and inorganic precipitation, active transport, and intracellular compartmentalization, while major constituents of cell walls, e.g., chitin and melanin, have significant metal-binding abilities (Gadd, 2004a, b, 2005, 2006). Other properties lead to metal solubilization from organic and inorganic sources (Gadd, 2007a).
Fungi can also transform certain metals, metalloids (elements with properties intermediate between those of metals and non-metals, e.g., arsenic, selenium, and tellurium), organometallic compounds and metal radionuclides by, e.g., oxidation, reduction, methylation, and dealkylation (Gadd, 1993b, 2004a, b). Such transformations alter chemical speciation and may modify mobility and toxicity. For example, methylated selenium derivatives are volatile and less toxic than inorganic forms while reduction of metalloid oxyanions, such as selenite or tellurite to amorphous elemental selenium or tellurium respectively, results in immobilization and detoxification. All the mechanisms by which fungi (and other microorganisms) effect changes in metal speciation and mobility are survival determinants but also components of biogeochemical cycles for metals, and many other associated elements including carbon, nitrogen, sulfur, and phosphorus (Gadd, 2004a, 2006, 2007a, 2008). They may be simply considered in terms of metal mobilization or immobilization mechanisms.

Metal mobilization

Metal mobilization from rocks, minerals, soil, and other substrates can be achieved by protonolysis, resulting in carbonic acid formation from respiratory CO₂, complexation by excreted metabolites and Fe(III)-binding siderophores, and methylation which can result in volatilization. In addition, other excreted metabolites with metal-complexing properties, e.g., amino acids, phenolic compounds, and organic acids may also play a role. Fungal-derived carboxylic acids can play an important role in chemical attack of mineral surfaces and these provide protons as well as a metal-chelating anion (Burgstaller and Schinner, 1993). Oxalic acid can act as a leaching agent for those metals that form soluble oxalate complexes, including Al and Fe (Strasser et al., 1994). Solubilization mechanisms can also have consequences for mobilization of metals from toxic metal containing minerals, e.g., pyromorphite (Pb₅(PO₄)₃Cl), contaminated soil, and other solid wastes (Sayer et al., 1999; Fomina et al., 2004, 2005b, c). Fungi can also mobilize metals and attack mineral surfaces by redox processes: Fe(III) and Mn(IV) solubility is increased by reduction to Fe(II) and Mn(II) respectively. Reduction of Hg(II) to volatile elemental Hg(0) can also be mediated by fungi (Gadd, 1993b).

Removal of metals from industrial wastes and by-products, low grade ores and metal-bearing minerals by fungal “heterotrophic leaching” is relevant to metal recovery and recycling and/or bioremediation of contaminated solid wastes (Burgstaller and Schinner, 1993; Gadd, 2007a, b). Although fungi need a source of carbon and aeration, they can solubilize metals at higher pH values than thiobacilli and could perhaps become important where leaching with such bacteria is not possible and in bioreactors. Leaching of metals with fungi can be effective although a high level of organic acid production may be necessary. Other possible applications of fungal metal solubilization are the removal of unwanted phosphates, and metal recovery from scrap electronic and computer materials (Brandl, 2001).

The ability of fungi, along with bacteria, to transform metalloids has been utilized successfully in the bioremediation of contaminated land and water. Selenium methylation results in volatilization, a process which has been used to remove selenium from the San Joaquin Valley and Kesterson Reservoir, California, using evaporation pond management and primary pond operation (Thomson-Eagle and Frankenberger, 1992).

Metal immobilization

Fungal biomass provides a metal sink, either by metal biosorption to biomass (cell walls, pigments and extracellular polysaccharides), intracellular accumulation and sequestration, or precipitation of metal compounds onto and/or around hyphae (Gadd, 1993a, 2000b, c, 2001a, b, c, 2007a; Baldrian, 2003; Fomina et al., 2007b, c). Fungi are effective biosorbents for a variety of metals including Ni, Zn, Ag, Cu, Cd, and Pb, and this can be an important passive process in both living and dead biomass (Gadd, 1990, 1993a; Sterflinger, 2000). The presence of chitin, and pigments like melanin, strongly influences the ability of fungi to act as sorbents. In a biotechnological context, fungi and their by-products have received considerable attention as biosorbent materials for metals and radionuclides (de Rome and Gadd, 1987; Gadd and White, 1989, 1990, 1992; White et al., 1995). Fungi can also precipitate several inorganic and organic compounds, e.g., oxalates, oxides, and carbonates, and this can lead to formation of biogenic minerals (mycogenic precipitates) as discussed previously (Gadd, 2007a).

Halide transformations

Fungi have the ability to produce a variety of atmospheric methyl halides. This ability is widespread in both free-living and symbiotic fungi, and is dependent on substrate concentrations and community composition (Redeker et al., 2004). The production of chloromethane (CH₃Cl) by wood-rotting fungi, e.g., Phellinus spp., may be particularly significant with one estimate of annual global input to the atmosphere from this source being 160,000t of which 75% is released from tropical and subtropical forests (Watling and Harper, 1998). Filamentous fungi may also contribute to the global circulation of stable iodine and also the long-lived radioiodine, 129I (half-life: 1.6 × 10⁷ years), released from nuclear facilities into the environment (Ban-nai et al., 2006).

Fungal symbioses in mineral transformations

One of the most remarkable adaptations of fungi for exploitation of the terrestrial environment is their ability to form mutualistic partnerships with plants (mycorrhizas) and algae or cyanobacteria (lichens). Symbiotic fungi are provided with carbon by the photosynthetic partners...
(photobionts), while the fungi may protect the symbiosis from harsh environmental conditions (e.g., desiccation, metal toxicity), increase the absorptive area, and provide increased access to mineral nutrients.

Lichens
Lichens are fungi that exist in facultative or obligate symbioses with one or more photosynthesizing partners, and play an important role in many biogeochemical processes. The lichen symbiosis formed between the fungal partner (mycobiont) and the photosynthesizing partner (algal or cyanobacterial photobiont) enables lichens to grow in practically all surface terrestrial environments: an estimated 6% of the Earth’s land surface is covered by lichen-dominated vegetation (Haas and Purvis, 2006). Lichens are pioneer colonizers of fresh rock outcrops. Globally, lichens play an important role in the retention and distribution of nutrient (e.g., C, N) and trace elements, in soil formation, and in rock weathering. Alteration of bedrock minerals and synthesis of biominerals in the proximity of lichens gives rise to different chemical microenvironments and emphasizes their participation in mineral nutrient cycling (Banfield et al., 1999). Lichens can accumulate metals such as lead (Pb), copper (Cu), and many other elements of environmental concern, including radionuclides, to high levels. They can also form a variety of metal-organic biominerals, especially during growth on metal-rich substrates. For example, on copper-sulfide bearing rocks, precipitation of copper oxalate (moolooite) can occur within the lichen thallus (Purvis, 1996; Purvis and Halls, 1996).

Mycorrhizas
Nearly all land plants depend on symbiotic mycorrhizal fungi (Smith and Read, 1997). Two main types of mycorrhizas include endomycorrhizas where the fungus colonizes the interior of host plant root cells (e.g., ericoid and arbuscular mycorrhizas) and ectomycorrhizas where the fungus is located outside plant root cells. Mycorrhizal fungi are involved in proton-promoted and ligand-promoted metal mobilization from mineral sources, metal immobilization within biomass, and extracellular precipitation of mycogenic metal oxalates (Fomina et al., 2004, 2005b). Biogeochemical activities of mycorrhizal fungi lead to changes in the physicochemical characteristics of the root environment and enhanced weathering of soil minerals resulting in metal release. Ectomycorrhizal mycelia may respond to the presence of different soil silicate and phosphate minerals (apatite, quartz, potassium feldspar) by regulating their growth and activity, e.g., colonization, carbon allocation, and substrate acidification (Rosling et al., 2004a, b).

During growth, mycorrhizal fungi often excrete low molecular weight carboxylic acids (Martino et al., 2003; Fomina et al., 2004). The weathering of hornblendes, feldspars, and granitic bedrock in certain soils has been attributed to oxalic, citric, succinic, formic, and malic acid excretion by ectomycorrhizal hyphae which can produce micro- to millimolar concentrations of these organic acids. Ectomycorrhizal fungi (Suillus granulatus and Paxillus involutus) can release elements from apatite and wood ash (K, Ca, Ti, Mn, Pb) and accumulate them in the mycelia (Wallander et al., 2003). Ericoid mycorrhizal and ectomycorrhizal fungi can dissolve a variety of cadmium, copper, zinc, and lead-bearing minerals including metal phosphates (Leyval and Joner, 2001; Martino et al., 2003; Fomina et al., 2004, 2005b). Mobilization of phosphorus is generally regarded as one of the most important functions of mycorrhizal fungi.

Summary
The biogeochemical importance of fungi is significant in several key areas. These include organic and inorganic transformations, nutrient and element cycling, rock and mineral transformations, bioweathering, mycogenic mineral formation, fungal–clay interactions, and metal–fungi interactions. While such transformations can occur in both aquatic and terrestrial habitats, it is in the terrestrial environment where fungi probably have the greatest influence especially when considering soil, rock, and mineral surfaces, and the plant-root–soil interface. Of special significance are the mutualistic symbioses, lichens and mycorrhizas. Geochemical transformations that take place can influence plant productivity and the mobility and specification of toxic elements, and are therefore of considerable socioeconomic relevance. Some fungal transformations have beneficial applications in environmental biotechnology, e.g., in metal and radionuclide leaching, recovery and detoxification, and xenobiotic and organic pollutant degradation. They may also result in adverse effects when these processes are associated with the degradation of foodstuffs, natural products, and building materials, including wood, stone, and concrete.

Bibliography


GEYSERITE

A dense, banded or laminated variety of sinter that forms at and near the vents of geysers and some hot springs. See entries “Sinter” and “Hot Springs and Geysers” for further reading.

GLASS

See entry “Basalt (Glass, Endoliths).”

GLAUCOPHYTES

Glaucophytes (glaucocystophytes or glaucocystids) are colorless freshwater protists containing plastids derived from primary endosymbiosis (see also entry Symbiosis) that is, they are still surrounded by a peptidoglycane wall, which may be viewed as reminiscent of their cyanobacterial origin. Previously, these primitive plastids were also called “cyanelles.” For details, please refer to entry “Algae (Eukaryotic).”

Cross-references
Algae (Eukaryotic)

GOLD

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Synonyms
Aurum (Latin)

Definition
Precipitation/Bioaccumulation of gold is the precipitation of fine-grained gold colloids by microbial activities.

Nugget. A natural gold grain (>5 mm, >200 mg).

The impact of the use of microbial activity for the solution and precipitation of gold was undervalued for a long period, thus affording the opportunity for economic and environmentally friendly gold bio-processing. Furthermore, the current high price of gold has led to intensive research into the precipitation of gold.

Some iron and sulfur oxidizers (e.g., Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans) contribute to the disintegration of important gold ores (Nordstrom and Southam, 1997). In an environment of gold-bearing rocks, the dissolved gold content is 40 times higher than in comparable non-gold-bearing areas (Benedetti and Boulégue, 1991). It has been shown that the dissolution of gold is dominated by the gold thiosulfate complex $\text{Au(S}_2\text{O}_3\text{)}_2^{3-}$ which is composed of the above-mentioned bacteria and Actinomycetes (e.g., Streptomyces fradiae) (Nordstrom and Southam, 1997; Sand et al., 2001). However, $\text{Au(S}_2\text{O}_3\text{)}_2^{3-}$ is only stable until the sulfur-oxidation is complete (Lengke and Southam, 2005). Subsequently the gold thiosulfate complex is degraded by $A.\;\text{thiooxidans}$. Thereby gold accumulates in the form of fine-grained colloids inside the bacterial cell and on the surface of the cell membrane. Comparable with the results of Southam and Beveridge (1996) with Bacillus subtilis 168, hexagonal-octahedral gold crystals are generated out of the colloids after some months (Figure 1).

As a result of the toxicity of an increasing content of ionized gold, most of the microorganisms that can generate gold die on accumulation of gold. However, various microorganisms, such as bacteria (e.g., Escherichia coli, Pseudomonas maltophilia, Plectonema boryanum), Archaea (e.g., Pyrococcus furiosus), Actinomycetes (e.g., Streptomyces cinamomensis), fungi (e.g., Neurospora sitophila, Fusarium oxysporum) and yeasts (e.g., Endomycopsis fibuligera, Sporobolomyces salmonicolor) from different environments (e.g., hydrothermal, supergene), are partially resistant to the toxicity induced by the accumulation of gold (Kashefi et al., 2001; Nakajima, 2003; Lengke et al., 2006). These bioaccumulation processes are related to various microbial activities (e.g., detoxification, energy generation, passive adsorption). In the laboratory, Pseudomonas aeruginosa biofilms demonstrate a higher resistance to the toxicity of gold than planktonic $P.\;\text{aeruginosa}$ cells and form solid gold at the base of the biofilms (Karthekeyan and Beveridge, 2002). Reith et al. (2006) isolated a 16SrDNA sequence from natural samples. This sequence was only found on gold grains

Gold, Figure 1 A secondary electron (SE) image of a hexagonal gold crystal from the secondary gold deposit Silberkuhle near Korbach, Germany.
Gold, Figure 2 Laser scanning micrograph (ex. 488 nm, em. 543 nm) of microorganisms (green) on a gold grain (red) from the Eder River, Germany. Eubacteria labeled by oligonucleotide probe EuB-CY3.

and not in the surrounding sediments. With a similarity of 99%, the sequence correlated to Cupriavidus (Ralstonia) metallidurans. Ninety percent of the bacteria in the laboratory experiment died because of the toxicity of AuCl₄⁻. The surviving cells adapted themselves to the high concentration of Au(III) ions and precipitated gold. In addition to morphological studies (Bischoff, 1997), this provides evidence for bioaccumulation of secondary gold (e.g., nuggets, gold grains) by bacteria like C. metallidurans (Figure 2).

The basal biochemical microbial processes of the biomineralization of gold are not completely understood yet. Precipitation of gold provides the cellular defense of C. metallidurans and is controlled by a coupling of efflux, reduction and possibly methylation of gold complexes (Reith et al., 2009). Reith et al. (2007) have published a comprehensive review of the geomicrobiology of gold.

Summary
Different microorganisms are partially resistant to dissolved gold. Various microbial activities precipitate gold. Biofilms of Cupriavidus metallidurans accumulate gold naturally and contribute to the formation of secondary gold.

Bibliography

Gondwanaland, Formation
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Definition
Gondwanaland or “Gondwana” is the name for the southern half of the Pangaea supercontinent that existed some 300 million years ago. Gondwanaland is composed of the major continental blocks of South America, Africa,
Arabia, Madagascar, Sri Lanka, India, Antarctica, and Australia (Figure 1). The name “Gondwana” is derived from a tribe in India (Gonds) and “wana” meaning “land of.” Gondwanaland is superficially divided into a western half (Africa and South America) and an eastern half (India, Sri Lanka, Madagascar, Antarctica, and Australia).

The archetypal view of Gondwanaland assembly was an Ediacaran–Cambrian-age coalescence of East Gondwana (India, Sri Lanka, Madagascar, Australia, and Antarctica) with West Gondwana (South America and Africa) along the Mozambique Belt (labeled East African Orogen in Figure 1). “Pan-African” tectonothermal belts outside of the Mozambique Belt (~500–600 Ma) were well-known at the time, but they were often treated as zones of ensialic activity rather than sites of continental collision. Although this rather simplistic view of Gondwanaland assembly is now strongly debated, the polyphase assembly model outlined below is not completely accepted (see Yoshida, 2007).

A new view of Gondwanaland assembly began with the work by Stern (1994) who demonstrated clear evidence of juvenile arc development and accretion in the Arabian–Nubian shield and continental collision to the south in Kenya and Tanzania. In the early 1990s, work by Dalziel (1991), Hoffman (1991), and Moores (1991) hinted that the assembly of Gondwanaland followed the breakup of an earlier supercontinent called Rodinia (see Chapter Breakup of Rodinia). Although the exact continental configuration of Rodinia is still debated (for example, Meert and Torsvik, 2003), the assembly of West Gondwana is more or less viewed as a polyphase accretion of formerly disparate blocks while East Gondwana is generally treated as a coherent unit from ~1,100 Ma until its breakup in the Mesozoic (Meert, 2003). More recently, Fitzsimons (2000), Meert (2003), Collins and Pisarevsky (2005), and Kelsey et al. (2008) noted that at least two “Pan-African” age mountain belts cut across the East Antarctic shield and juxtapose distinct crustal fragments thought to comprise the East Antarctic “Grenvillian craton.” The existence of these belts preclude a united East Gondwana and favor a polyphase accretion of major blocks to form Greater Gondwanaland in the Cambrian. In this scenario, the assembly of Gondwanaland was accomplished along three major orogenic belts known as the Brasiliano, East African, and Kuungan orogenies (Figure 1). Additional data supporting a polyphase assembly of East Gondwana are derived from paleomagnetic and geochronologic studies.

No matter what the exact model of Gondwanaland assembly is, the formation of this supercontinent followed
shortly after the extreme glaciations during the late Neoproterozoic (Hoffman et al., 1998) and was nearly synchronous with the rapid evolutionary pulse that coincides with the Ediacaran and Cambrian radiations. Final assembly of Gondwanaland and a circum-Gondwanaland mountain chain is thought to have provided nutrients to the oceanic realm and also resulted in a large $^{87}$Sr/$^{86}$Sr spike due to the erosion of the mountains (Squire et al., 2006). Therefore, links between the assembly of the Gondwanaland supercontinent and biological evolution are forwarded by numerous studies (Meert and Lieberman, 2008).

Bibliography


Cross-references

Breakup of Rodinia
Critical Intervals in Earth History
Ediacaran Biota
Origins of the Metazoa
Snowball Earth
Trace Fossils: Neoproterozoic

GREAT OXYGENATION EVENT (GOE)

The term “Great Oxygenation Event” (GOE, also known as “Great Oxidation Event,” “oxygen catastrophe,” or “oxygen crisis”) describes a critical environmental change in Earth history that resulted from the appearance of diatomic oxygen ($O_2$), a waste product of oxygen photosynthesis, in the atmosphere. The GOE occurred around 2.4 billion years (Ga) before present, close to the boundary between the Archean and the Proterozoic (2.5 Ga). For details, please refer to “Critical Intervals in Earth History.”

GREEN ALGAE

Green algae are photosynthetic eukaryotes with simple plastids (derived from primary endosymbiosis) that are mostly microscopic and rarely more than a meter in greatest dimension. The approximate 6,000 extant species show an enormous diversity of growth habit and fine details of their cellular architecture. Green algae occur as unicellular or colonial, microscopic or macroscopic, motile planktonic, as well as benthic attached forms. Some (e.g., Dasycladales) are important carbonate producers in aquatic environments, others live in symbiotic associations with fungi to form lichens in terrestrial settings. Green algae living as endosymbionts inside heterotrophic organisms are a common phenomenon. The order Charales, in which full tissue differentiation occurs, is considered to include the closest relative of higher plants. For details, please refer to entry “Algae (Eukaryotic).”

GUILD

Metabolically related populations, e.g., sulfate-reducing microorganisms. See entry “Microbial Communities, Structure, and Function” for further reading.
HABITAT

The physical location or dwelling place of a particular organism, where an individual ecotype can be found or isolated from. See entry “Microbial Communities, Structure, and Function” for further reading.

HALOBACTERIA – HALOPHILES

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Definition


Introduction

Saline waters dominate the earth, with the oceans holding 97% of the planet’s water (a total of $1.338 \times 10^9$ km$^3$; http://ga.water.usgs.gov/edu/earthwherewater.html). Saline inland seas, groundwater, and saltwater lakes hold another 0.9–0.94%, which exceeds the volume of the world's available freshwater ($\sim 1.1 \times 10^7$ km$^3$). The definition of a hypersaline environment is one that possesses a salt concentration greater than that of seawater (>3.5% w/v). Water-containing environments are usually described as thalassohaline or athalassohaline (see Chapters “Saline Lakes,” “Soda Lakes”). Thalassohaline waters are marine-derived and therefore contain, at least initially, the seawater composition; with increasing evaporation, the concentration of various salts is changing. Athalassohaline waters may also receive influx of seawater; however, the chemical composition is mainly determined by geological, geographical, and topographical parameters.

Interactions between halophilic microorganisms and geological materials have been recognized early in the history of mankind and continue to impact human endeavors, including the exploration of energy sources, the storage of waste, the search for extraterrestrial life (see Chapter “Deep Biosphere of Salt Deposits”). For example, the evaporation of seawater for the purpose of making salt and the concomitant development of red color of brines, which is due to pigmented microorganisms, have been described already 2,700 years ago (Oren, 2002). It was also observed early that the presence of microorganisms in solar salterns enhances the precipitation and the yield of salt (Oren, 2002). Halite, the mineral of sodium chloride, primarily forms from the evaporation of seawater. Subsurface deposits of halite occur in many areas and are far more abundant than was previously recognized. The advent of widespread oil drilling revealed a frequent association of ancient salt deposits and the presence of the biogenic energy sources, oil and methane (Sassen et al., 1994). The potential impact of viable halophilic microorganisms in salt deposits (see Chapter “Deep Biosphere of Salt Deposits”), some of which are being used as storage sites for long-lived radioactive isotopes such as transuranic wastes, has been the focus of much research. The striking discovery of extraterrestrial halite in meteorites (see Chapter “Deep Biosphere of Salt Deposits”) and recently, in salt pools on Mars (Osterloo et al., 2008) make a search for halophilic microorganisms on other planets or moons plausible.
Saline environments and their inhabitants
Organisms occur within a range of salinities, from distilled water to saturated salt solutions, and may be loosely classified as non-, slightly, moderately, or extremely halophilic, requiring about 0.2 M, 0.2–0.85 M, 0.85–2.5 M, and 2.5–5.2 M NaCl, respectively, for growth. The largest saline environment, seawater, contains 31–38 g of total dissolved salts per liter, nearly 80% of which is NaCl. Hypersaline environments are characterized by higher salinities than seawater. The oceans are inhabited by a great diversity of organisms; when seawater is evaporating, the biological diversity decreases with increasing concentration of salt. Macroscopic organisms such as salt-tolerant fish (e.g., *Cyprinodon variegatus* and *Menidia beryllina*) are no longer found at concentrations of more than about 11% salt (1.88 M NaCl); copepods (fleas), turbellarian worms, and some rotifers tolerate 15–17% salt. Interestingly, several invertebrates such as brine shrimp (*Artemia salina*) or brine flies (*Ephydra cinerea*) are capable of survival at NaCl concentrations of 30–33% (~5 M; Javor, 1989). However, the dominating organisms in saturated brines are extremely halophilic microorganisms which grow optimally in the presence of 2.5–5.2 M NaCl and are generally unable to grow in less than 1.7 M NaCl. They can occur at such high cell densities (up to $10^8$ colony forming units per milliliter) that they cause brines to turn bright red or purplish. Figure 1 shows a commercial salt-producing facility where seawater is evaporating in a series of ponds. The colors of the pools are caused by carotenoid pigments, which are contained in the membranes of haloarchaea, or by the chlorophylls of halophilic algae or cyanobacteria. Typical haloarchaea in such communities are species of the genera *Halobacterium*, *Halorubrum*, and *Halococcus*, halophilic bacteria such as *Ectothiorhodospira halochloris*, *Salinibacter ruber*, cyanobacteria (e.g., *Aphanoece halophytica*, *Phormidium* sp. and *Schizothrix arenaria*), and green algae (e.g., *Dunaliella salina* and *Asteromonas gracilis*). Figure 2 shows two characteristic haloarchaeal morphologies – *Halobacterium salinarum* NRC-1 grows as single rod-shaped cells; *Halococcus salifodinae* cells are roundish and tend to aggregate into large clusters. Crude solar salt contains large numbers of viable bacteria and *archaea* (about $10^5$–$10^6$ colony forming units per milliliter; Oren, 2002). When using this salt for the preservation of fish, meat, and hides, damage in the form of red discoloration of the goods was noted under conditions of moisture and elevated temperatures. These observations started the early research on halophilic microorganisms in the 1920s–1930s. Only much later – in the 1980s – was it recognized that most halophiles from such sources belonged to the archaea. The haloarchaea are not pathogenic; in fact, some are used in traditionally fermented foods in Asia for human consumption (Oren, 2002).

There exist other hypersaline environments which are not directly derived from seawater and are called athalassohaline (see above and Chapter “Soda Lakes”); often they are remnants of prehistoric inland lakes, such as the Dead Sea and the Great Salt Lake. Additional larger lakes include Mono Lake in California, soda lakes in Egypt and China, Lake Magadi in Kenya, and Lake Eyre in Australia. The chemical composition varies depending on the geology of the surroundings, but overall salinity is usually between 200 and 340 g/l of dissolved salts. Soda lakes are an example of naturally occurring alkaline environments which may also be hypersaline, and are characterized by the presence of large amounts of sodium carbonate and correspondingly minute concentrations of Ca$^{2+}$ and Mg$^{2+}$. The pH of alkaline lakes is usually in the range of 10–11; in some evaporation ponds, it can rise up to 12 (Oren, 2002). Microbial isolates from alkaline hypersaline lakes include the archaea *Natronomonas* sp., *Natronococcus* sp., *Natrialba* sp., and bacteria of the genera *Halomonas*, *Chromohalobacter*, *Spirulina* (the latter is known as nutrient for humans and animals) and others still to be characterized (Javor, 1989; Oren, 2002).
From the Deep Lake in the Vestfold Hills of Antarctica, a psychrophilic archaeon, Halorubrum lacusprofundi, was isolated. Deep Lake has a salinity that is ten times higher than that of seawater (3.6–4.8 M). The lake remains ice-free throughout the year, with water temperatures fluctuating between +10 and −15°C and remaining below 0°C for ~8 months of the year. The microbial diversity in Deep Lake is extremely low and is dominated by haloarchaea, with apparently low numbers of Dunaliella sp. and bacteria (of the beta- and gamma-proteobacteria) present in the sediment (Cavicchioli, 2006).

Less explored and unusual hypersaline habitats are represented by anoxic brines near the bottom of the sea, for example in the Mediterranean deeps, which contain saline brines almost saturated with magnesium chloride, and where a wide diversity of novel archaea and bacteria was detected (van der Wielen et al., 2005).

Another environment for halophilic microorganisms are ancient salt deposits; recent reports have described the presence of haloarchaea in Triassic and Permian salts (summarized in McGinity et al., 2000; Stan-Lotter et al., 2004). These viable microorganisms were consistently isolated from surface-sterilized crystals obtained from freshly blasted rock salt or deep drilling bore cores, suggesting that these were survivors from the original depositional event (see Chapter “Deep Biosphere of Salt Deposits”).

Finally, halophilic viruses have been detected, which were released from bacteria and archaea; in addition, free virus particles were found by electron microscopical examination of the Dead Sea and in Spanish saltern ponds (Oren, 2002). The viruses (also called bacteriophages) were equally dependent on high concentrations of salt for their structural stability. There is little information on the role of viruses in natural environments; suggestions which were made involve ecological functions in controlling the sizes of microbial communities (Oren, 2002). A website with pictures of haloviruses from the laboratory of M. Dyall-Smith is available: http://www.microbiol.unimelb.edu.au/people.dyallsmith/research/haloviruses/

Classification and genomics
Halophilic means salt-loving and this term, often as the prefix hal- or halo-, has been in use since the 1930s. Therefore, members of the bacteria as well as the separate prokaryotic group archaea, which was identified only in the 1970s, may contain such prefixes in their names. Some confusion may arise from this nomenclatural convention, since, for example, the genus with the name Halobacterium belongs to the halophilic archaea (or haloarchaea), not to the bacteria.

The number of recognized haloarchaeal genera has increased to 26, according to the International Committee on Systematics of Prokaryotes (http://www.the-icsp.org) and the number of validated species at this time is 86. Main taxonomic criteria for the identification and recognition of haloarchaea are the sequence of the 16S rRNA genes and the composition of their characteristic membrane polar lipids, which are unique ether-linked phosphoglycerides (see Fendrihan et al., 2006, for a recent overview). The complete list of required and recommended criteria for the formal identification and recognition of haloarchaeal species was proposed by Oren et al. (1997). For halophilic bacteria belonging to the large family Halomonadaceae, a similar list of specific characteristics, which has been revised recently, can be consulted (Arahalk et al., 2007).

Five genomes of haloarchaea have been completely sequenced, that of Halobacterium salinarum NRC-1, the related strain Halobacterium salinarum R1, Haloarcula marismortui, Natronomonas pharaonis, and Haloquadratum walsbyi, and one genome of a halophilic bacterium (Salinibacter ruber). The website http://www.ncbi.nlm.nih.gov/sites/entrez lists all genome projects, whether complete or still in progress, including references
and detailed descriptions. Comparisons of genomes continue to provide insights about similarity and differences between archaea and bacteria and about the early evolution of prokaryotes. They have, for example, proven that the adaptation mechanism of keeping a high internal salt concentration (see next paragraph) is not unique to archaea, as was thought before, but is used also by *Salinibacter ruber*; this common property as well as some other characteristics suggests the possibility of early lateral gene transfers between prokaryotes (Veilleux et al., 2007).

**Adaptations to high salinity**

Most halophiles are unable to survive outside their high-salt native environments. Some species of the haloarchaea are so fragile that when placed in distilled water they lyse within minutes from the change in osmotic conditions (Grant, 2001). This reflects a general adaptation of the cells’ entire intracellular machinery to high concentrations of cations. Halophilic and halotolerant organisms have to expend energy to exclude salt from their cytoplasm to avoid protein aggregation (“salting out”). Two different strategies are known to be used to prevent desiccation through osmotic movement of water out of their cytoplasm, and both work by increasing the internal osmolarity of the cell. In the first, organic compounds, termed “compatible solutes,” are accumulated in the cytoplasm; this strategy has been found in many halophilic bacteria and also in some archaea, yeasts, algae, and fungi (Galinski, 1995). Compatible solutes are neutral or zwitterionic and include amino acids, sugars, polyols, betaines, and ectoines, as well as derivatives of some of these compounds; they can be synthesized de novo or taken up from the environment (Galinski, 1995; Grant, 2004). The second adaptation involves the selective import of K⁺ ions into the cytoplasm, which can reach internal concentrations of 5 M. This adaptation is restricted to the moderately halophilic bacterial order *Halanaerobiales*, the haloarchaeal family *Halobacteriaceae*, and the extremely halophilic bacterium *Salinibacter ruber* (Oren, 2002). Most proteins of haloarchaea and also of *Salinibacter* contain a large excess of acidic amino acids (glutamate and aspartate) and a low content of basic amino acids (lysine and arginine; Lanyi, 1974; Oren, 2002); these features are thought to represent a specific adaptation to high levels of salt (Mevarech et al., 2000; Oren, 2002). In contrast, the action of compatible solutes is much less specific, with little or no adjustment of intracellular macromolecules required, which is in line with the observation that these compounds act as general stress protectants against heating, freezing, and drying (see Oren, 2002, and references therein).

**Conclusions**

Halophilic organisms occur in all major groups on the tree of life (see Oren, 2007) and even halophilic viral representatives are known. Halophilic microorganisms in particular may be found on earth in almost every hypersaline environment – tropical to polar, terrestrial to submarine, acidic to alkaline, or aerobic to anaerobic (Javor, 1989). Initially a little-studied group of microorganisms, halophilic bacteria and archaea have proven to be of interest for life sciences and earth sciences as well; their investigation has resulted in major insights for molecular biology, genomics, biogeochemistry, and – most recently – astrobiology.

**Bibliography**


### Cross-references
- Archaea
- Astrobiology
- Deep Biosphere of Salt Deposits
- Extreme Environments
- Hypersaline Environments, Terrestrial
- Saline Lakes
- Soda Lakes

### Haptophytes

Haptophytes (Haptophyta, Prymnesiophyta) are unicellular chlorophyll a and c containing algae with complex plastids derived from secondary endosymbiosis (“red lineage”) (see entry “Symbiosis”). They occur principally as solitary free-living motile cells that possess two smooth flagella, unequal in length in the Pavlovophyceae and more or less equal in the Prymnesiophyceae. Haptophytes inhabit littoral, coastal, and oceanic waters and are important primary producers in many aquatic environments. Within the haptophytes, the so-called coccolithophores (coccolithophorids) evolved the ability to control the intracellular calcification onto organic plates and the assembly of the mature calcium carbonate (calcite) scales (coccoliths) at the cell surface. These forms have been significantly contributing to the deposition of calcium carbonate in marine waters since the Mesozoic, today accounting for about a third of the total marine CaCO₃ production. In modern and ancient sediments, haptophyte-derived organic matter contributions can be specified by distinct lipid biomarkers (alkenones), whose unsaturation patterns also provide a paleo-thermometer for past surface water temperatures (see entry “Biomarkers (Molecular Fossils)”). For further details, please see entry “Algae (Eukaryotic).”

### Heavy Metals


### Histochemistry

**Definition**

*Histology* is the biological science concerned with the minute or microscopic structures of cells, tissues, and organs of animals, plants, and fungi, in relation to their function, and any organismic constructions produced by living individuals or symbiotic assemblies of living organisms. 

*Biopsy* is the sample of an organism or part of a tissue that is processed for histological investigations.

*Fixation* serves the inhibition of all vital processes by special chemicals that also preserve and prepare organic matter for further histological or morphological investigations.

*Mineralization* is the process by which organic components of organisms are impregnated or replaced by inorganic material and converted to mineral matter.

*Staining* is the chemical reaction of particular laboratory chemicals (stains and dyes) with tissue components and materials of the samples.

### Introduction

The term *histology* was coined by C. Mayer in 1819 as a part of the scientific discipline comparative anatomy (Mayer, 1819). Histological investigations, concerned with the minute structures of cells, cell organelles, intracellular fiber arrangements, and tissue components and organs in relation to their function, are usually done by light microscopy. Since the invention of the microscope by Leeuwenhoek in 1668 (Palm and Snelders, 1983), the early histological technology (Gerlach, 1998) has experienced major improvement by the refinement of microscopes (Bozzola and Russell, 1999) and new methods of tissue preservation, sectioning devices, stains, and staining methods. Histochemical methods now permit to identify and localize intracellular components and enzymes within distinct and isolated samples (e.g., Bancroft and Gamble, 2007; Böck, 1989; Horobin and Kieman, 2002; Mulisch and Welsch, 2010). Therefore, the studied structures, tissues, complete organisms, or any organismic compound have to be preserved from enzymatic decay, and then cut into sections of just a few microns thickness. The histological sections can vary between ultra thin sections (40–80 nm), semi thin sections (0.1–3.0 µm), thin sections (3–20 µm), and thick sections (more than 20 µm). While ultra thin sections could be investigated only by transmission or scanning electron microscopy (TEM), the others can be investigated with any light microscope. Afterward, these sections are...
processed by various histochemical and biophysical techniques according to the aim of the study.

Geobiological samples usually do not consist of organic soft material, alone, like the above mentioned biological materials, but they are often composites of hard inorganic and mineralized organic material, with nearly all biological components converted to inorganic matter. Some geobiological samples are compositions of both, organic and inorganic materials, such as bio films growing on hard substrates, or sessile organisms that adhere to hard substrates like corals, sponges, lichens, or fungi. These samples therefore can seldom be histologically processed like biological material via paraffin embedding and sectioning with traditional microtomes. Special procedures and mechanical devices (like saw microtomes, annular saws or cutting/grindings systems) are necessary to produce sections of geobiological samples (e.g., Hoffmann et al., 2003). By means of special polishing machines, like the ultramill or lapping machines, sections or slices of these sections can be studied by distinct microscopic methods, for example under fluorescent light, X-ray-mappings and topographic element analyses (Janssens et al., 2000) can identify the outline of the biological compounds and their environment, and they may also serve as a biomarker; harsh biochemical treatment can isolate the organic from the inorganic components, but often destroy the original context. Histological analyses may provide tools to identify the biological components even if their former structural context has decayed (Wrede et al., 2008), or help to analyze particular processes or compositions (Neuweiler et al., 2007). Principally these results must be “calibrated” by means of histological investigations of recent tissues in order to understand organ structure and its function (Young and Heath, 2000).

This entry reviews the state of the histological methods and offers the modifications necessary to topographically investigate geobiological samples. For clarity, this latter information is given in italics.

Methods

Different from biochemical investigations in the broadest sense, histological analyses are concerned with samples, where the structures and their topographical context are preserved or known before biopsies are taken and only in a controlled way modified by procedures according to the specific aim of the study.

For histological processing of tissues or any other organic compounds, all vital functions have to be stopped by fixation, in order to preserve a distinct structure within or more often isolated from its natural environment. In geobiology this process often is “history,” but must be known to understand the given results of further analyses. Usually, the biological samples are embedded into a special medium which provides particular mechanical properties that allow sectioning in the appropriate thickness. The sections are then placed on glass slides or cover slips, afterwards stained by special chemicals and dyes that have affinities to particular tissues or histological structures. The staining allows the differentiation of cell structures, various types of cell surface and skeleton assemblies, filaments or fibrillar components (e.g., collagenous fibers, elastic fibers, or reticular fibers), tissues, and even complete organs or pathological and genetic modifications of the various structures. Under the microscope the results can be observed and – most often after a thorough comparison to reference samples – evaluated and interpreted.

In geobiology, the selective staining of minerals prevails, although protein staining has been successful in various studies. The major problem in geobiology is that mineralized samples are too hard for conventional section procedures and therefore special preparation methods are necessary (see also below, paragraph “Embedding”). Accordingly, often only the surface of sectioned samples is accessible to the staining which limits the differentiations of components. Structural analyses of biological compounds have to refer to similar structures or organs histologically known from recent specimen. Therefore, histology in geobiology has to refer to a biological histology (Robenek, 1995). Specialized methods are available which either demineralize the sample for showing the biological contents or dissolve the organic leftovers to investigate their “casts” in the inorganic surroundings. Often the resulting histologically processed specimens can be biophysically investigated, and topographic X-ray element mappings (Janssens et al., 2000) are often the method of choice to serve as biomarkers.

Fixation and infiltration

The process of fixation and infiltration of biological specimens is most crucial for the results and aims to stop any autolytic and bacterial decomposition. The most commonly used fixative is formalin; however, other fixatives (e.g., Bouin, Carnoy, Paraformaldehyde, potassium dichromate, chromic acid, or mercury-fixation) are necessary depending on the planned subsequent protocol. The choice of the fixative determines the results, since every fixative has assets and drawbacks for the preservation of particular histological structures and especially the binding sites for differential staining, e.g., in immunohistology. For biophysical analyses, the metal-free fixatives and also metal-free pre- and post-processing protocols are essential.

The fixation process, which is a chemical reaction, has to be controlled and stopped after a particular time that depends on type and size of the tissue, but also on environmental factors like temperature and air pressure. Afterwards the fixative must be removed following a special protocol, which takes care not to destroy structural characteristics (Böck, 1989).

Prior to embedding, the sample has to be slowly transferred to the final substance via several intermediate steps mixable with the respective embedding medium, e.g.,
for the paraffin/wax embedding dehydrants (ethanol, isopropanol, and methanol) and clearants (xylene, toluene, and xylene-replacing clearants).

In case of aerated tissues, it may be necessary to perfuse the sample with a prefix and process fixation and embedding continuously in a vacuum with fluid movement for a proper impregnation of all layers of the specimen.

In geobiology, fixation and conservation also plays a crucial role, since these chemical processes have to be balanced between the necessities for the mineralized and organic components of a geobiological sample (biofilms or water containing fossils; e.g., Desrochers et al., 2007). Every fixative generates chemical reactions, which sometimes may degrade or destroy either the organic or the mineralized components. Accordingly, empirical studies have to be performed before crucial samples are processed in order to ascertain whether the fixative may cause any problems for either the soft or hard tissues of a particular type of samples.

**Embedding**

Embedding of histological material is performed in order to provide a mechanical stable and hard entity, which can be sectioned to various coherent thin sections. The sections should enable a spatial reconstruction of the original sample after differentiating analyses. The composition of the specimen and the histological program are decisive for the embedding methods of choice. The most common material for tissue embedding is paraffin (wax-embedding), also embedding in celloidin and resin. If the tissues under study are more or less soft (which means without any calcareous or other mineralized structures), the paraffin embedding is the best choice. Celloidin embedding takes much time and is useful for very sophisticated questions (e.g., embedding of complete brains or very large organs). Plastic embeddings are the best choice if the biopsies contain mineralized structures, such as calcium carbonate, calcium phosphate, and silicate, or if organic components adhere to rocks or other hard substrates, like for example biofilms.

Therefore, these latter embedding methods lend themselves for geobiological samples, which then can be processed like biological material. For resin embedding (Howat et al., 2007) one can choose between epoxy-embedding, methyl-methacrylate-embedding, or glycol-methacrylate-embedding. Various products are commercially available (Obenauf et al., 2007), all of them have well-documented assets and drawbacks. The most drawbacks of plastic embeddings can be seen in their high costs of time and money. On the other hand, resin embedded samples can be sectioned, sawed, milled, or polished by use of various microtomes, saws, milling, or polishing machines.

**Sectioning**

Whole mount samples can only be investigated from their outer surface. Analyses inside the samples need dissection with preferably minor destruction of the structural context. In general, penetration of staining chemicals is enhanced afterwards, and microscopic and biophysical inspection is improved as the spatial resolution corresponds to the thickness of the section. Several problems may arise from inappropriate section procedures:

- The samples may be too brittle and break. This effect is reduced by pre-treatment procedures like decalcification or demineralization, also choosing another type of embedding medium may help possibly combined with different section techniques, e.g., a vibrating knife or sample cooling by a special refrigerant device.
- The sections are heavily distorted. A proper selection of the microtome, cutting angle, and cutting velocity may improve the result. The sections may stretch, for example, after floating on a warm water bath about 10°C below the melting point of the embedding wax.
- The requested thickness requires different blades, which cannot be used “universally,” even if section series of varying thickness are necessary for the protocol. For example, thick sections need a hard metal blade, while semithin- and ultrathin sections can be produced only by means of glass or diamond knives.

Biological samples, which are embedded in paraffin or resin, can generally be sectioned by a special device, a microtome. Some microtomes are equipped with a motor-driven support, both for controlling the section thickness as well as the force of the blade. Several types of microtomes are available, each optimized for variably embedded samples: Paraffin embedded samples can be sectioned by sled microtomes or rotary microtomes equipped with metal knives. Resin embedded samples need microtomes which have a special retraction mechanism, to avoid mechanical damage of the knife and the sample. The blades are either manufactured of especially hardened steel for sections between 5 and 30 µm, for semithin sections (2–5 µm) and ultra thin sections (about 40–100 nm) glass or diamond knives must be used.

**Hard materials, like mineralized geobiological probes, cannot be sectioned by conventional microtomes unless the samples have been pre-treated: decalcification or demineralization by different chemicals. While these procedures may cause uncontrolled damage, when applied to the unsectioned samples, preferably control material should be preserved to evaluate the original structural context. Therefore, careful special section devices like diamond milling, saw microtomes, annular saws, or section/grinding-systems especially optimized for this purpose should be applied. About 30 µm thick sections of rather large samples can be achieved by these methods. The sections or grinded pieces can then be further processed, and also partly preserved for subsequent evaluation.**

Another method, available since several years, is the laser microtome which may contact free isolate minute samples out of the otherwise untreated entity. The samples must neither be pre-treated nor embedded. **Section thickness ranges between 10 and 100 µm. Special**
devices allowing for the isolation of minute tissue pieces like a single cellular nucleus.

A satisfying section quality requires a long time of experience especially to decide for the most appropriate procedure. While continuously new embedding products are commercialized, only little has changed concerning the basic techniques of standard microtomy, which is taught as a component part of the education of laboratory and technical assistants.

Mounting

For further histological processing, the sections must be mounted, most often on glass slides or on grids coated with a special synthetic film.

The major difficulty is the choice of the best possible sticking procedures in order to prevent both, a floating resp. a loss of the sections during the staining protocol and undesired background staining by the glue. While before often unreliable "home-made" coating procedures, e.g., by chromic sulphate or polyllysine, were used, now various types of pretreated glass slides are commercially available, they have been optimized for selected histological procedures. Thick sections, e.g., sections that are produced by saw microtomes or cutting/grinding-systems, have to be glued to object slides by special sticking resins that, e.g., harden with ultraviolet light; in this aspect, histological sample preparation is quite similar to the preparation of geological thin ground-sections.

Biophysical analyses of geobiolocal samples require mounting according to the planned technique (see list of Handbooks of the suppliers). In, for example, X-ray investigations (micro fluorescence analyses), the mounting must be free of auto fluorescence; the sometimes long lasting measuring intervals need a stable non-vibrating mechanical stability.

Staining

Multiple histological staining methods allow identifying cellular structures, tissue components, and organ systems, also enzymatic processes and involved macromolecules. It is even possible to clearly differentiate between organic and inorganic material as well as decaying from living material by selective marking of minerals and molecules.

Stainings can be performed by use of natural stains and dyes or by chemicals often designed for certain projects. New fields of histological analyses have been launched by immunohistochemical stainings combined with fluorescent markers which are very selectively binding to particular proteins. Differential and double stainings, also section series with alternatively stained sections, have revealed important insight into basic cellular processes, information processing, and also pathological resp. genetic changes. The standard protocols are well documented including their modifications for the application to tissues of various origins.

For any staining it is necessary that the structures that should be stained are free of any embedding media. This means that for standard-histology the paraffin has to be removed by special procedures so that the staining chemicals can react with the tissues. If samples are embedded in resins, this process is more crucial and difficult; several resins cannot be removed and accordingly only the surface of the section provides reactive structures for staining.

The staining of cellular structures and tissues is based on chemical affinities of the stains with particular tissues resp. tissue components. In many cases, a particular pretreatment is necessary for adequate results. Pretreatments may be, for example, mordants, which modify the stain so that it reacts with the tissue or modifies the tissues so that the stain can react with the tissue. In some cases mordants generate a reaction that transformed the water soluble stain into a water- and alcohol-stable lacquer. This chemical reaction can, for example, be induced by metal ions like tungsten or molybdenum or by alkali ions like lithium, sodium, or barium (Böck, 1989; Burck, 1988).

Stainings are usually composed of three steps: in the first step cellular structures like nuclei, etc., are stained. In the second step cell plasma and some extracellular structures are stained, and in the third step the surrounding matrix is stained.

As a result of the staining, the nuclei, cells, various fibers, and other structures or chemical components in a tissue have particular colors that allow differentiation and several diagnostics. Some stainings also provide particular optical characteristics to tissues; they may for example provide polarizing effects to the tissue. Other procedures provide fluorescence-effects or particular ultra-violet-effects of the polished surface and allow further investigations (Figures 1 and 2).

While traditional stainings induce a chemical reaction within tissues and may stain in an unselective way (Horobin and Kiernan, 2002), immunohistology (Polak and Noorden, 2003) selectively marks specific epitopes, proteins, and molecules bound to membrane or subcellular structures. Traditional and immunohistochemical analyses under the light microscope meet the limits of resolution at magnifications of about 1,200×; therefore, immunohistological methods have been upgraded for analyses under the TEM, either by pre-embedding or post-embedding staining protocols.

While traditional stainings are used for topographic and micro anatomical investigations, immunohistological stainings are used to understand the functional context of molecular processes and to allocate the regions in which these processes take place in an organism or in a tissue.

These refined immunohistological staining methods have also been applied to sections of geobiological samples. The analyses could demonstrate biological compounds, thus serving as biomarkers, and in some cases even disclose the genetic origin of the fossilized organisms.
Histology, Figure 1 Cross section of a human long bone under different microscopic optics (thin-ground section of a non-demineralized sample). (a) Under through light with minimal color saturation; (b) same illumination with maximum color saturation; (c) differential interference contrast (After Nomarski). Already without staining procedures characteristic structural details and varying densities can be identified. (a–c same magnification.)

Histology, Figure 2 Section series through a chondral ossification zone of a demineralized porcine long bone after various differential histological staining procedures (Protocols according to Böck et al., 1989; Young and Heath, 2000). (a) Masson Goldner A; (b) Masson Goldner L; (c) Masson Goldner ResF A; (d) Masson Goldner ResF L; (e) Crossmon A; (f) Crossmon L; (g) Azan after Geidis; (h) Movat Pentachrome; (i) Safranin Fastgreen. The layers of young bone (upper area), cartilage (middle area), and secondary ossification area (below) can be analyzed concerning their different structural details (a–i same magnification).
Conclusion

Geobiology is defined as field of science which analyses the interaction of the environment with living matter. Mainly fossilized samples are investigated concerning their systematic relationship and – even more – the origin of this specific form of life. Research results can find their application not only in a better understanding of our life on earth, but also in exploring putative extraterrestrial biology. Another aspect is the transfer of basic sciences to various aspects of biomedical research like the synthesis or degradation of bones and teeth or the control of biogenic mineralization processes. All these research topics cannot be planned and performed or adequately evaluated without a thorough knowledge of the biological structures mainly based on the comparison of living and fossilized specimen. Histology delivers the methods to topographically analyze organisms, organs, tissues, and even molecules. Their modifications by environmental or biogenic processes can be recognized by the investigation of their complex interaction.

Perspectives

Histological investigations provide a huge field of research for biology and geobiology. Both fields have to be combined, since geobiological samples often can only be interpreted with reference to known biological samples. Some examples are histological sections of dinosaur bones or teeth that have to be compared to bones and teeth of recent organisms, others are the discoveries at special fossil-sites, like the Messel pit, the Burgess-Shale, the rocky Hunsrück region, etc., which may provide interesting information about fossilized microscopic structures. And even the investigation of recent samples like biofilms or coatings is of major relevance to understand their composition and emergence in order to discriminate between biogenic and abiotic origin.

Summary

Histology provides methods to topographically investigate the structure of cells, tissues, and organs down to the molecular level, especially in geobiology as reference to recognize, for example, the phylogenetic origin and age and also as a basis to analyze biological in comparison to bio geological turnover processes, which induce structural changes via environmental and climatic conditions.

Bibliography


Cross-references

Animal Skeletons, Advent
Animal Biocalcification, Evolution
Biofilms
Biomarkers (Molecular Fossils)
Calcification
Calcified Cyanobacteria
Carbonate Environments
Fluorescence-In-Situ-Hybridization (FISH)
Immunolocalization
Metalloenzymes
Metals, Acquisition by Marine Bacteria
Microbial Biominalization
Microbial Communities, Structure, and Function
Microbial Mats
Organomineralization
Scanning Probe Microscopy (Includes Atomic Force Microscopy)
Sponges (Porifera) and Sponge Microbes

**HOT SPRINGS AND GEYSERS**

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**Definition**

*A hot spring is a* discharge of hot (>35–40°C) water from a vent at the Earth’s surface.

*A geyser is a* hot spring characterized by intermittent, turbulent discharges of boiling water and steam.

*A sublacustrine hot spring* is a hot spring that discharges from the floor of a lake.

**Introduction**

A hot spring is characterized by discharge of hot water from a vent. There is, however, no universally accepted definition of “hot” and the temperature for distinguishing a “warm spring” from a “hot spring” remains contentious (Pentecost et al., 2003). In general usage, a hot spring is one with vent water temperature between about 40°C and boiling point (Renaut and Jones, 2000). It must be remembered, however, that boiling temperature changes with altitude; thus, boiling in Yellowstone National Park occurs at ~92°C, whereas in New Zealand geothermal areas, which lie closer to sea level, it is at ~100°C.

The term “geyser” is derived from “Geysir,” located in southwest Iceland. First mentioned in historical records from 1294, the Icelandic word “geysir” means “gusher” or “one who rages.” Accordingly, a geyser is regarded as hot spring that is characterized by intermittent, turbulent discharges of boiling water and steam (Bryan, 2005, 2008).

Geyser and hot springs are end members of a continuous spectrum that includes “intermittent springs” that experience periodic overflows but never erupt, and “pulsating springs,” “perpetual spouters,” or “ebullient springs” that are characterized by continual eruptions. Some hot springs discharge on the floors of lakes and in caves.

**Distribution**

Hot springs and geysers are found on every continent except mainland Antarctica (Waring, 1965). Many hot springs and geysers, known from historical records, have ceased activity because of natural and anthropogenically induced changes to their plumbing systems. Yellowstone National Park, USA., El Tatio located high in the Andes of northern Chile, the Taupo Volcanic Zone on the North Island of New Zealand, Kronotski National Park in Kamchatka in eastern Russia, and Iceland are the largest known geothermal fields with numerous hot springs and geysers. Bryan (2008), for example, estimated that more than 50% (~500) of known geysers are located in Yellowstone National Park, with another 385 in the other four major geothermal areas. Low-enthalpy hot springs that discharge at maximum temperatures of about 35–60°C are much more common.

**The plumbing system**

The hot water discharged by a hot spring or geyser is the final, surface expression of a complex system that involves water, a heat source, and an intricate underground plumbing system. The water is derived from meteoric waters that have seeped deep into the Earth’s crust, supplemented in some regions by connate, magmatic, or metamorphic waters. Irrespective of its origin, some spring or geyser water may come from depths of ~4 km where it has been magmatically heated to temperatures of >225°C. In other settings, however, meteoric waters may only penetrate to shallow depths before being expelled from a low-enthalpy spring vent. The plumbing system of a hot spring is unrestricted and allows continuous movement of water to the surface. In contrast, a geyser has a restriction within its plumbing system, commonly a sinter caprock that precludes continuous vertical flow. The configurations of these plumbing systems remain unknown because of the difficulties in accessing them. Nevertheless, schematic depictions of vast underground, water-filled chambers are probably incorrect. Instead, it seems that the plumbing systems are formed of complex networks of faults, fractures, small cavities, and strata with variable permeability. Geyser are less common than hot springs because of the special plumbing system requirements.

Water trapped below a restriction in a geyser plumbing system will be heated by steam that is moving upwards from deeper parts of the system. The reservoir will then empty as the trapped superheated water is violently discharged. Such an eruption will continue until the reservoir is emptied. The magnitude, frequency, and duration of the eruption depend on reservoir size, the rate of heat transfer, and the rate at which water refills the system once emptied by the eruption. By necessity, therefore, geysers can only exist if the bedrock can withstand the pressures created by the steam and if the bedrock is permeable enough to allow rapid reservoir replenishment.
**Water acidity and composition**

Most springs and geysers discharge alkaline water with a pH $>7$. Most high-enthalpy geothermal areas (e.g., Taupo Volcanic Zone of New Zealand), however, include springs that discharge acidic waters with a pH $<7$ and commonly $<3$. The acidic spring waters usually form through oxidation of $\text{H}_2\text{S}$ and (or) $\text{SO}_2$ that move upward with steam released upon subsurface boiling and are then absorbed by shallow groundwaters. The pH of the spring water is important because it controls the type of biota living on the discharge apron and the minerals that are precipitated in and around the spring vent.

The composition of the spring water is mainly a function of reactions that take place between the bedrock and the water as it circulates through its plumbing system. In this context, the composition of the bedrock is critical because that will determine the elements that can be transferred to the groundwater.

**Precipitates**

The type of precipitate that forms in association with a hot spring or geyser is fundamentally controlled by water composition. Thus, waters containing $\text{SiO}_2$ may produce opal-A ($\text{SiO}_2\cdot n\text{H}_2\text{O}$) (Walter, 1976; Cady and Farmer, 1996; Jones et al., 1997), waters containing $\text{CaCO}_3$ may give rise to calcite and (or) aragonite (Renaut and Jones, 1997), and acidic waters containing $\text{Fe}$ may produce jarosite [$\text{KF}_3(\text{SO}_4)_2(\text{OH})_6$], lepidocrocite ($\gamma$-$\text{FeOOH}$), and (or) hydrous ferric oxide (mainly goethite: $\alpha$-$\text{FeOOH}$) (Jones and Renaut, 2007).

**Silica**

Water discharged from hot springs and geysers commonly has a high content of dissolved $\text{SiO}_2$ (e.g., Geysir with $\sim$500 ppm $\text{SiO}_2$). With cooling and evaporation, the waters become supersaturated with respect to amorphous silica, and non-crystalline opal-A containing 1.5–15 wt% $\text{H}_2\text{O}$ (as molecular water and hydroxyl) is precipitated. Siliceous sinters precipitated around hot spring and geyser vents have been called “geyserite.” Morphologically similar to stromatolites because of their thin laminae and botryoidal structures, geyserite was considered abiogenic because it lacked evidence of permineralized microbes and grew in hot waters that were presumed sterile (Walter, 1976). Scanning electron microscope imaging of “geyserite” collected from the vent areas of many springs and geysers on the North Island of New Zealand, Yellowstone, and elsewhere, however, revealed the presence of well-preserved microbes (Cady and Farmer, 1996; Jones et al., 1997, 2001) and thereby demonstrated that some “geyserite” was not abiogenic.

Sinter precipitated in hot springs and geysers commonly contains significant quantities of other elements, including $\text{Fe}$, $\text{Al}$, $\text{Au}$, $\text{Ag}$, and $\text{As}$. Orange siliceous sinters on the submerged shelf around Champagne Pool, Waiotapu (Figure 1b), for example, are rich in $\text{As}$, $\text{Sb}$, $\text{Tl}$, and $\text{Hg}$, and contain $\sim$100 ppm $\text{Au}$ and $\sim$330 ppm $\text{Ag}$. Such trace element concentrations commonly appear to be related to microbial adsorption and complexation.

**Calcite/aragonite**

Folk (1994) suggested that calcite as opposed to aragonite precipitation in hot spring and geyser systems is controlled primarily by water temperature (calcite $< 45^\circ\text{C}$ > aragonite) and (or) $\text{Mg}:\text{Ca}$ ratio (calcite $< 1:1$ > aragonite). Nevertheless, both calcite and aragonite are found around some hot springs and geyser vents where water temperatures are $>45^\circ\text{C}$. Spring waters discharged with high $\text{PCO}_2$ may, through rapid $\text{CO}_2$ degassing, quickly attain very high levels of supersaturation with respect to $\text{CaCO}_3$. This commonly seems to be responsible for calcite and aragonite precipitation even if the waters are $>75^\circ\text{C}$ and have a $\text{Mg}:\text{Ca}$ ratio of $<1:1$. Bizarre and unusual crystal morphologies also seem to be consequence of the high saturation levels.

**Fe-rich precipitates**

Non-crystalline hydrous ferric oxides (HFO), lepidocrocite, alunite, jarosite, pyrite, and clay minerals (kaolinite) are commonly precipitated in acidic, hot spring systems (Figure 1e). Some non-crystalline HFO contains high concentrations of other elements, including $\text{As}$, $\text{Mn}$, $\text{Cu}$, $\text{Zn}$, $\text{Cd}$, $\text{Pb}$ (Jones and Renaut, 2007). Precipitation of the different Fe-rich minerals appears to be related to subtle changes in the pH and sulfate ($\text{SO}_4^{2-}$) of the spring waters.

**Biota**

Hyperthermophilic microbes, which thrive in waters with a temperature $>75$–$80^\circ\text{C}$, include Bacteria and Archaea (Sheehan et al., 2005). Most Archaea are in the form of cocci, rods, or discs (Stetter, 1996, his Table 3). These non-distinctive morphologies coupled with the problems of laboratory culturing due to their poorly known growth requirements and nutritional needs have made species identification difficult. 16S rRNA sequencing, however, has shown that a diverse array of hyperthermophilic Bacteria, and Archaea thrive at high temperatures and extremes of pH, redox state, and salinity (Stetter, 1996; Kvist et al., 2005). Among the Bacteria, *Aquifex pyrophilus* and *Thermotoga maritima* thrive up to 95°C and 90°C, respectively. Among the Archaea, members of *Pyrobaculum*, *Pyrodictium*, *Pyrococcus*, and *Methanopyrus* survive in temperatures up to 100°C. The thermoacidophile *Sulfolobus*, for example, thrives in sulfur-rich hot springs at a pH of 2–3 and temperatures up to 90°C.

An easy way of collecting microbes that live in the dangerous environs of hot springs/geysers is to place sterile glass slides in the water for 24–72 h. Microbes quickly colonize such substrates and remain attached once the slides are extracted from the hydrothermal waters.

**Microbe preservation**

Mineralized microbes are commonly found in precipitates that form in hot spring and geyser systems (Figure 2).
Such mineralization, which may involve replacement of the organic tissues and (or) encrustation around the microbes, must take place before the microbes are lost through decay. Microbe preservation is common in non-crystalline precipitates (e.g., opal-A, HFO) but rare in crystalline precipitates (e.g., calcite, aragonite, jarosite), possibly because the former generally precipitate at a faster rate than the latter and organic materials are less prone to destruction by crystal growth.

The microbes can play an active role (i.e., they mediate precipitation through modification of their microenvironment) or a passive role (i.e., they act as templates for precipitation by having nucleation sites on their surfaces) in their own mineralization. Experimental work and interpretation of naturally mineralized microbes indicate that microbes are probably passive during their own mineralization. There is, however, some evidence that some microbes may exert some influence over the initial pattern of opal-A precipitation (Jones et al., 2004).

HOT SPRINGS AND GEYSERS, Figure 1 Hot springs and geysers in the Taupo Volcanic Zone on the North Island of New Zealand (a, b, e, f) and El Tatio, northern Chile (c, d). (a) Prince of Wales Feathers and Pohutu during eruptive phase. Note silica precipitates on surrounding Geyser Flats, Whakarewarewa, Rotorua. (b) Margin of Champagne Pool showing exposed siliceous sinters (right) and submerged shelf covered with orange, gold- and silver-rich siliceous sinter. Water has a constant temperature of 75°C and pH of 5.4. Waiotapu geothermal area. (c) Siliceous sinter forming around margin of hot-spring pool, El Tatio. (d) Streamers of filamentous microbes in outflow channel from hot-spring pool, El Tatio. (e) General view of spring in northern part of Waiotapu geothermal area, water temperature of ~80°C and pH of 2.1–2.4. Submerged reddish-brown precipitates formed largely of noncrystalline As-rich hydrous ferric oxide, poorly crystalline lepidocrocite, and crystalline jarosite. (f) Black coating around small hot spring (T = 100°C, pH = 2.0) in Kerosene Creek area, north part of Waiotapu geothermal area formed of bitumen generated by the spring system.
SEM imaging commonly reveals microbes that appear to be superbly preserved (Figure 2). It is, however, very difficult to identify such microbes in terms of extant taxa because most of the taxonomically important features are lost and DNA is generally not preserved. Thus, identifications must rely solely on morphological features such as general configuration, length, width, and the presence or absence of septa. The problem is compounded by the fact that most extant taxa are defined by DNA sequencing and may not have been imaged to show their morphology.

Conclusion

Hot springs and geysers are spectacular geological features that encompass a wide range of environments that are commonly characterized by extreme conditions (high T, low pH). Although opal-A, calcite/aragonite, and various Fe-rich deposits are the most common minerals precipitated from hot spring and geyser waters, these spring deposits commonly contain significant amounts of other elements, including Au, Ag, As, S, and many others.

Many hot spring and geyser systems are inhabited by a diverse array of microbes (archaea, bacteria, cyanobacteria, fungi, diatoms) that flourish in the warm discharge waters. Such microbes are frequently preserved, typically in opal-A, and thereby provide some insights into the nature of the microbial communities that inhabit these complex environs. Such fossils have received considerable attention, as they are commonly considered analogous to silicified microbes that are found in much older deposits, especially those in the Precambrian.

Bibliography


Sinter mass and number, exists on Earth principally in combination with oxygen (in $\text{H}_2\text{O}$) or carbon (in hydrocarbon and organic compounds). The diatomic form, $\text{H}_2$ – although present in only trace abundance in the modern oceans and atmosphere – has played a central and evolving role in prebiotic and origin of life chemistry, in linking geochemical and biological cycles, and in shaping the structure, function, and interaction of microbial populations.

**Geochemistry of $\text{H}_2$**

**Geosphere**

$\text{H}_2$ is produced by the interaction of water and rocks through a variety of mechanisms (see review in Hoehler, 2005). Among processes that occur in widespread crustal rocks, perhaps the best known and most quantitatively important involves the reduction of water to $\text{H}_2$ by the ferrous iron components of rocks, which can be generalized as

$$2(\text{FeO})_{\text{rock}} + \text{H}_2\text{O} \rightarrow (\text{Fe}_2\text{O}_3)_{\text{rock}} + \text{H}_2,$$

with $(\text{FeO})_{\text{rock}}$ representing the ferrous iron component of igneous rock and $(\text{Fe}_2\text{O}_3)_{\text{rock}}$ representing the ferric iron component of alteration minerals (e.g., magnetite). The capacity for $\text{H}_2$ generation via this mechanism is greatest in minerals with high ferrous iron contents, such as the ultramafic minerals olivine and pyroxene, where the process is called serpentinization. Basalts, with a lower ferrous iron content, have a smaller capacity to produce $\text{H}_2$, and granites (where ferrous iron-bearing phases are typically present as minor components) smaller still. The rates of hydrogen generation by water–rock reaction depend significantly on both temperature and the exposure of fresh surface area to water.

$\text{H}_2$ may also be generated via the radiolytic cleavage of water by $\gamma$, $\beta$, or $\alpha$ radiation produced during decay of isotopes in the host rocks (Spinks and Woods, 1964). Although oxidative species generated simultaneously during the process have potential to recombine with the hydrogen, radiolysis is nonetheless believed responsible for elevated $\text{H}_2$ concentrations (up to millimolar levels) in fluid emanations from a number of continental rock units. This mechanism is generally most important for granites, in which the content of the principal radiogenic elements U, Th, and K is generally highest among major crustal rock types.

Fracturing of silicate rocks may also serve to liberate $\text{H}_2$ that is formed either in solid-state reactions involving trace water impurities in the mineral lattice (Freund et al., 2002) or by direct reduction of water at the fractured surface (Kita et al., 1982).

Among these processes, serpentinization offers the greatest potential capacity for $\text{H}_2$ generation (per unit volume of rock), by orders of magnitude. However, multiple factors – including mineral composition, fluid flow and chemistry, temperature regime, and mechanical processing – ultimately determine which mode of lithogenic $\text{H}_2$ production may prevail in a given environment.

### Cross-references

- Extreme Environments
- Hydrothermal Environments, Fossil
- Hydrothermal Environments, Terrestrial
- Microbial Biomineralization
- Microbial Silicification – Bacteria (or Passive)
- Sinter

### HYDROGEN

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**Synonyms**

Dihydrogen; $\text{H}_2$

**Definition**

A diatomic molecule consisting of two atoms of hydrogen or its heavier isotopes.

**Introduction**

Hydrogen, the most abundant element in the universe by mass and number, exists on Earth principally in combination with oxygen (in $\text{H}_2\text{O}$) or carbon (in hydrocarbon and
Atmosphere

H₂ is a trace component of the modern atmosphere, with an abundance of about 0.5 ppm (Levine, 1985) and a residence time of less than 1 year (Atkinson et al., 1979), but likely higher levels in the early atmosphere would have had important geochemical and biological implications. Atmospheric H₂ abundance reflects a balance of sources and sinks. Major source terms (excluding anthropogenic sources) include geological efflux (e.g., volcanic and hydrothermal vent emissions) and biological production. Sinks include oxidative atmospheric reactions, biological consumption, and escape to space. The low abundance of H₂ in the modern atmosphere reflects the dominance of oxidative reactions and biological consumption. The early, prebiotic Earth largely lacked such sinks and had a geological source term that was expectedly larger as a result of higher rates of geothermal emanation and a potentially more reducing character in the early mantle and crust (leading to higher H₂ concentrations in geothermal emansations). Estimates of atmospheric H₂ abundance at this time depend principally on theories regarding the oxidation state of the early mantle and crust, and consideration of the factors constraining H₂ escape to space. Even at the low end, these estimates generally predict atmospheric H₂ abundance of hundreds to thousands of parts per million, 3–4 orders of magnitude higher than in the present day.

H₂ itself is considered photochemically inert in the lower atmosphere (Levine, 1985), but it participates in a variety of reactions involving the radical products of photolysis. By serving as a sink for the oxidizing OH radical (Atkinson et al., 1979), and influencing the abundance of the atmospheric reductants H, HCO, and H₂CO (Canuto et al., 1983), H₂ directly affects the oxidation state of the atmosphere. As such, its relative abundance in the early atmosphere may have significantly affected redox speciation in the volatile nitrogen, carbon, and sulfur systems. In turn, this balance (e.g., the CH₄/CO₂ ratio, in the carbon system) affects the atmospheric radiation budget (through differing efficiencies of IR absorption), the potential for formation of photochemical hazes (Sagan and Chyba, 1997), and the formation and stability of simple organics in atmospheric chemical processes.

Above and beyond its role in buffering the oxidation state of the atmosphere through chemical interactions, escape of hydrogen to space can contribute to the irreversible oxidation of the planet, by carrying electrons out of the system (Walker, 1977). This process occurs at low rates in the modern, oxidized atmosphere, where the low abundance of H₂ results in low escape rates. However, H₂ escape from an early atmosphere containing orders of magnitude higher H₂ concentrations may have contributed substantially to the overall oxidation of the planet.

H₂ in prebiotic and origin of life chemistry

Prebiotic chemistry

The emergence of life must depend significantly on development of an inventory of abiotically generated compounds that can serve as building blocks for biochemistry. Two of the most potentially important endogenous sources for such compounds – synthesis in hydrothermal vent settings and in atmospheric chemical processes – are known or inferred to depend strongly on H₂ for the yield and nature of compounds formed. In vent settings, H₂ from water-rock reactions is implicated as the reductant in Fischer–Tropsch type synthesis of methane, higher hydrocarbons, and simple organic acids, and is predicted to support the synthesis of amino acids and other prebiotic compounds. Miller–Urey type spark discharge experiments that simulate atmospheric chemical processes have a similar dependence that is demonstrated experimentally. For example, the yield of amino acids from simpler precursors in such experiments depends directly on the abundance of H₂, regardless of whether reduced or neutral starting materials (i.e., CH₄/NH₃ (Miller and Urey, 1959), or CO₂/N₂ (Schlesinger and Miller, 1983)) are used. Given its significance in prebiotic synthesis and atmospheric redox and photochemistry on the early Earth (see above), H₂ likely played an important overall role in establishing the environmental and chemical context from which life emerged.

Origin of life

Although the specific chemistry leading to the origin of life is not known, H₂ is proposed in some scenarios to have been a key component of the earliest metabolism (Wächtershauser, 1993). The oxidation of H₂ serves a potentially important dual purpose with respect to such metabolism, in yielding both reducing power and H⁺. Reducing power is necessary for generating organic biomolecules when the carbon source is CO₂. The establishment of H⁺ gradients (specifically, across membranes) can be used to store energy for subsequent coupling to biochemistry. Such dual-purpose chemistry underpins the metabolism of a wide range of modern organisms, including those thought to represent Earth’s earliest life. The widespread availability of H₂ in geothermal emansations (e.g., Zobell, 1947; Elderfield and Schulz, 1996) and, presumably, in the prebiotic oceans and atmosphere would have made it a ubiquitous and reliable substrate on which to base the first metabolic chemistry.

H₂ and biology

The reversible oxidation of molecular hydrogen, H₂ ⇌ H⁺ + 2e⁻, is among the most widely utilized reactions in microbial metabolism. Hydrogenases, the enzymes which catalyze this transformation, are present in all three phyllogenetic domains of life (Fenchel and Finlay, 1995; Vignais et al., 2001; Schwarz and Friedrich, 2003). They are particularly widely represented in the Bacteria and Archaea, including the most deeply branching and least derived lineages (Vignais et al., 2001; Schwarz and Friedrich, 2003), suggesting that, exclusive of a possible role in origin of life chemistry, H₂ metabolism has been an important contributor to biochemistry since the very early stages of evolution.
Since its emergence in metabolism, H₂-based redox chemistry has come to be associated with an extremely broad range of microbial metabolic strategies, including oxygentic and anoxygentic phototrophy, anaerobic chemolitho- and chemoheterotrophy, and aerobic chemolithotrophy (Schwarz and Friedrich, 2003). H₂/H⁺ can serve as electron donor, acceptor, or both, in these processes. This broad metabolic utility and versatility casts H₂ in a highly central role in the ecology of microbial communities, and in the geochemistry they mediate.

Lithotrophic communities
Organisms capable of H₂ metabolism can potentially be supported directly by geochemical production of H₂ (by, e.g., water–rock reaction or radiolysis). As such, they have potential to exist independently of energy input from light or the direct products of photosynthesis, oxygen, and organic matter. Such communities are therefore frequently envisioned as important contributors to Earth’s earliest biosphere, to the rock-hosted subsurface biosphere, and, potentially, to subsurface life on other worlds. H₂-based, photosynthesis-independent communities have been documented or inferred for a range of systems and H₂-producing mechanisms, including vent fluids sourced in serpentinitizing systems, basalt-hosted aquifers, and continental groundwaters bearing a signal of radiolytic H₂ production.

Heterotrophic communities
Systems driven by anaerobic decomposition of organic matter typically host a great diversity of H₂-cycling organisms. Under anaerobic conditions, the complete degradation of complex organic molecules cannot be accomplished by a single organism. Instead, the overall process is mediated, in a series of individual steps, by communities of microorganisms having different metabolic capabilities (Schink, 1988). The individual steps are linked through the transfer of electrons from one group of microbes to another, with H₂ serving as one of the most commonly employed molecular carriers of electrons. The pool of H₂ in such systems is typically characterized by low concentrations (sub-nanomolar to micromolar) and rapid turnover, with resultant residence times frequently in the range of seconds (Hoehler et al., 2002). Combined with a strong influence on the thermodynamics of reactions that produce or consume it (resulting from its typically high stoichiometric coefficient in such reactions), this effect casts H₂ as a key regulator of degradation pathways, community structure, and carbon and energy flow in such environments. Specifically, fluctuations in H₂ concentration are documented, in a process known as “interspecies H₂ transfer,” to affect the H₂-cycling reactions by inhibition or stimulation, alteration of products, or even reversal (see review in Hoehler, 2005).

H₂ thus occupies a central role in anoxic ecosystems. Anoxia prevailed globally prior to about 2.4 billion years ago and continues to prevail in most organic-bearing aqueous environments (including ocean, lake, and wetland sediments and animal digestive tracts). In the past and present, such systems represent a major control on the redox chemistry of the oceans and atmosphere, and the ultimate biological filter on material passing into the rock record.

Phototrophic communities
Most of the known photosynthetic microorganisms, representing both anoxygenic and oxygentic photosynthesis, possess a capability to engage in H₂ metabolism (Schwarz and Friedrich, 2003; Vignais et al., 1985). Most anoxygenic photosynthesizers, including representatives of the purple sulfur, purple non-sulfur, green sulfur, and green non-sulfur bacteria, can utilize H₂ as an electron donor for photosynthetic carbon fixation, and several are also capable of utilizing H₂ as an electron donor for the chemical reduction of inorganic oxidants. Some representatives of the cyanobacteria (oxygentic photosynthesizers) are similarly capable when operating in an anoxygenic photosynthetic mode of metabolism. H₂ is produced obligately during the conduct of nitrogen fixation and can also be photo-produced by the nitrogenase enzyme complex independently of nitrogen fixation. These capabilities, which occur widely among the cyanobacteria and anoxygenic photosynthesizers, are among the principal mechanisms being explored for biological production of H₂ as an alternative energy source. H₂ is also generated as a product of fermentation, a capability demonstrated in some cyanobacteria and purple sulfur bacteria. The fermentative mode of H₂ production appears to dominate the H₂ cycle in a variety of cyanobacterial “mats,” resulting in significant fluxes of H₂ out of the system (Hoehler et al., 2001; Hoehler et al., 2002). During the 1–2 billion years of Earth’s history in which cyanobacterial mats dominated biological productivity on Earth, such fluxes may have contributed significantly to atmospheric H₂ levels and, by enhanced rates of H₂ escape to space, the overall oxidation of the planet.

Eukarya
Although not as widespread as in the Bacteria and Archaea, H₂ metabolism is also found in the Eukarya. In a variety of deep-branching anaerobic Eukaryotes, metabolism is tied to, or dependent on, an H₂-based symbiosis with Bacterial or Archaeal partners (see review in Fenchel and Finlay, 1995). The driver of most of these symbioses is an H₂-generating organelle, the hydrogenosome, which appears in some cases to be derived from a mitochondrion. These hydrogenosomes carry out fermentation, rather than oxidative phosphorylation, of pyruvate – and thereby produce H₂. Microbial symbionts (which may include methanogens, sulfate reducers, and (in one case) photosynthetic purple non-sulfur bacteria) increase the energetic yield of this fermentation by consuming the end product H₂. The symbiosis significantly enhances the growth of the host, and is, in some cases, so evolved that methanogen symbionts have lost their cell walls, and exhibit synchronization of cell
division with the host. Among photosynthetic Eukarya, green algae exhibit an active H₂ metabolism, and are considered a promising potential source of “biohydrogen” for alternative energy systems.

Summary

Hydrogen has played an important role in Earth’s geochemistry and biology since the earliest stages of the planet’s history. H₂ is produced by a variety of geochemical processes and is postulated to have been a significant component of the early, prebiotic atmosphere, where it had direct or indirect effects on atmospheric redox chemistry and radiation budget, and contributed to planetary oxidation by escape to space. Prebiotic and origin of life chemistry may have significantly involved H₂, and its prominent use among deeply branching organisms in the tree of life reflects, at very least, a key role in metabolism since the biosphere’s early stages. In the modern biosphere, H₂ biochemistry is associated with a broad variety of metabolic strategies, in all three domains of life. Although it now represents a trace component of the oceans and atmosphere, the significance of H₂ for planetary chemistry persists through its central role in the microbial world.

Bibliography


Cross-references

Acetogens
Anoxygenic Oxidation of Methane with Sulfate
Astrobiology
Chemolithotrophy
Early Earth
Fermentation
Hydrothermal Environments, Terrestrial
Microbial Communities, Structure, and Function
Microbial Degradation
Origin of Life
Photosynthesis
Sulfate-Reducing Bacteria
Symbiosis
Terrestrial Deep Biosphere

HYDROTHERMAL ENVIRONMENTS, FOSSIL

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Synonyms
Black and White Smoker communities; Volcanogenic massive sulfide deposits – VMS

Definition
“Black smokers” represent extreme hydrothermal activities that are commonly located in deep sea environments
related with mid-ocean ridges and/or oceanic crust fault systems. Hydrothermal vent systems result from hydrothermal circulation of fluids controlling the transfer of energy and various matter from the interior to the surface of the oceanic crust, respectively the sediment surface. This hydrothermal circulation is a fundamental geological process and has been permanently influencing the chemistry of the oceans, and in consequence, also the composition of the entire earth crust. Seafloor hydrothermal activity has a major impact on the chemistry and life processes of the oceans (Parson et al., 1995; Humphris et al., 1995; van Dover, 2000). This entry focuses on volcanicogenic massive sulfide deposits (VMS) and not on sedimentary exhalative deposits (SEDEX) – however, the boundary between both environments is not very strict. Massive sulfide deposits, hydrothermally altered rocks, and special communities of organisms are an important record of this process. Ancient seafloor hydrothermal activity is recorded in massive sulfides from the 3.5 GY-old Pilbara region of Western Australia (Barley, 1992). The new findings of hydrothermal vents with microbialites from 1.43 GY-old Precambrian rocks in northern China (Li and Kusky, 2007) are also of great importance due to very well-preserved filamentous microbial communities forming massive sulfidic microbialites (Li and Kusky, 2007).

Geobiological notes
Hydrothermal vents have become one of the most fascinating fields of research in marine sciences. The recognition of complex vent communities consisting of organisms dependent on chemolithotrophy and extreme conditions of very high temperature, pressure, and chemistry at the ocean ridges was a great discovery (Humphris et al., 1995). There are many reasons to believe that life originated at hydrothermal vent environments in the late Hadean. However, only few publications are dealing with the fossil record of hydrothermal environments connected with preserved organism communities.

There are about 20 known Phanerozoic VMS sites with preserved hydrothermal vent communities, ranging from the Silurian to the Eocene. Most of these sites are found in the Ural Mountains and on Cyprus, some of them in Ireland, California, Philippines, and New Caledonia. All occurrences are linked to remains of oceanic crust (ophiolites). The investigation of vent systems is still a challenge, because the sites are normally small and most of them were destroyed during subduction of oceanic crust.

The vent communities contain assemblages of inarticulate and rhynchonellid brachiopods, gastropods, bivalves, and also monoplacophorans (Little et al., 1998; Kiel and Little, 2006). Remains of worm tubes are very characteristic and may be related to alvinellid polychaetes and pogonophorid-vestimentiferan chemotrophic worms. Most of the fossil taxa of the vent sites are endemic. However, the diagenetic loss of the original shell material makes it very difficult to classify the worm tubes in a modern taxonomic scheme. Surprisingly, no arthropods were found at the fossil sites, whereas arthropods are very common in modern vent environments. There is an ongoing discussion on the origin of the vent fauna. New groups of organisms may have immigrated from the ambient environment and adapted to the extreme conditions of the vent sites and vice versa, some of them have left this environment. The origin of the vent faunas is thoroughly discussed in Kiel and Little (2006) and Little and Vrijenhoek (2003). Kiel and Little (2006) suggested that the mollusk seep faunas (hydrothermal vents and cold seeps) have a significantly longer evolutionary history than normal marine mollusks and show a tight taxonomic relationship with deep-sea mollusk faunas. The oldest known Phanerozoic hydrothermal vent is of Silurian age (Little et al., 1998). The discovery of the Mesoproterozoic (1.43 GY) hydrothermal vents in northern China was of great importance due to very well-preserved filamentous microbial communities forming massive sulfidic microbialites (Li and Kusky, 2007).

Conclusion
Fossil hydrothermal vent systems are known since the early Archaean and represent important geobiological environments. These environments have a significant key role in biological evolutionary processes. However, only few (ca. 20) sites are known worldwide in the Phanerozoic with preserved vents faunas. The oldest known fossil hydrothermal vent system with a metazoan community is known from the Silurian of the Ural Mountains (Little et al., 1998). Many of the vent organisms probably have a significantly longer evolutionary history than normal marine taxa (Kiel and Little, 2006).

Bibliography


Cross-references
Cold Seeps
Deep Biosphere of the Ocean Deep Sea
Extreme Environments
Hydrothermal Environments, Marine
Iron Sulfide Formation
Origin of Life

HYDROTHERMAL ENVIRONMENTS, MARINE

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Definition
Marine hydrothermal environments are one of the most extreme environments on Earth, yet they support highly productive biological communities over a wide range of physical and chemical environments. The following review is a general discussion of the types of microorganisms found in these different environments preceded by brief descriptions on hydrothermal fluid generation, mineral deposit formation, and microbial metabolism.

Introduction
The discovery of deep-sea hydrothermal vents and the lush biological communities associated with them in the late 1970s ushered in a new era for marine biologists. Prior to this discovery, life in the deep-sea was thought to be dependent on the settling of detrital material from the productive, overlying surface waters. While this is true for much of the ocean basins, the level of production observed at these newly discovered vent sites was too great to be supported by these mechanisms alone. It soon became evident that life in these environments was supported by chemosynthetic primary production in which microorganisms harnessed the abundant geochemical energy available in the hydrothermal fluids. This challenged one of the basic ecological premises that all ecosystems on Earth were dependent on light energy and driven by photosynthetic primary production.

Since those initial revelations, research efforts have focused on assessing the microbial diversity and attempting to understand the intimate associations between microbial productivity, geology, and geochemistry in marine hydrothermal environments. This article is a general discussion of the trends that are beginning to emerge from these efforts. Included in the discussion are overviews of the geologic setting of marine hydrothermal systems, the generation of hydrothermal fluids from different geologic settings, and the types of structures formed.

For additional in-depth reviews, the Geophysical Monographs published by the American Geophysical Union (AGU) are valuable resources (Humphris et al., 1995; German et al., 2004; Wilcock et al., 2004; Christie et al., 2006).

Geological setting
Most seafloor hydrothermal vent systems are associated with extensional tectonic activity and heated by magmatic heat as it is convected into the crust (Seyfried and Mottl, 1995). The most well-studied areas of hydrothermal circulation are along divergent plate boundaries where basaltic seafloor is made and mid-ocean ridges (MOR) arise (Figure 1). Hydrothermal circulation along MORs results from the active heating of seawater that percolates through newly formed basaltic crust. Hydrothermal venting is also commonly found along convergent plate margins where an oceanic plate is subducted beneath a continental plate forming island arcs and back-arc basins (Christie and Fisher, 2006). Vents along back-arc basins are actively heated in the same way as those along MOR, although the fluids tend to be more heterogeneous due to the variability in magma composition and additional inputs from the subducting plate (GAOM et al., 2006). A third location where seafloor hydrothermal systems can be found is at intraplate volcanic hot spots. Volcanic hot spot systems are not directly associated with tectonic plate margins, but are actively heated as plumes of molten magma push up through the mantle and crust (Seyfried and Mottl, 1995).

The recently discovered Lost City vent field more than 15 km off axis of the Mid-Atlantic Ridge (MAR) represents a new type of seafloor hydrothermal system as it appears to be at least partially heated by exothermic reactions between mantle derived peridotite and seawater (Kelley et al., 2001). These reactions have a dramatic impact on the composition of the hydrothermal fluids, which in turn affects the microbial community as discussed in the following sections. Although Lost City represents the first vent type of its kind discovered, similar systems are believed to exist along margins of slow- and ultraslow-spreading ridges where uplifting of ultramafic massifs are common.

Hydrothermal fluids
Seawater heated in contact with subsurface rocks dramatically alters the chemical composition of the end member fluids. The degree of alteration is influenced by several factors including the initial composition of the seawater, the type and structure of the host rock, the presence of sediment overlaying the host rock, and the type, depth, and size of the heat source (reviewed in Tivey, 2007). Understanding these interactions and how they affect the composition of hydrothermal fluids is important as the fluids set the physiological parameters and provide the metabolic menu for colonizing microbes. For comparison, representative compositions of hydrothermal fluids from different settings are presented in Table 1.
Basalt hosted hydrothermal fluids

Water–rock reactions begin as cold seawater infiltrates shallow basalt in the recharge zones (Figure 2). Initial low temperature reactions (≤60°C) result in the removal of alkali metals from seawater and the oxidation of basaltic minerals (i.e., olivine and plagioclase), with the concomitant leaching of silica (Si), sulfur (S), and sometimes magnesium (Mg) from the minerals (reviewed in Alt, 1995). Magnesium is eventually completely removed from the fluids as Mg-rich clays precipitate at temperatures above 150°C. Anhydrite (CaSO₄) precipitation and seawater sulfate (SO₄²⁻) reduction also occur in the recharge zone at temperatures above 150°C, resulting in the complete removal of SO₄²⁻ from the fluids. As the fluid continues through the crust, reactions with iron-bearing minerals such as olivine and pyroxene result in highly reducing fluids with elevated concentrations of hydrogen gas (H₂) (Tivey, 2007). Upon reaching the reaction zone where fluids are heated upward of 400°C, the fluid obtains its chemical signature by leaching S and metals (copper-Cu, iron-Fe, manganese-Mn, zinc-Zn) from the surrounding rocks (Alt, 1995). Phase separation may also occur in the reaction zone if temperature and pressure are greater than 407°C and 298 bars, respectively (Von Damm, 1995). Phase separation can have a significant impact on the composition of end member fluids as volatiles such as hydrogen-sulfide (H₂S) tend to partition in the vapor phase while metals such as Fe become enriched in the liquid (brine) phase. Gases such as helium (He), methane (CH₄), and carbon dioxide (CO₂) may also be added to the hydrothermal fluids as volatiles from the underlying magma (Alt, 1995). In total, these reactions result in end member fluids that are acidic (pH 2.8–4.5), highly reduced, rich in H₂S and metals, and heated up to 400°C. The fluid is also extremely buoyant relative to seawater and is forced back to the seafloor through the discharge zone and may emerge as diffuse or focused flow depending on the degree of subsurface mixing during the ascent (Figure 2).

Other factors, such as differences in host rock and sediment cover, can contribute to the chemical composition of end member fluids. The impact that different host rocks have on fluid chemistry is best illustrated by the ultramafic hosted Rainbow vent field along the MAR.
Hydrothermal Environments, Marine, Table 1  Representative chemical compositions of hydrothermal fluids from different geologic settings

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Deep seawater</th>
<th>Mid-ocean ridge</th>
<th>Back-arc basin</th>
<th>Sediment hosted</th>
<th>Rainbow vent field</th>
<th>Lost City</th>
</tr>
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<tbody>
<tr>
<td>T (°C)</td>
<td>2</td>
<td>&lt;405</td>
<td>278–334</td>
<td>100–315</td>
<td>365</td>
<td>&lt;91</td>
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<td>pH (25°C)</td>
<td>8</td>
<td>2.8–4.5</td>
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<td>0</td>
<td>0</td>
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<td>255–790</td>
<td>412–668</td>
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<td>548</td>
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<td>Na, mmol/kg</td>
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<td>210–590</td>
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<td>6.5–89</td>
<td>160–257</td>
<td>67</td>
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<td>10.5–79</td>
<td>13.5–49.2</td>
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<td>1.10–5.98</td>
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<td>0.035–0.5</td>
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<td>13</td>
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<td>CO₂, mmol/kg</td>
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<td>3.56–39.9</td>
<td>14.4–200</td>
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<td>N/A</td>
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<td>&lt;0.02–0.652</td>
<td>0.148</td>
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</table>

Modified after Tivey (2007). bdl below detection limit

(Douville et al., 2002). Fluids from Rainbow vent field are lower in pH, have much higher Fe concentrations, are enriched in H₂ and CH₄, and are depleted in H₂S relative to fluids from most MOR systems (reviewed in McCollom, 2007). These differences in composition are related to the same serpentinization reactions that fuel the Lost City vent field (see below) although the heat sources differ. Sediments overlying the host rock can also affect the composition of end member fluids (German and Von Damm, 2004). In general, sediment-hosted systems generate fluids that are higher in pH (5.1–5.9) and lower in metal contents than those from unsedimented systems. Chemical differences largely depend on the amount and type of organic matter present in the sediments (Tivey, 2007).

Lost City hydrothermal fluids

Hydrothermal fluid generation at Lost City begins much in the same way as fluid generation at MOR systems, with the infiltration of seawater into rock. However, the rock is not basalt but rather mantle-derived peridotite which reacts with seawater to form serpentine (Kelley et al., 2001). Serpentinization reactions result in the hydration of minerals such as olivine and produces large amounts of heat, H₂ and CH₄. This heat is believed to at least partially drive hydrothermal circulation at Lost City although other heat sources have been suggested (Kelley et al., 2001; Allen and Seyfried, 2004). Regardless of the heat source, the end member fluid chemistry is dramatically different than MOR vent systems and is indicative of seawater–rock reactions below 150°C. The most striking differences are the alkaline pH (≈10–11), the lower temperatures (up to 93°C), the elevated calcium (Ca) content, and the low concentrations of metals and H₂S found in the fluids (Kelley et al., 2001, 2005). Methane and H₂ concentrations are also elevated relative to basalt-hosted MOR systems (Table 1).

Hydrothermal structures

MOR sulfide deposits

When acidic hydrothermal fluids rich in reduced chemical species interact with cold, oxygenated seawater, minerals rapidly precipitate out of solution forming large deposits (reviewed in Juniper and Tebo, 1995). Mineral phases found in these deposits include sulfides, sulfates, oxides, hydroxides, silicates, and carbonates. Deposit formation can occur in the subsurface as the fluids ascend through oceanic basalt and mix with infiltrating seawater or directly on the seafloor as high temperature fluids erupt into bottom seawater. Subsurface precipitation of minerals generates stockwork deposits in the shallow crust and results in low-temperature venting on the seafloor. Low-temperature fluids are generally low in metal sulfides and as a result, precipitate oxides upon reaching the seafloor. In contrast, fluids that have not been diluted and cooled in the subsurface rapidly precipitate metal sulfides such as pyrite (FeS₂), chalcocite (CuFeS₂), and sphalerite (ZnS) upon mixing with seawater. Precipitation of metal sulfides results in the appearance of “black smoke” and ultimately forms massive sulfide deposits (>60% sulfide minerals). Several types of sulfide features are found in vent fields, with the most spectacular being the black smoker chimney (Figure 3a). Other sulfide structures generated at marine hydrothermal vents include diffuse flow beehives, flanges, and complex flow sulfide mounds (Van Dover, 2000). In all cases, active sulfide features provide extreme physical and geochemical gradients that
sustain diverse microbial populations even after fluid flow has ceased.

Lost City carbonate deposits
In contrast to the sulfide structures found along basalt-hosted MOR systems, hydrothermal fluids venting at Lost City produces large white chimneys and flanges composed primarily of aragonite (CaCO$_3$) and brucite (Mg(OH)$_2$) (Figure 3b) (Kelley et al., 2001). Active structures are highly porous and emit fluids from complex networks of centimeter sized channels (Kelley et al., 2005). Extinct structures are less porous resulting from the replacement of aragonite with calcite (CaCO$_3$) and the dissolution of brucite. The largest structure at Lost City, Poseidon, is over 60 m tall, making it the tallest seafloor hydrothermal feature yet discovered (Kelley et al., 2001).

Microbial metabolism
The hydrothermal fluids provide the energy and nutrients for a thriving microbial ecosystem. Additionally, the porous sulfide deposits are excellent habitats for the metabolic diversity of microorganisms.

**Terminology**
Energy sources for microbial metabolism include light-dependent reactions (less important at deep-sea vents) and light-independent chemical reactions and are described by the prefixes photo- and chemo-, respectively. Energy sources are further classified depending on whether the source of electrons is inorganic (-litho-) or organic (-organo-). For carbon sources, the suffix -autotroph describes an organism that obtains carbon from an inorganic source (CO$_2$), while the term -heterotroph describes an organism that obtains carbon from an organic source. For example, a microorganism that is able to gain energy and electrons from H$_2$ and use this energy to fix CO$_2$ is referred to as a chemolithoautotroph, while humans who require organic carbon and energy sources are described as chemoorganoheterotrophs, as are many microorganisms. Some microorganisms are not obligatory autotrophs and will assimilate organic carbon substances if they are available. The term mixotroph is used to describe these organisms which appear to be common in most environments.

Microorganisms also have varying requirements for oxygen (O$_2$) as an oxidant. Some organisms have an
obligate need (aerobes) while others cannot tolerate O2 at all (anaerobes). Furthermore, some organisms require low levels of O2 (microaerophile) while others can switch between aerobic and anaerobic metabolisms (facultative anaerobe).

At deep-sea vents, the heat and pH preference provides an additional habitat selection. All microbes have a preferred temperature range at which optimal growth is achieved. Psychrophiles have optimal growth ≤15°C, mesophiles grow optimally between 25 and 40°C, thermophiles and hyperthermophiles grow optimally between 45 and 80°C, and ≥80°C, respectively.

The pH gradients at deep-sea vents can vary from as low as pH 2.5 to over pH 8.0, as the acidic hydrothermal fluid mixes with the cold seawater. Thus, there are habitats of low pH where acidophiles thrive; however, by far the majority of habitats at vents available for microbial colonization are circumneutral (pH >6 <8), and these organisms are therefore referred to as neutrophiles.

Energy sources
Seafloor hydrothermal ecosystems are supported almost entirely by chemoautotrophic primary production fueled by the chemical disequilibrium between the oxygenated seawater and the reduced hydrothermal fluids. Microorganisms capture this energy by way of reduction/oxidation (redox) reactions in which seawater typically provides the oxidant (O2, SO42−, NO3−, CO2) and the hydrothermal fluids provide the reductant (H2, H2S, HS−, S0, S2O32−, Fe2+). Redox reactions generate energy that is used to fix CO2 into cellular material which then provides organic carbon for various heterotrophic microbes and animals that make up the rest of the community. Some of the most thermodynamically favorable reactions found in marine hydrothermal environments are presented in Table 2. Reactions are expressed in terms of the change in free energy (∆G), where the more negative the value the more energy an organism will gain by catalyzing the reaction. Calculated values suggest that aerobic oxidation of reduced sulfur compounds (HS−, S0, S2O32−) are the most energetically favorable and potentially the most significant reactions in terms of biomass production in marine hydrothermal environments. It is important to note, however, that thermodynamic calculations can be misleading as they do not consider the bioavailability of certain reactants and potential kinetic inhibition under different environmental conditions (Takai et al., 2006). Several past studies have attempted to model the overall energetics in a variety of marine hydrothermal systems considering such factors (McCollom and Shock, 1997; McCollom, 2000; McCollom, 2007). An interesting conclusion from a theoretical study is that four times as much energy per kg of hydrothermal fluid is available to H2 oxidizers in ultramafic hosted systems than to sulfur oxidizers because of the increased concentrations of H2 (McCollom, 2007). This suggests that H2 oxidizers may be more important than sulfur oxidizers in ultramafic hosted systems (McCollom, 2007). A recent report lends support to

![Photographs of active chimney structures from different hydrothermal environments.](image-url)
this hypothesis, as the diversity of H₂-oxidizing bacteria was found to increase as the concentration of H₂ gas increased in vents sampled from the ulamafic hosted Logatchev vent field along the MAR (Perner et al., 2007). These studies speak to the tight coupling of geochemistry and microbial primary production in marine hydrothermal environments.

**Microbial habitats and diversity**

Seafloor hydrothermal systems provide several habitats over a wide range of physical environments that sustain distinct microbial communities. Such environments include active and inactive vent structures, biotic and abiotic surfaces receiving hydrothermal fluid inputs, inside vent animals (endosymbionts), and hydrothermal vent plumes (Figure 2) (reviewed in Karl, 1995a; Van Dover, 2000; Kelley et al., 2002; Takai et al., 2006). Many of these environments are exposed to varying inputs of hydrothermal fluids, resulting in fluctuating temperatures and chemistry. Below is a brief discussion of each of these habitats and their associated microbial biodiversity. These biodiversity assessments are based on growing and characterizing isolates under laboratory conditions (culturing techniques) and through molecular phylogenetic approaches that are culture-independent. This latter approach is based on extracting genomic DNA from environmental samples, and then amplifying a diagnostic phylogenetic marker for Bacteria and Archaea, the small subunit rRNA gene (16S rRNA), using the polymerase chain reaction.

**Active sulfide structures**

Using culture-dependent and culture-independent techniques, a rich diversity of Bacteria and Archaea have been found associated with active sulfide structures (reviewed in Jannasch, 1995; Karl, 1995b; Kelley et al., 2002; Takai et al., 2006). Free-living microorganisms supported by active sulfide structures include mesophilic, thermophilic, and hyperthermophilic chemolithoautotrophs, heterotrophs, and mixotrophs. Organisms can be anaerobes, facultative anaerobes, microaerophiles, and obligate aerobes.

In general, Bacteria are the most numerically abundant microorganisms associated with active sulfide structures (reviewed in Jannasch, 1995; Karl, 1995b; Kelley et al., 2002; Takai et al., 2006). Their abundance can range anywhere from 35 to 99% of the microbial population, depending on the method used to measure abundance, the type of vent feature, and where within a chimney structure one examines (Harmsen et al., 1997; Takai and Horikoshi, 1999; Takai et al., 2001; Hoek et al., 2003; Schrenk et al., 2003; Nakagawa et al., 2006).

**Within the Bacteria, members of the epsilon-Proteobacteria have consistently been found to dominate microbial communities in deep-sea hydrothermal environments (Reysenbach et al., 2000; Campbell et al., 2001; Hoek et al., 2003; Nakagawa et al., 2005).** These free-living deep-sea epsilon-Proteobacteria are mesophilic to moderately thermophilic. Recently, several representatives have been obtained in pure culture that are chemolithoautotrophic capable of oxidizing H₂ and sulfur compounds while using O₂, NO₃⁻, or sulfur compounds as terminal electron acceptors (reviewed in Campbell et al., 2006). Like in cave environments, the epsilon-Proteobacteria play an essential role in sulfur cycle at deep-sea vents.

Other commonly identified but less abundant bacterial members of the free-living microbial communities include the thermophilic Aquificales and Thermotogales (reviewed in Miroshnichenko and Bonch-Osmolovskaya, 2006). The Aquificales are globally distributed in marine
hydrothermal environments but are restricted to active sulfide structures and hydrothermal fluids due to their thermophilic lifestyle. They are generally chemolithotrophic and chemooorganotrophic capable of oxidizing H$_2$, S$^0$, and S$_2$O$_4^{2-}$ either aerobically or anaerobically (NO$_3^-$ or sulfur compounds) (Reysenbach, 2001a). The Thermotogales, on the other hand, are strictly anaerobic, thermophilic heterotrophic fermenters (Reysenbach, 2001b).

As stated above, Archaea are less abundant in active sulfide structures, yet they play a key biogeochemical role especially at the higher temperatures. Some of the most exhaustive studies on microbial diversity at deep-sea vents have focused solely on the Archaea revealing a high phylogenetic and metabolic diversity within this domain (Takai and Horikoshi, 1999; Takai et al., 2001; Nercessian et al., 2003; McCliment et al., 2006). Most of the Archaea found here are thermophilic to hyperthermophilic and are thus restricted to areas most influenced by hydrothermal fluids. Within the Archaea, the two main phyla, the Crenarchaeota and Euryarchaeota, are well represented, while the enigmatic “Korarchaeota” and “Nanoarchaeota” are rarely detected (reviewed in Takai et al., 2006).

Crenarchaeota commonly detected at active sulfide structures include cultivated members of the Desulfurococcales (e.g., Pyrodictium and Pyrolobus) and several uncultivated groups such as the deep-sea hydrothermal vent crenarchaeotic group (DHVC1), and the marine group I (Takai and Horikoshi, 1999; Takai et al., 2006). Pyrodictium is an example of a hyperthermophilic mixotrophic organism with the ability to use either H$_2$ or complex organics to reduce elemental sulfur (reviewed in Reysenbach et al., 2002). Pyrolobus is also a hyperthermophilic but, unlike Pyrodictium, Pyrolobus is an obligate chemolithoautotroph that can only use H$_2$ to reduce nitrate (NO$_3^-$), thiosulfate, (S$_2$O$_4^{3-}$) or O$_2$.

Euryarchaeota commonly found at marine hydrothermal systems include the hyperthermophilic methanogens (Methanocaldococcus spp.), the hyperthermophilic SO$_4^{2-}$-reducing Archaeoglobales, and a variety of thermophilic fermentative Thermococcales species (Takai and Horikoshi, 1999; Takai et al., 2006). While these are readily grown in the laboratory, their relative abundance in the environment has been shown to vary from one site to another (Reysenbach et al., 2000; Takai et al., 2001; Hoek et al., 2003; Nercessian et al., 2003; McCliment et al., 2006). Several lineages often detected at vent sites around the globe have been classified as the Deep-sea Hydrothermal Vent Euryarchaeotal Groups (DHVEG) (Takai and Horikoshi, 1999; Reysenbach et al., 2000; Takai et al., 2001; Hoek et al., 2003; Nercessian et al., 2003). Several subdivisions exist within this group but at least one lineage, the DHVEG-2, appears to be endemic to deep-sea vents. Little was known about this lineage until Moussard et al. (2006) showed optimal activity and thermostability of a DNA polymerase belonging to this lineage which suggested a thermophilic lifestyle. Thermophily was confirmed when the first isolate was obtained in pure culture (Reysenbach et al., 2006). The organism, Aciduliprofundum boonei, is a heterotrophic thermoadocophile capable of fermenting peptides and reducing Fe and S. Isolation of this organism was significant because it is the first obligate acidophile isolated from a deep-sea vent and the first member of the DHVEG-2 lineage to be grown. Prior to this discovery, the absence of acidophiles from a deep-sea vent had always puzzled microbiologists as hydrothermal fluids are acidic, and geochemists had predicted the presence of acidic microhabitats within sulfide chimneys (Von Damm, 1995; Tivey, 2004).

Inactive sulfide structures
Hydrothermal fluid flow through individual sulfide structures is temporary as subsurface flow paths constantly change and chimneys collapse. Inactive sulfides are subjected to low-temperature oxidative weathering in which the reduced sulfur moiety is converted to soluble SO$_4^{2-}$ and the metals, mostly Fe, are converted to insoluble oxyhydroxide crusts (Juniper and Tebo, 1995). Oxidation occurs abiotically as oxygenated seawater penetrates the rock, but can also be mediated by S- and Fe-oxidizing bacteria (reviewed in Edwards, 2004; Edwards et al., 2005). While microbial mediated sulfide weathering in terrestrial systems has been studied for decades, this area of research is in its infancy in the deep sea (Edwards et al., 2003a, b; Edwards, 2004; Rogers et al., 2003; Suzuki et al., 2004). Culturing studies have resulted in the isolation of several psychrophilic, neutrophilic Fe-oxidizing bacteria belonging to the alpha- and gamma-divisions of the Proteobacteria from sulfide rocks collected along the Juan de Fuca Ridge (Edwards et al., 2003a). A follow-up study with one of the gamma-Proteobacteria isolates showed that the organism promoted the dissolution of native sulfide minerals under anaerobic conditions (Edwards, 2004). Although the mechanism of dissolution remains unclear, these studies illustrate that Fe-oxidizing bacteria are associated with extinct sulfide minerals on the seafloor and can actively promote the dissolution of these minerals at least in the laboratory.

Carbonate structures of Lost City
Microbial communities found in active carbonate structures at the Lost City vent field differ from those found in active sulfide structures due to the contrasting physical and geochemical conditions. Inner portions of carbonate chimneys in contact with hydrothermal fluids tend to be covered by thick microbial biofilms dominated by single Archaeal phylotype belonging to the Methanosarcinales (Schrenk et al., 2004; Brazelton et al., 2006). The Methanosarcinales are the most metabolically diverse of all the methanogens, with various representatives known to generate CH$_4$ from acetate and H$_2$/CO$_2$ or by dismuting methyl compounds (Boone et al., 2001). Members of the Methanosarcinales also participate in a nonobligatory syntrophic relationship...
with SO$_4^{2-}$-reducing bacteria that results in the anaerobic oxidation of methane (AOM) in oceanic cold seep environments (Orphan et al., 2001). A second archaeal phylotype belonging to the closely related ANME-1 was identified in weakly flowing and extinct carbonate chimneys (Brazelton et al., 2006). Like the Methanosarcinales, the ANME-1 are involved in AOM but appear to mediate this process without a SO$_4^{2-}$-reducing partner and at lower temperatures (Orphan et al., 2002). As the Methanosarcinales and the ANME-1 found at Lost City were only identified by molecular techniques, it is difficult to speculate on whether they are producers or consumers of CH$_4$.

In contrast to the Archaea, Bacteria appear to be more diverse in active and inactive carbonate chimneys (Brazelton, et al., 2006). The most diverse bacterial group at Lost City is the gamma-Proteobacteria which includes close relatives of known chemolithoautotrophic S- and HS-oxidizers (e.g., *Thiomicrospira* ssp.) and several methylo trophic (CH$_4$-oxidizing) genera (e.g., *Methylobacter*, *Methylomonas* and *Methylophaga*). Other chemolithoautotrophic S-oxidizing bacteria belonging to the epsilon-Proteobacteria are also found at Lost City.

Perhaps the most interesting trend in bacterial diversity observed at Lost City is not what is present but what has not yet been detected. Specifically, neither any known thermophilic H$_2$-oxidizing Bacteria (i.e., *Aquificales*; common in most deep-sea and terrestrial hot spring environments) nor members of the delta-Proteobacteria (the SO$_4^{2-}$-reducing syntrophs found in other AOM environments), were identified in carbonate chimneys (Brazelton et al., 2006). The lack of bacterial H$_2$ oxidizers is unexpected as geochemical modeling suggests that H$_2$ oxidation provides four times as much energy per kg of hydrothermal fluid than S oxidation in ultramafic hosted systems (McCollom, 2007). This suggests that available energy may not be the main constraint driving which microbial metabolisms can be supported in particular environments. It is important to note, however, that relatively few studies have been conducted at Lost City and that these observed trends may be due to the lack of adequate sampling or incorrect inferences based solely on 16S rRNA gene similarity.

**Biotic and abiotic surfaces**

Microbes are opportunistic organisms and will colonize any surface where growth substrates are provided. In areas influenced by hydrothermal fluids, this includes the sulfide deposits as described above and surfaces of invertebrate animals (reviewed in Van Dover, 2000). Some of these relationships are commensal as the host does not appear to gain an advantage by being colonized while others truly are symbiotic (episymbiosis), with both the host and microbes benefiting. An example of a commensal relationship is found between the giant tube worm *Riftia pachyptila* and the diverse microbial communities that colonize the outside surfaces of its chitinous tube (López-García et al., 2002). Most of the microbes identified on the outside of the tube are members of the ubiquitous epsilon-Proteobacteria and are likely oxidizing the reduced sulfur compounds in the hydrothermal fluids. An episymbiotic relationship is found on the polychaete worm *Alvinella pompejana* (Van Dover, 2000). *Alvinella pompejana* lives in tubes on the sides of black smoker chimneys where water temperatures can exceed 70°C (reviewed in Desbruyeres et al., 1998). It has a fully functional digestive system and appears to feed upon microbial mats inside of its tube as well on sulfide surfaces. The worm also houses diverse microbial communities on its dorsal surfaces including microaerophilic H$_2$S-oxidizing members of the epsilon-Proteobacteria. Although the exact nature of this relationship is unknown, it is thought that the bacteria may detoxify H$_2$S or provide nutritional supplement to the worm.

In addition to surfaces of vent animals, microbes often colonize rocks and sediments surrounding hydrothermal vents. These environments are primarily sustained by hydrothermal fluids flowing diffusely through the underlying porous sediments. The most well-studied and possibly most diverse microbial mat system found in marine hydrothermal environments is in the sediment-hosted Guaymas Basin. Microbes found here include thermophilic/mesophilic methanogens and methanotrophs (Dhillon et al., 2005), SO$_4^{2-}$-reducing Bacteria (Kallmeyer and Boetius, 2004), H$_2$S-oxidizing gamma-Proteobacteria (Nelson et al., 1989), and even spore forming Mn-oxidizers (Dick et al., 2006). Much of this diversity is fueled by the presence of underlying sediments which provides different combinations of organic/inorganic substrates typically not found in non-sediment-hosted systems.

**Endosymbionts**

Several invertebrate animals form obligate symbiotic relationships in which the bacteria are hosted inside the animal cells. The most well-studied example is in the giant tube worm *Riftia pachyptila* (reviewed in Minic and Hervé, 2004). As adults, *Riftia* completely lack a mouth, the digestive system and anus and are sustained by chemoa trophic H$_2$S-oxidizing Bacteria belonging to the gamma-Proteobacteria (Cavanaugh et al., 1981; Felbeck, 1981; Jones, 1981; Minic and Hervé, 2004). In this relationship, *Riftia* provide the bacteria with inorganic carbon, O$_2$, H$_2$S, and NO$_3^-$ and, in turn are provided with organic carbon. Other invertebrate organisms such as the giant clam *Calypgoena magnifica* and the mytilid mussels *Bathymodiolus* spp. also house H$_2$S-oxidizing endosymbionts in their gill tissues (Van Dover, 2000). The symbiotic relationship between mussels and bacteria, however, is not obligate as they retain some of their filter feeding capabilities. In addition, certain species of *Bathymodiolus* have methanotrophic (CH$_4$-oxidizing) endosymbionts and even dual symbionts with both sulfur- and CH$_4$-oxidizing bacteria.
Hydrothermal plumes

Upon reaching neutral buoyancy, hydrothermal vent plumes carrying elevated concentrations of S, metals (Fe and Mn), H₂, and CH₄ extend hundreds of meters laterally into the water column. Not surprising, elevated biomass has been detected in plumes, suggesting that these environments are areas of high productivity (reviewed in Winn et al., 1995). Methane oxidation has been measured in plume waters collected along the Juan de Fuca Ridge, further supporting microbial activity in vent plumes (DeAngelis et al., 1993). More recently, Sunamura et al. (2004) used culture-independent techniques to assess the diversity of microbes in plumes over the Suiyo Seamount. Members of the gamma- and epsilon-Proteobacteria were dominant in the plume waters and are likely using reduced sulfur compounds because of their close phylogenetic relationship with known sulfur-oxidizers (Sunamura et al., 2004).

The first aerobic anoxygenic photosynthetic bacteria from the deep sea were isolated from plumes (Yurkov and Beatty, 1998). These organisms belong to the alpha-Proteobacteria and appear to support heterotrophic growth with photosynthesis under laboratory conditions. Their presence in deep-sea hydrothermal environments seems puzzling because of the lack of sunlight, but high-temperature fluids (≈350°C) have been detected to emit photons capable of supporting facultative photosynthetic bacteria (Van Dover et al., 1996). Their role and significance in the environment, however, remain unknown.

Conclusions

Although we know significantly more about the diversity of microorganisms from deep-sea vents since their discovery in the late 1970s, we still have a rudimentary understanding of their ecology and geobiology. Recently, the discovery of acidophiles (Reysenbach et al., 2006), high-temperature nitrogen fixation (Mehta and Baross, 2006), and high-temperature Fe reduction (Kashefi and Lovley, 2003) provided additional insights into the roles microbes play in biogeochemical cycling at vents. Furthermore, studies are now being undertaken to explore the temporal changes that occur in the microbial communities as the available carbon and energy sources change (e.g., Pagé et al., 2008). Whether these changes are due to the in situ biological activity or due to the geochemical constraints of the hydrothermal fluids and mixing and subsurface reaction zones is still unknown. It is clear that deep-sea vents represent one of the few places on Earth where the “bio” is so tightly coupled to the “geo.”

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Cross-references

Acidophiles

Archaea

Bacteria

Begggiatoa

Chemolithotrophy

Cold Seeps

Deep Biosphere of Sediments

Deep Biosphere of the Oceanic Deep Sea

Extreme Environments

Fe(II)-Oxidizing Prokaryotes

Fe(III)-Reducing Prokaryotes

Hot Springs and Geysers
Hydrothermal Environments, Fossil
Hydrothermal Environments, Terrestrial
Iron Sulfide Formation
Manganese (Sedimentary Carbonates and Sulfides)
Methanogens
Ores, Microbial Precipitation and Oxidation
Origin of Life
Sulfide Mineral Oxidation
Sulfur Cycle
Thiomargarita
Thiotrophic Bacteria

**HYDROTHERMAL ENVIRONMENTS, TERRESTRIAL**

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**Definition**

*Hydrothermal Environments, Terrestrial.* Areas on the Earth’s surface that are under the influence of geothermal waters, steam, and associated gases discharged from hot springs, geysers, and fumaroles.

**Introduction**

Terrestrial hydrothermal environments are those settings where fluids discharge either at or close to the land surface at a temperature that is significantly above the local ambient air temperature. The hydrothermal processes transfer heat and dissolved matter to the surface in a liquid or vapor (gas) phase. Those fluids originate at variable depths below the Earth’s surface and have a wide range of temperature and chemical composition. Visible features of terrestrial hydrothermal environments include hot springs, geysers, fumaroles, and steam vents. Travertine (calcite and aragonite: CaCO₃) and sinter (mainly opal-A: SiO₂.nH₂O), precipitated from thermal water, commonly form mounds and terraces around many spring and geyser vents. In contrast, chemical reactions between acidic hydrothermal fluids and the host bedrock and soils usually produce extensive hydrothermal alteration that generates a very different type of landscape.

Since the pioneering studies of life in hydrothermal environments, begun in the 1960s by Brock (1978) and colleagues, interest in these environments has increased significantly for many reasons (Bock and Goode, 1996; Renaut and Jones, 2000; Jones and Renaut, 2003a). The extremophiles that inhabit modern high-temperature settings, which include thermophiles and thermoacidophiles, have implications for understanding the origin of life on the Earth and potentially on other planets. Many of the rocks precipitated around hot springs havestromatolitic fabrics and contain numerous fossilized microbes. Many recent studies have tried to determine if such microbes directly influence mineral precipitation, or are simply trapped in essentially abiotic mineral deposits. From a geological perspective, surface hydrothermal environments can provide clues to subsurface processes including the potential for epithermal ore mineralization and for geothermal energy development (Pirajno, 1992; Bogie et al., 2005).

**Geothermal fields**

The geothermal gradient, a measure of the increase in temperature with depth in the Earth’s crust, has an average value of 25–30°C km⁻¹, but this may rise to several hundred °C km⁻¹ in regions of volcanic activity. Geothermal systems are found in regions with normal and slightly elevated geothermal gradients, but high-temperature systems are most common in regions of active tectonics and volcanism near plate margins (Figure 1). Although geothermal fields can extend over several thousand square kilometers, the individual surface areas of hydrothermal discharge tend to be localized and of limited extent within those domains.

Using stable isotopic evidence, Craig (1963) confirmed that most hydrothermal fluids are meteoric in origin. Some hydrothermal systems are partly fed by connate (formation) waters that were buried along with the host sediments; other hydrothermal fluids may derive from juvenile (magmatic) and metamorphic waters, but these are usually minor components if present (Nicholson, 1993; Giggenbach, 1994).

The fluids that feed high-temperature (high-enthalpy) liquid-dominated hydrothermal systems derive mainly from cool meteoric water that has circulated to a few kilometers depth (up to ~5 km) via faults, fractures, and permeable horizons. The heated fluids react with host rocks, acquire solutes, and typically evolve to become saline (3,000–5,000 mg kg⁻¹) and chloride-rich, forming geothermal reservoirs with temperatures of up to ~400°C. Those fluids then rise convectively and may boil with the decreasing pressure during ascent to create a two-phase (steam + liquid) zone below the land surface. The liquid phase may discharge at the surface as hot springs, whereas the vapors released on boiling (steam separation) may discharge at the surface as fumaroles. Rising hot fluids may mix with shallow groundwaters before surface discharge, which may modify their composition and temperature. Not all geothermal systems are of high-temperature type. In low-temperature (low-enthalpy) systems, the reservoir temperature may be <150°C, and the fluids may not boil before being discharged as springs.

**Thermal fluids**

Geothermal fluids are commonly classified according to their dominant anion, into chloride waters, sulfate waters, and bicarbonate waters (Table 1) (Henley and Ellis, 1983; Nicholson, 1993). Waters of mixed anion composition are also found. The composition of the discharged fluids
profoundly affects the characteristics of the terrestrial surface environment, including any mineral deposits and hydrothermal alteration, and the biota that inhabit the areas of hydrothermal discharge.

Chloride waters (also termed “alkali-chloride” or “neutral chloride” waters)

These saline waters typify the deep geothermal fluids in most high-temperature geothermal systems and where discharged at the surface, they commonly overlie permeable zones of major thermal upflow (Nicholson, 1993). They have near neutral pH and are chloride-rich with variable SO$_4^{2-}$ and HCO$_3^-$ concentrations. Chloride waters are typically rich in dissolved silica and have Na$^+$ and K$^+$ as the dominant cations. Carbon dioxide is usually the main dissolved gas with much lower amounts of H$_2$S.

At the land surface, chloride fluids commonly discharge from hot and boiling springs, often with clear bluish-green waters, and from most geysers (Figure 2a). Such fluids characteristically precipitate large quantities of silica sinter (see Chapter Sinter). If the fluids are rich in HCO$_3^-$, they may boil at greater depths and may...
Precipitate both sinter and travertine upon CO₂ degassing at the land surface.

Spectacular examples of geysers and springs that eject chloride waters are found in most of the major geothermal sites, including those in Yellowstone National Park (Bryan, 2008), the Taupo Volcanic Zone of North Island, New Zealand (Ellis and Mahon, 1977; Jones and Renaut, 2003b), El Tatio in northern Chile (Cusicanqui et al., 1976; Fernandez-Turiel et al., 2005), and Iceland (Torfason, 1985). The sinter that precipitates around many geyser and spring vents and on their discharge aprons as mounds and terraces is commonly colonized by diverse, varicolored, microbial communities.

**Sulfate waters ("acid sulfate waters")**

Sulfate-rich waters form through condensation of geothermal gases in shallow oxygenated groundwater. Steam, gases, and other volatiles (e.g., NH₃, As, B) that were originally dissolved in deep geothermal fluids can separate from chloride waters when they undergo boiling at depth, and rise toward the surface. The steam-heated acidic waters commonly boil at the water table because the separated steam contains high enthalpy from the deeper geothermal fluid.

**Sulfate, the dominant anion, is formed by oxidation of condensed hydrogen sulfide (Nicholson, 1993):**

\[
\text{H}_2\text{S(g)} + 2\text{O}_2(\text{aq}) = 2\text{H}^+(\text{aq}) + \text{SO}_4^{2-}(\text{aq}).
\]

The protons produced may be supplemented by condensation of CO₂ to create acidic waters:

\[
\text{CO}_2(\text{g}) + \text{H}_2\text{O(l)} = \text{H}_2\text{CO}_3(\text{aq}) = \text{H}^+(\text{aq}) + \text{HCO}_3^-(\text{aq}) = 2\text{H}^+ + \text{CO}_3^{2-}(\text{aq}) .
\]

The pH is typically between 2.8 and ~5. Chloride is usually present in minor amounts. Bicarbonate is usually absent because it is lost from solution as CO₂ gas. These acidic fluids, typically present above the water table close to the land surface (<100 m depth), are usually found around the margins of geothermal fields at sites distant (few hundred meters to several kilometers) from the main chloride-rich upflow.

Acidic waters form distinctive surface environments characterized by hot springs, turbid boiling mud pools, or steaming ground commonly with extensive pits and collapse features resulting from hydrothermal alteration (Figure 2b). The acidic waters leach the host rocks

**Hydrothermal Environments, Terrestrial, Figure 2** Variations in modern hydrothermal environments according to water chemistry. (a) Hot springs, geysers, and extensive silica sinter coating a fault scarp at Orakeikorako, New Zealand. The fluids are alkaline chloride waters. (b) Muddy hot springs and fumaroles at Myvatn, northern Iceland. The fluids are acid sulfate in composition. Hydrothermal alteration of young volcanic rocks has produced extensive clays and Fe oxides at the land surface. (c) Boiling hot spring and perpetually spouting hot spring (right) rimmed by calcite travertine on the shoreline of Lake Bogoria, Kenya. The spring water is of sodium bicarbonate composition. (d) Small geyser discharging mixed acid chloride–sulfate waters (pH: 2.1–2.6) near Hakereteke Stream, north Waiotapu, New Zealand. Thin Fe-rich sinter covers the substrate.
producing extensive clays (mainly kaolinite), commonly with alunite, alunogen, jarosite, Fe-oxhydroxides, pyrite, cristobalite, and native sulfur. Little sinter forms in acidic waters, but alteration of volcanic rocks by sulfuric acid can leave behind abundant opaline silica in place, termed “silica residue” (Rodgers et al., 2002). Well-developed examples of acid sulfate water discharge are found at Tikitere (New Zealand), Myvatn (northern Iceland), and Norris Geyser Basin (Yellowstone National Park).

**Bicarbonate waters**

Bicarbonate-rich hot waters are formed mainly when steam and gases (mainly CO$_2$) condense in shallow groundwaters. Although most common around the margins of geothermal fields, they may also form in sites distant from volcanic activity. They often form where geothermal fluids are in contact with limestone or volcanic bedrock. Such fluids normally have a near-neutral pH when discharged. The initial acidity of the rising CO$_2$-rich fluids is neutralized by hydrolysis reactions with bedrock that consume protons (H$^+$) leaving waters dominated by HCO$_3^-$ and Na$^+$. Sulfate and chloride have variable concentrations. At the land surface, these fluids discharge from springs that range from warm to boiling and most have clear water. Travertine deposits, composed of calcite and (or) aragonite, commonly form around the vent and downslope of these springs (Figure 2c) where sufficient Ca$^{2+}$ is available. Sinter is generally absent, but minor amounts can form from very hot and boiling bicarbonate waters. Hydrothermal alteration at the surface is generally very minor, though more extensive at depth.

Carbonate deposits associated with such springs may be localized around their vents or form vast discharge aprons. Examples include the large terrace mounds, fissure ridges, and related features at Pamukkale in Turkey, Mammoth Hot Springs in Wyoming, and at many sites in central Italy (Bargar, 1978; Chafetz and Folk, 1984; Pentecost, 2005). The travertines precipitate from bicarbonate waters over a wide temperature range, from ambient to boiling. Most carbonate precipitation has been attributed to rapid loss of CO$_2$ upon discharge of the spring waters at the land surface (Renaut and Jones, 1997; Pentecost, 2005). Microorganisms, principally cyanobacteria, may play a role in some carbonate precipitation through photosynthetic removal of CO$_2$, but at high temperatures most precipitation is probably driven by abiotic degassing of carbon dioxide.

**Mixed waters**

Waters that contain two anions with similar concentrations form by several different processes. Sulfate–chloride waters typically have a pH of ~2–5, but can have a pH as low as 0. They originate through subsurface mixing of sulfate and chloride waters, oxidation of H$_2$S in chloride waters, near-surface condensation of volcanic gases in meteoric waters, or dissolution of sulfate-bearing bedrock by migrating thermal chloride waters (Nicholson, 1993). Such waters discharge as warm to hot springs, commonly with thin sinter deposits and sulfate-bearing minerals around the vent. Native sulfur may also be present.

Examples of acidic sulfate–chloride springs are found in the northern part of the Waiotapu geothermal area of North Island, New Zealand (Figure 2d) (Jones and Renaut, 2006). These springs discharge waters a pH of 2.1–2.6 at temperatures >90°C. Spicular sinters containing silicified microbes have precipitated around these springs together with kaolinite, jarosite, and gypsum.

Dilute chloride–bicarbonate waters form where chloride waters have been diluted by groundwater or bicarbonate water (Nicholson, 1993). They typically are found in springs of variable temperature around the margins of thermal upflow zones, but waters of this composition also discharge in low-temperature systems. The fluids are typically Cl-dominated, have variable HCO$_3^-$ concentrations, and a neutral pH of 6–8. Minor travertine and (or) sinter may form around the vents, but many of these springs lack surface deposits.

**Types of geothermal systems**

The environmental characteristics of terrestrial hydrothermal sites are strongly controlled by the type of geothermal system that supplies their fluids. Many classifications of geothermal systems have been proposed (e.g., Heiken, 1982; Henley and Ellis, 1983; Pirajno, 1992; Nicholson, 1993). Kühn (2004) recognized two major groups. In static systems, heat may be transferred to the surface by conduction without convective fluid movement. In dynamic systems, heat is transferred to the surface by moving fluids undergoing convective circulation.

**Static (conductive) geothermal systems**

Static geothermal systems depend on above-average conductive heat flow in the crust. In static magmatic systems, fluids can be heated by granitic plutons. Other conductive systems are hosted by porous sedimentary rocks in deep sedimentary basins. Static systems generally have little impact at the surface. However, trapped formation waters, which are typically saline, chloride-rich and have temperatures of ~70–150°C, can be released by tectonic movements and discharge at the surface as thermal springs with variable temperature and salinity, depending on the degree of mixing with shallow groundwaters. Many examples are known from Europe, especially Germany (Kühn, 2004).

**Dynamic (convective) geothermal systems**

Most high-temperature geothermal systems are linked to magmatic intrusions (volcano-plutonic and volcanic), which provide the heat that drives the convective circulation. In such systems, reservoir temperatures are commonly >150°C at depths of a few kilometers. Such systems are most common in sites of geologically young volcanism and in tectonically active regions such as areas of faulting, rifting, and caldera collapse, and at plate
margins in general. As in other hydrothermal systems, the waters are predominantly meteoric, and the ascending fluids commonly boil before or during surface discharge. The hydrothermal systems vary with tectonic location and type of volcanism (Pirajno, 1992). Many are associated with silica-rich and andesitic volcanism, whereas others are associated with basaltic volcanism.

Silica-andesitic systems
High-temperature hydrothermal systems associated with silica-rich volcanism are highly variable. Some systems are liquid dominated, producing springs and geysers, whereas others are vapor dominated, generating mainly fumaroles and steam vents at the land surface.

In regions of low relief (Figure 3a), magmatic systems supply heat and gases to meteoric groundwater and give rise to convective columns that can allow deep chloride-rich waters to flow directly to the surface (e.g., Taupo Volcanic Zone, New Zealand). The deep fluids may discharge as springs and geysers above the main upflow or may move laterally some distance away from upflow column. Steam zones commonly develop in sites marginal to the main upflow. Oxidation of H₂S and other gases in groundwater produces acid sulfate waters, generating fumaroles and steam vents. Condensation of CO₂ in groundwater may produce neutral to alkaline bicarbonate springs around the margins of the field from which travertine may be formed. These types of system commonly have fumaroles, steaming ground, and both acid and alkaline springs located near each other because of the low topographic relief and limited potential for lateral flow of fluids near the land surface.

In areas of high relief (Figure 3b), such as andesitic stratovolcanoes, a thick two-phase zone (liquid—steam—gas) commonly develops below the land surface. At high elevations, fumaroles, steam vents, and acid sulfate springs fed by condensate are common features. Deep chloride-rich waters normally do not reach the surface but may flow laterally and mix with groundwater or cooled, descending sulfate waters before discharging as hot springs on the volcano flanks or other areas of relatively low relief. Numerous examples are found in Indonesia, the Philippines, and Japan.

Some silicic systems are vapor dominated (e.g., Lardarello, Italy; The Geysers, California). The deep thermal reservoir contains steam, probably fed by saline boiling water, most of which is contained in convective cells capped by low-permeability rocks. Fluids escaping the reservoir produce steaming ground and fumaroles at the surface and some thermal springs similar to those in high-relief regions. Surface fluids can be of acid sulfate or bicarbonate type.

Basaltic systems
High-temperature basaltic systems occur at mid-oceanic ridges (see Chapter Hydrothermal Environments, Marine) and in continental rifts. Many tectonic and hydrogeological settings are possible (Pirajno, 1992), but in most examples hydrothermal fluids rise along permeable faults discharging at hot springs and geysers on the rift basin floor (Figure 2c), including subaqueous discharge on the floors of lakes. Fluid compositions are variable and depend partly on the bedrock composition, which may be volcanic, basement rocks, or valley-fill sediments.

Low temperature systems
Low-temperature geothermal systems are common and of diverse origins. Most are characterized by dilute waters that are discharged as springs with maximum temperatures of about 30–60°C. They form in regions of both normal and above-average heat flow. They are common where meteoric fluids return to the surface having been heated to temperatures above ambient surface temperature. These springs commonly occur in regions of deep faulting and folding, in regions of tectonic uplift where hot rocks have been elevated to higher levels in the crust, and from the residual heat of cooling plutons (Nicholson, 1993). Meteoric fluids circulate at depth through faults, fractures, and permeable rocks. The chemical composition of the fluids reflects such factors as the bedrock mineralogical composition, depth and temperature of fluid circulation, the rates of recharge, and degree of mixing with near-surface waters before discharge. Such low-enthalpy systems are common in western Canada, Europe, Australia, and on most continents.

The surface environment in hydrothermal areas
Areas of hydrothermal discharge are distinctive features of the landscape. Where they are extensive, they can influence the local ecology at all scales, from large mammals to microbes. The impact of hydrothermal discharge at the land surface ranges from negligible, where dilute warm springs flow quietly from small vents at temperatures little above ambient, to major, where high-temperature and acidic fluids destroy vegetation, precipitate mineral deposits, or flood the landscape. In general, areas of neutral to alkaline hydrothermal discharge are mainly construct with new rocks (sinter, travertine, and others) precipitated from hydrothermal fluids upon an existing landscape (Figure 2a, c). In contrast, where the fluids are mainly acidic, the net impact on the surface environment is often destructive and the landscape commonly becomes nearly barren and pock-marked by craters that result from collapse due to hydrothermal alteration of the host bedrock or hydrothermal explosions (Figure 2b, d). The main hydrothermal surface features are hot springs and geysers, fumaroles, steam vents, and mudpots. Some hydrothermal springs also discharge on the floors of lakes.

Hot springs
Hot springs are sites where geothermally heated groundwater discharges at the land surface (see Chapter Hot Springs and Geysers). There is no universally accepted definition of a hot spring (Pentecost et al., 2003). Some definitions place the lower temperature limit at an
Hydrothermal Environments, Terrestrial, Figure 3 General models of liquid-dominated magmatic geothermal systems showing relationships between subsurface fluid evolution and associated surface environments. (a) Liquid-dominated system in a low-relief setting. Inset expands the upper left half of the figure to show how surface environments and deposits vary spatially according to the type of fluid being discharged. (b) Liquid-dominated system in a high-relief (stratovolcano) setting. Adapted from figures by Henley and Ellis (1983) and Nicholson (1993).
spouters (Figure 4e), erupt jets of water and steam con-
tination of a true geyser. Some springs, termed perpetual
odically but discharge quietly and lack the forceful ejec-
ting, USA (Bryan, 2005). Well-known geysers include
geo) (Jones and Renaut, 2003b). Intermittent springs flow peri-
2008), and Pohutu at Whakarewarewa in New Zealand
Old Faithful in Yellowstone National Park (Bryan,
Fumaroles are common features of active volcanoes,


taneous, but lack the periodicity of geysers.


ter views (see Chapter 
Sinter

). Mineral precipitation commonly begins around pool mar-
rines to form terraces (rimstone dams and pools),
ines, and a range of other spring deposits
Jones and Renaut, 2003a; Pentecost, 2005). At some
ings, including many geysers, vents may grow upward
ring a range of conical mound deposits (Figure 4d).
Microorganisms are commonly fossilized in the spring
some have been implicated in mineral precipitation.

Fumaroles (steam vents) and mudpots
Fumaroles are vents or openings in the ground that dis-
charge hot water vapor (steam) and (or) volcanic gases.
Fumaroles are common features of active volcanoes,


arbitrary value (e.g., human body temperature: ~37°C; or
some fixed value: 20°, 35°, 50°C, etc.), whereas others
define a hot spring by its temperature relationship to local
ambient surface water temperature, mean air temperature,
or a similar environmental factor. As generally used, the
term hot spring is given to those springs that discharge at
between ~35°C and the local boiling point.

Individual hot springs vary greatly in size (<10 cm to
~80 m in vent diameter), depth, shape, and discharge.

fluctuate rapidly due to a sudden pressure reduction
in the plumbing system to withstand high fluid pressures gener-
ated by boiling. Most eruptions result from the rapid gen-
eration of steam due to a sudden pressure reduction
(Browne and Lawless, 2001). The explosive release of
vapor may lead to break up of sinter or other rocks near
the land surface. Blocks become strewn across the land-
scape, new vents may be formed, and old vents may
become inactive because of the changes in the subsurface
plumbing system.

The surface environment at high-temperature hydrother-
mal sites occasionally undergoes major disturbances due
to hydrothermal eruptions. Such eruptions have several
causes but are typically related to the inability of the
plumbing system to withstand high fluid pressures gener-
ated by boiling. Most eruptions result from the rapid gen-
eration of steam due to a sudden pressure reduction
(Browne and Lawless, 2001). The explosive release of
vapor may lead to break up of sinter or other rocks near
the land surface. Blocks become strewn across the land-
scape, new vents may be formed, and old vents may
become inactive because of the changes in the subsurface
plumbing system.

The impact of fumaroles on the landscape and envi-
ronment reflects their abundance, gas composition, tem-
perature, and location. Fumaroles can form on steep and
low-angled slopes. On steep slopes, they typically emerge
from cracks in the substrate, cm to m in scale, which may
be linear, irregular, or funnel-shaped openings where
gases have hydrothermally altered the bedrock. On low
ground, fumaroles may pockmark the landscape with
series of pits (Figure 4f), some meters in diameter. Min-
erals (fumarolites) are commonly precipitated around
the vents as rock coatings, many of which are sublimates
from the fumarole gases. This diverse array of minerals
includes opaline silica, native sulfur, jarosite, Fe-
monoxide, ammonia, boron and fluorine compounds are
present in some gases. The predominant steam is mainly
superheated groundwater, whereas much of other gas is
magnetically sourced. Solfataras are areas where fuma-
robes discharge large volumes of sulfur gases; mofettes dis-
charge carbon dioxide, sometimes methane, from
fumaroles at temperatures below boiling point of water
but above those of ambient air. Mofette gases trapped in
valleys and depressions can cause asphyxiation.

Rising steam and gases may heat the ground and con-
dense near the land surface or may heat groundwater at shal-
low depth. The condensed steam and heated water may boil
at the water table to produce areas of steaming ground. If
H2S is present in the vapor phase, the resulting sulfuric acid
commonly attacks the bedrock and soils and may generate
abundant clay minerals, especially kaolinite, producing
mudpots (Figure 4g). Forceful eruption of viscous mud
can produce mud volcanoes (Figure 4h). The mineralogy
of mudpots has received little attention, but includes
a similar suite of minerals to other acid sulfate springs.
Hydrothermal Environments, Terrestrial, Figure 4 Surface features in terrestrial hydrothermal environments. (a) Hot spring with sinter pool-rim dam. Oyster Pool (pH 4.5; T: 65°C), Waiotapu, New Zealand. (b) Hot spring (pH 8.8; 99°C) emerging from fractured trachyphonolite bedrock at Chemurkeu, Lake Bogoria, Kenya. (c) Low-enthalpy, dilute bicarbonate hot spring (pH: 6.6; T: 36°C) at Loboi, Lake Bogoria, Kenya. (d) Clepsydra Geyser, Yellowstone National Park. (e) Perpetually spouting hot spring (pH: 8.7; T: 98.5°C) with mound of subfossil travertine, Lake Bogoria, Kenya. (f) Large (3 m wide) fumarole vent at Sol de Manya geothermal field, southern Bolivia. (g) Boiling mudpool at Sol de Manya geothermal field, southern Bolivia. (h) Mud volcano, Tikitere, near Rotorua, New Zealand.
The Waimangu geothermal area on North Island, New Zealand, experienced major hydrothermal explosions on April 1, 1917 that created Frying Pan Lake, with further eruptions in 1924 and 1973 (Lloyd and Keam, 1974). The 1973 eruption, for example, distributed ejecta, which consisted of hydrothermally-altered angular blocks of country rock (up to 30 cm diameter) and fine debris, over an area of \( \frac{1}{24} \) km\(^2\).

**Sublacustrine hydrothermal discharge**

Thermal fluids can discharge directly from lake floors either as underwater springs or as steam and gas vents. Diffuse upward-moving steam and gases may also condense in lake water through permeable sediments without point-sourced vents. Sublacustrine springs are most common on the floors of caldera (crater) lakes, continental rift, and other tectonic lakes, and in lakes produced by subsidence, collapse, or hydrothermal eruptions in areas of acidic hydrothermal discharge. Their impact on life in the lake depends on the respective chemistry and temperatures of the lake water and thermal fluids, the rate and volume of thermal fluid discharge, and lake size, morphology, and climate, which can control seasonal stratification and hydrological closure. Like submarine springs, sublacustrine hot springs may host local communities of thermophilic organisms, and biofilms or microbial mats commonly cover the vent surfaces. Sublacustrine sinter, travertine (tufa), and other mineral deposits can precipitate around vents at sites where thermal fluids mix with lake water, undergo cooling, or exsolve noncondensable gases. Spring deposits include spires, chimneys, mounds, crusts, and various microbialites. Sublacustrine spring deposits commonly incorporate lacustrine organisms, especially planktonic diatoms and freshwater sponges, which can help distinguish them from morphologically similar subaerial spring deposits when examining ancient deposits.

Well-known examples of sublacustrine spring systems include those in Crater Lake (Oregon), Yellowstone Lake (Wyoming), Lakes Tanganyika, Kivu and Bogoria in the East African Rift, and Lake Taupo (North Island, New Zealand). Hot springs on the floor of Lake Taupo at depths of \(~140\) m, for example, have produced small chimneys formed of opal-A and various Mn-, Fe-, and Hg-precipitates (de Ronde et al., 2002). Crater lakes in active and dormant volcanoes commonly receive discharge from acidic springs and fumaroles that produces acid lake waters.

**Life in terrestrial hydrothermal environments**

Terrestrial hydrothermal environments host many organisms, but some, notably thermophiles (\(~45^\circ-80^\circ\)C) and hyperthermophiles (\(>80^\circ\)C), are unique to hydrothermal settings (see Chapter *Extreme Environments*). Although the ecology of hydrothermal environments has been studied extensively (e.g., Brock, 1978; Reysenbach et al., 2001; Robb et al., 2007; Wiegel, 2007), new discoveries continue to be made regularly. The distribution of microorganisms at hot springs and geysers is controlled mainly by temperature, pH, and water chemistry. Brock (1978) first established the main temperature tolerance ranges for the main groups of organisms in hot-spring systems (Figure 5).

Proximal areas under the influence of near-boiling water ejected from hot springs and geysers were once regarded as being sterile. The ornate and banded silica deposits commonly found around geyser vents were named “geyserite” because they were thought to have formed abiotically (Walter, 1976). It has since been shown that a diverse community of microorganisms inhabit these very hot waters (Blank et al., 2002) and that geyserite commonly contains abundant silicified microbes (Jones et al., 1997). Identifying these microbes is a challenge because many are difficult to culture and their

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**Figure 5**

Upper temperature tolerance limits of selected organisms in hydrothermal environments (modified from Brock, 1978).
morphological diversity is low. DNA analyses, however, show that these extremophiles are biologically diverse (Barns et al., 2004). The hyperthermophiles are dominantly archaea, and some are also thermoacidophiles. In the hottest (anoxic) waters, the archaea are mainly chemolithoautotrophs and chemooorganotrophs, and include coccoïd, rod-shaped, and filamentous forms (e.g., Pyrolobus, Pyrococcus, Methanothermus). Hyperthermophilic bacteria (e.g., Aquifex, Geothermobacterium), some of which are sulfate and hydrogen oxidizing species, also thrive in waters up to boiling point. Viruses also inhabit these high-temperature settings. Thin microbial films locally coat immersed rock and soil surfaces around the edge of the vents (Figure 6a), and filamentous stringers of hyperthermophiles are sometimes seen attached to the substrate in proximal outflow channels.

As water temperature decreases downstream from the spring and geyser vents, the species diversity usually increases. At temperatures between 75 and ~40°C, microbial mats are commonly well developed in neutral and alkaline waters (Figure 6b). Varicoloured (green, orange, brown) microbial mats built by photosynthetic cyanobacteria often cover the substrate in sites that remain almost constantly moist from thermal outflow, and are commonly several cm thick (Figure 6c). Small coniform stromatolites form in some low-energy pools (Figure 6d, e) (Jones et al., 2002). Common genera include Oscillatoria, Synecococcus, Phormidium, Anabaena, and Spirulina. Chloroflexus, a green sulfur bacterium and anoxygenic phototroph, is commonly associated with these mats. Extensive extracellular polymeric gels are produced by some of these communities. Heterotrophic bacterial communities normally underlie the surface layers of these mats (Brock, 1978). Organisms common in waters cooler than ~45°C include the cyanobacteria Calothrix and Pleurocapsa, which often build nodular mats, eukaryotic algae, diatoms, fungi, and protists, some of which become important components of microbial mats.

Acid thermal areas, including areas around some fumaroles (and solfataras), usually have distinctive acidophilic communities. In acidic hot springs where the temperature is <60°C, green mats built by the unicellular red alga Cyanidium caldarium are often present (Figure 6f) (Brock, 1978), together with a range of bacteria (e.g., Thiobacillus). Below pH 5, cyanobacteria are less abundant and mats composed of diatoms and fungi become common. Stromatolitic sinters built by diatoms, for example, are present at Frying Pan Flats at Waiotapu, New Zealand, where the pH is <3 (Figure 6g) (Jones et al., 2000).

Invertebrates, such as mat-grazing ostracods, are common in some hot spring waters that are <45°C. Insects are common at some hot springs and include spiders, beetles, and flies. Adult and larval Ephydrya spp., for example, feed on microbial mats in hot spring effluent (Figure 6h). Few vertebrates are restricted to thermal aquatic environments, but fish (Alcolapia grahami) inhabit hot spring pools with temperatures up to 46°C at Lake Magadi, Kenya (Figure 6i).

Environmental impact of terrestrial hydrothermal activity

The environmental consequences of terrestrial hydrothermal activity are highly diverse, as a few examples can illustrate. High concentrations of metals such as As that are present in effluent can be absorbed by vegetation in thermal areas with variable effects (Koch et al., 1999). High concentrations of fluoride in dilute hot-spring outflow are a problem (fluorosis) for animals and humans in parts of China, Kenya, Tanzania, and elsewhere (e.g., Xu et al., 1995). Toxins in thermophilic cyanobacterial mats have been blamed for deaths (e.g., Krienitz et al., 2003) of birds. Fumarole gases are commonly toxic and have killed animals and vegetation in many locations (e.g., Baxter et al., 1999). Effluent from geothermal sites can contaminate freshwater aquifers and surface waters. In many cases, the impact of hydrothermal activity is negative from a human perspective, but this is partly balanced by the strong appeal of hot springs, geysers, and fumaroles as tourist attractions.

Temporal and anthropogenic changes in hot spring and geyser activity

Hydrothermal features at the land surface are typically ephemeral, with activity lasting from months up to several hundred thousand years (see Chapter Hydrothermal Environments, Fossil), but renewed activity may recur at the same location following volcanic and tectonic events (e.g., faulting) or climate change. Hot springs, geysers, and fumaroles may continue to function provided the basic requirements of a heat source, reliable fluid source, and permeable fluid pathway to the surface are met. Variations in heat source typically occur over long timescales (e.g., magmatic cooling), but water supply and fluid pathways (permeability) to the land surface can vary over much shorter timescales and induce frequent and shorter-term changes in hydrothermal activity.

The fluid recharge is especially sensitive to climate. Sturchio et al. (1993), for example, showed that periods of sinter formation in the arid northern Kenya Rift during the Pleistocene could be correlated with phases of humid climate. Springs that discharged chloride waters and precipitated sinter when water tables were regionally high during wetter periods are now acidic fumaroles or have become extinct. Even very short-term climate changes can have an impact. The activity of geysers and hot springs on the shoreline of Lake Bogoria in Kenya, for example, responds rapidly to minor changes in lake level (<30 cm) that are induced by El Niño rains (Renaut et al., 2005).

Anthropogenic activity can also cause substantial changes to the level of the water table over periods of a few years, as examples from New Zealand demonstrate. Development of the Wairakei geothermal power plant in the late 1950s led to a large fall in the water table, subsidence, and the loss of at least 100 geysers (Glover, 1965; Bryan, 2008). Decreasing geyser activity and other
Life in terrestrial hydrothermal environments. (a) Vent of small geyser (former well bore) at Tokaanu, New Zealand. Despite the boiling water spray and overflow, a diverse microbial community inhabits the area at the vent and their remains are fossilized in the sinter. (b) Thick (up to 10 cm) microbial mats near the vent of a perpetually spouting, Na–HCO₃ hot spring at Lake Bogoria, Kenya (shown in Figure 2c). The mats (*Chloroflexus* and several cyanobacterial species) receive spray from the spring but are absent where water temperature is constantly >70°C. (c) Microbial mats (cyanobacteria) in shallow outflow (pH: 8.8; T: 40–85°C) from a Na–HCO₃ hot spring at Lake Bogoria, Kenya. (d), (e) Small coniform stromatolites, built by *Phormidium* growing in Kirihoro spring pool (pH: 7.5; T: 38°C), Tokaanu, New Zealand. (f) Green subaqueous *Cyanidium* mats adjacent to glassy spicular sinter in an acidic (pH 2.6; T: 48°C), shallow spring pool near Hakereteke Stream, north Waiotapu, New Zealand. (g) Soft mats built mainly by diatoms and fungi in acidic outflow channel (pH: 2.6; T: 41°C) from Frying Pan Flat, Waiotapu, New Zealand. (h) Thick accumulation of pupae of *Ephydra* sp. (e) along the edge of a small alkaline hot spring (v) (pH: 8.6; T: 47°C), Lake Magadi, Kenya. The ephydrids feed on the mats on the discharge apron. (i) *Alcolapia grahami* swimming in the vent pool (pH: 9.9; T: 46°C) of an alkaline hot spring, Lake Magadi, Kenya.
changes in the Whakarewarewa geothermal area over several decades have been attributed to natural changes in the subterranean plumbing system and to increased water extraction in nearby Rotorua. The history of Geysir in Iceland is known from 1,294 AD because its behavior has been recorded in Icelandic literature. Historical sources show that the geyser activity has varied considerably, with periods of regular discharge alternating with periods of quiescence with eruptions. At Geysir, earthquakes rather than climate variability often seem to cause changes in its patterns of eruption (Tórason, 1985; Jones et al., 2007).

In some instances, changes in the subsurface aquifer and plumbing systems may trigger renewed activity after years of inactivity. The sudden, renewed activity of hot springs in residential areas can, for example, have a radical impact. This has happened recently (2000–2001) in Rotorua, New Zealand, where house owners awoke to find hot springs and mudpools in what used to be their back gardens, and a hydrothermal eruption (February 2001) sent mud 100 m into the air and created a pool 10 m in diameter in a local park.

Conclusions
Terrestrial hydrothermal environments occupy a small percentage of the Earth’s surface but they provide a wealth of information on both geological and biological processes. These environments cover almost the entire spectrum of pH (0 to >10 in some alkaline hot springs) and water temperature range, and it is commonly possible to examine alkaline and highly acidic environments within a few kilometers of each other. For the geologists, they provide clues to mineral–water reactions occurring below the Earth’s surface and the origin of some ore minerals. For the biologists, they provide opportunities to study a wide range of extremophiles in natural environments that, with care and permission, are generally accessible. With the discovery of possible siliceous hydrothermal deposits and highly acidic environments on Mars, the scientific interest in these environments is currently high and is likely to continue.

Bibliography


**Cross-references**

*Astrobiology*

*Extreme Environments*

*Hot Springs and Geysers*

*Hydrothermal Environments, Fossil*

*Hydrothermal Environments, Marine*

*Sinter*

**HYPERSALINE ENVIRONMENTS**

ICHNOLOGY

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Definition
Ichnology is the study of fossilized, generally macroscopic, animal–sediment interactions. This includes morphological, textural, and local compositional effects related to the animal’s activities. Similar studies in modern sediments are referred to as neoichnology.

Overview
Ichnological studies comprise a wide range of animal–sediment interactions. The scope of animal–sediment interactions was broadly established by Seilacher (1967). He showed that various animal activities, including motility, dwelling, grazing, farming, scavenging, predation, and sessile feeding, are represented by ichnofossils (also referred to as trace fossils).

Bioturbation is the displacement of sediment by animals. In marine environments, common mediators of bioturbation include worm-form animals (such as nemerteans, polychaetes, and hemichordates), marine crustaceans, bivalves, motile echinoderms, sponges, and sea anemones.

Ichnofossils can form from impressions on the sediment surface, intrusion into the sediment, or from excavation of sediment. The former can be thought of as tracks and trails, whereas intrusions and excavations are generally represented by burrows, shafts, and tunnels. Biodeformation structures may result from intrusive activities in loose or fluid-rich sediment. Intrusive, excavatory, and biodeformational structures impact the sedimentary and geochemical character of the surrounding sediment. Traces also incorporate, rework, and redistribute organics in sediment. This influences the geochemical zonations associated with sedimentary surfaces, and in this regard, bioturbation can be viewed as a diagenetic process.

Biomechanical alteration of sediment texture
Burrowing organisms significantly alter the physical character of a substrate. The changes involved include redistribution of grain sizes (vertically and laterally), modification of grain size, sediment compaction, and sorting (summarized in Bromley, 1996). The physical manifestations of bioturbation are variable depending on the intrusive process employed by the animal (i.e., inversion, compression, excavation, or backfill).

In clastic sediments, the most important result of bioturbation is the rearrangement of coarse versus fine grains. This results in an alteration of permeable pathways in the sedimentary media that can influence diagenetic reactions. In carbonate sediment, grain-size reduction probably represents the most significant substrate modification (Chow and Longstaffe, 1995). Reduction of substrate grain size permits a larger surface area to interact with diagenetic fluids and provides more nucleation sites in a given volume.

Biochemical alteration of sediment texture
An important result of bioturbation is the incorporation of localized, concentrated organic material in the form of mucus or feces. Burrow-associated organics induce a geochemical microenvironment that may extend several centimeters, or even decimeters, into the substrate. In well-aerated sediments, burrow linings comprising agglutinated, fecal or primary organic material contain the most concentrated local sources of organic material. These burrows therefore provide excellent substrates for bacterial colonization. Grazing and mining of intact organic remains further lead to their disintegration and introduce larger surface areas for microbial respiration. Moreover,
the ingestion of refractory organic carbon can lead to the excretion of more labile fecal pellets (Lee, 1992).

With most intrusive bioturbation, the sediment–water interface increases in surface area as a result of infaunal animal colonization. The larger (and deepened) sediment–water interface alters chemical fluxes between the sediment and water column. Animals that pump water through their burrow (bioirrigation) increase the rate of chemical flux. Most notably, irrigation of worm burrows with oxygenated seawater results in increased downward diffusion of O2, which then affects the oxidation of redox-sensitive elements. For the aforementioned reasons, the accepted scheme of biogeochemical zones in sediment is an oversimplification. Bioturbation influences the solid phase and pore-water properties of sediment by increasing the transport of diagenetic reactants and products across the sediment–water interface. Consequently, surficial sediments cannot be considered a homogeneous medium characterized by one-dimensional vertical diffusion. They are, instead, heterogeneous and intensively mixed with a complex spatial distribution of biogeochemical zones that are difficult to accurately resolve (Aller, 1980).

Metal ion enrichment in ichnofossils
Cation enrichment in burrow linings is the result of sorptive processes. Metals concentrate in burrow linings in three ways: (1) adsorption, as oxide or oxyhydroxide coatings on to the high-energy surfaces provided by fine-grained particles (silt and clay); (2) as sulfide or phosphate phases under reducing conditions; or (3) associated with organic material (as adsorbed coatings or forming organo-metallic complexes) (Over, 1990). The relative importance of each type of enrichment mechanism varies with the depth of burrow penetration, the grain size/composition of the sediment, and bottom-water and pore-water composition.

Ichnofossils and cementation
Trace fossils commonly influence local cementation patterns by changing the sediment texture, its redistribution, and local composition (Gingras et al., 2004). They also facilitate the accumulation of microbial communities, and through their chemoheterotrophic metabolisms, the microbes can alter pore-water chemistry and saturation states. Ichnofossils also possess higher metal concentrations which can serve as nucleation sites for authigenic mineral formation. Although ichnofossils have been strongly linked to the distribution of microorganisms, there is no existing model associating trace fossil diagenesis to microorganic process.

Summary
The presence of ichnofossils/bioturbation in a sedimentary substrate has the effect of altering sediment distribution and permeability pathways. Moreover, the redistribution and stowage of organics in and around ichnofossils strongly influence the distribution of microorganisms associated with the sedimentary surface. This has an impact on the nature and distribution of biochemical zonations in sediment. The mechanical and (bio)chemical nature of trace fossils influences diagenetic processes.

Bibliography

Cross-references
Biosignatures in Rocks Mat-Related Sedimentary Structures Microbial Biomineralization Microbial Degradation Organomineralization Pore Waters Sediment Diagenesis – Biologically Controlled Trace Fossils: Neoproterozoic

IMMUNOLOCALIZATION

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Synonyms
Immunocytochemistry; Immunodetection; Immunohistochemistry; Immunolabeling

Definition
Immunolocalization: Technique using specific antibodies to localize macromolecules (proteins, polysaccharides) within biological material (subcellular fractions, cells, tissues, biofilms).

Introduction
Immunolocalization is based on the detection of specific targets (“antigens”; mostly proteins or polysaccharides) in biological samples (cells, tissue, biofilms) by antibodies
as probes. Regularly, antibodies of IgG subtype (Figure 1a) and IgM and IgA subtypes are used. The antibodies may be monoclonal (i.e., identical molecules that recognize only one epitope, which is a short stretch of amino acids or sugar molecules) or polyclonal (a set of antibodies directed against a mostly undefined variety of epitopes on the surface of a larger molecule or a cellular component). Specific non-covalent binding is brought about by the hypervariable region of the antibody (paratope), exactly fitting to an epitope. The hypervariable regions are the tip parts of the Y-shaped arm of the antibody (Figure 1a). All other parts, including the Fc-part, are structurally identical within one antibody subclass. Localization of the antigen is only possible when the specific (or primary) antibody ("probe") binds to a marker. A variety of antibody-marker systems are available for different applications (Figure 1b; see below). The marker may be coupled by different methods to the probe (Hoppert, 2003):

- By direct coupling of the marker to the probe prior to use in a localization experiment. The detection procedure requires just one binding step. The method is also termed direct immunolocalization.
- By binding of a secondary probe (mostly a secondary antibody) to the Fc-part of a primary antibody after this primary antibody is already bound to the specific target (two-step procedure). The secondary probe is coupled to the marker. The method is also termed indirect immunolocalization.
- By binding of tertiary probes to the secondary probe (for signal amplification). Tertiary probes are responsible for binding or deposition of the respective marker.

**Immunolocalization in light and electron microscopy**

For immunolocalization in (fluorescence) light microscopy, fluorescent dyes are used as markers (immunofluorescence microscopy). The marker consists of the dye covalently coupled to a protein (e.g., the secondary antibody) that binds to the primary marker, the antigen-specific antibody. It is also possible to detect more than one target in a sample by the use of marker-probe systems with specificities for different targets coupled to fluorescence markers with discernable absorption–emission spectra. Access to the antigen in tissues or embedded samples can be achieved by (cryo-) sectioning or by permeabilization (Goldenthal et al., 1985). For (nonfluorescent) bright field microscopy, marker systems that catalyze enzymatic reactions are used, leading to deposition of dark-stained precipitates at the antigen binding sites (Ramos-Vara, 2005).

For electron microscopy, probes are coupled to colloidal gold as an electron dense marker. As stated for light microscopy, the marker can be directly coupled to the primary antibody, but regularly, a secondary system is combined with a primary antibody. Also, tertiary systems for signal amplifications are used. In equivalence to multiple detections of targets in light microscopy, it is possible to detect more than one target in a sample by use of marker-probe systems with discernable particle sizes of gold colloids. Particle sizes between 5 and 25 nm are used for experiments at the cellular level. Small gold particles may also be enlarged with silver or gold salt solutions for easier localization (Humbel et al., 1995). For epitope mapping in macromolecules, 1-nm markers ("nanogold") are available (Hainfeld and Powell, 2000).

Several modifications of the original immunogold marker technique can increase the resolution and sensitivity of the method. Dodecaborane clusters may be coupled to antibodies or Fab-fragments via polylysine dendrimers. The boronated antibodies have a lower detection limit in immunolocalization experiments than conventional gold conjugates. Detection of boron is performed by element-specific imaging in energy-filtering transmission electron microscopy.
Techniques like catalyzed reporter deposition (“CARD”) use tertiary markers. CARD is a technique for amplification of a signal, originally designed for Western blotting, but modified for light and electron microscopy. In the first step, a specific primary antibody is coupled to the target. Then, in a second step, a secondary, biotin-tagged antibody is coupled to the primary antibody. A streptavidin–peroxidase conjugate is then bound to the biotin tag. Peroxidase catalyzes the deposition of tyramides indirectly. The tyramide itself is bound to a detectable marker (e.g., again biotin), and may be localized by the use of streptavidin-coupled colloidal gold for detection in the electron microscope (Mayer and Bendayan, 1997). For light microscopy, fluorescent dye-coupled tyramides are used (Wasielewski et al., 1997).

Related techniques
Besides the widely used antibodies, other specific probes for localization of a target may be used. Table 1 gives an overview of the relevant techniques. Lectin proteins have defined specificities to short carbohydrate stretches and are therefore an alternative to antibodies for detection of polysaccharides. They are mainly used for detection of glycosylated residues or polysaccharides on cell surfaces and/or in microbial biofilms (Kämper et al., 2004).

Especially when macromolecules are present in high concentrations, a target based on a substrate-specific enzyme may be applied. Enzyme–substrate interaction may also be detected with a substrate inducing the precipitation of an electron-dense marker to localize an enzyme activity.

Equivalent to in situ hybridization in light microscopy, the electron microscopic approach can allow high-resolution mapping of specific mRNA. The technique

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### Immunolocalization, Table 1 Techniques for the localization of biological macromolecules by light (LM) and electron microscopy (EM)

<table>
<thead>
<tr>
<th>Problem</th>
<th>Specimen treatment</th>
<th>Marker system (technique; references)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localization of specific proteins (antibody-gold/antibody-fluorescent probe) or carbohydrate complexes (lectin-gold/lectin-fluorescent probe) inside cells (LM/EM)</td>
<td>Ultrathin sections of resin-embedded samples subjected to immunolocalization</td>
<td>Antibody-colloidal gold, lectin-colloidal gold (Roth et al., 1980; Horisberger, 1985; Danguy et al., 1988)</td>
</tr>
<tr>
<td>Localization of specific proteins on the cell surface or on subcellular particles (EM)</td>
<td>Incubation of cells or subcellular fractions with the marker system prior to resin embedding or direct adsorption on specimen support films</td>
<td>Antibody-colloidal gold, lectin-colloidal gold (Ackar, 1988; Rohde et al., 1988)</td>
</tr>
<tr>
<td>Localization of single protein epitopes and their relative position to each other on the surface of the object (EM)</td>
<td>Homogeneous protein preparations (especially large proteins or viruses) subjected to localization of epitopes</td>
<td>Antibody, Fab-fragment, (epitope-labeling; Hermann et al., 1991)</td>
</tr>
<tr>
<td>Detection of enzyme activities (EM)</td>
<td>Incubation of cells or subcellular fractions with the marker system</td>
<td>Specific enzyme substrate coupled to precipitation of electron dense marker (Hayat, 1973ff; Wohlrab and Gossrau, 1992)</td>
</tr>
<tr>
<td>Detection of macromolecular components acting as enzyme substrates (e.g., starch, lipids) (EM)</td>
<td>Labeling of enzyme substrates with gold particles coated with the relevant enzymes in ultrathin sections of samples</td>
<td>Enzyme-colloidal gold (Bendayan, 1985)</td>
</tr>
<tr>
<td>Localization of metabolites in the cell (EM)</td>
<td>Incubation of metabolically active cells with the precursor, embedding in conventional resin or by application of cryotechniques, treatment with a specific photoemulsion</td>
<td>Metabolic precursors (e.g., sugars or aminoacids) labeled with radioactive markers (autoradiography; Gregg and Reznik-Schüller, 1984)</td>
</tr>
<tr>
<td>Localization of specific DNA or RNA (preferably mRNA) sequences in cells (LM/EM)</td>
<td>Fluorescent in situ hybridization for light microscopy, hybridisation of DNA/RNA of fixed samples with the marker system followed by embedding and sectioning, detection of the marker system by autoradiography or with streptavidin-colloidal gold for electron microscopy</td>
<td>RNA or DNA oligonucleotide labeled with different markers (in situ hybridization; Amann et al., 1995; Egger et al., 1994)</td>
</tr>
<tr>
<td>Comparison of immunolocalization targets from overview (low resolution) to subcellular structures (high resolution) (EM)</td>
<td>Ultrathin (and semithin) sections of resin-embedded samples subjected to immunolocalization</td>
<td>Antibody-colloidal gold and antibody-fluorescent probe, lectin-colloidal gold and lectin-fluorescent probe (Takizawa and Robinson, 2003).</td>
</tr>
</tbody>
</table>
may be combined with specific signal enhancement methods (CARD, see above) and is suitable for high-resolution in situ hybridization (Cheung et al., 1999).

For microautoradiography, radiolabeled markers, incorporated into cell structures, are located by exposure to photographic emulsion. The deposition of silver bromide grains on the photoemulsion is detectable by light and electron microscopy. The technique is demanding and difficult to reproduce, though it gives information on processing and distribution of metabolites that cannot be obtained, on a structural level, by other methods (Gregg and Reznik-Schüller, 1984).

**Correlative microscopy**

The technique of correlative light/electron microscopy allows examination of the same sample at micrometer scale for overview images and at nanometer scale for imaging of features at high resolution. Regularly, (fluorescence) light microscopy and electron microscopy of samples is combined. The marker/probe systems are either suitable for both microscopic techniques at the same time or for combinations of different systems that are detectable either by light or by electron microscopy.

Quantum dots (qdots) are colloidal semiconductor nanocrystals and are detectable in the electron microscope, either as electron dense particles or via element specific imaging. Since qdots exhibit specific absorption/emission spectra in the light, they are also detectable as fluorescent markers. When qdots are coupled to primary antibodies, they are marker-probe systems suitable for both imaging techniques (Nisman et al., 2004). Though it is possible to visualize ultrathin sections by (fluorescence) light microscopy, sections of several micrometer in thickness (semithin sections) are regularly more suitable. It is possible to obtain nearly identical features of a specimen when one ultrathin and one semithin is consecutively cut from a specimen. Semithin and ultrathin sections are then processed in different ways, either with marker-probe-systems for immunofluorescence or for immunoelectron microscopy (Figure 2; Takizawa and Robinson, 2003, Wrede et al., 2008).

Correlation of data from immunolocalization may also be performed with other microscopic techniques, such as Raman microscopy, scanning electron microscopy, or atomic force microscopy.

**Conclusions**

It has already been understood in the first half of the past century that, besides comprehending biochemical or molecular reactions in organisms, the understanding of their organization is of equal importance (Hopkins, 1933). Tools for understanding organization of these processes have been developed consecutively, when methods became available to keep organisms alive or at least well-preserved down to molecular scale. The techniques revealed that the presumption of some early biochemists was right, i.e., metabolic pathways, growth and cell division, even of apparently non-compartmented bacteria, are organized in high ordered, large macromolecular complexes. Future direction of localization techniques will include the application of marker systems for high resolving microscopy systems that will allow observation of living cells down to nanometer scale (Donnert et al., 2006).

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IRON ISOTOPES

Please refer to “Isotope Fractionation (Metal).”

IRON SULFIDE FORMATION

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Definition

There has been a great deal of research into the formation of sedimentary iron sulfide minerals because their history is intertwined with the biogeochemical cycles of iron, sulfur, carbon, and oxygen (Berner, 2001). Iron sulfides occur in sediments from a wide range of depositional environments, from the deep sea to the nonmarine, and the principal mineral in the rock record is pyrite (FeS2, cubic). Its dimorph marcasite (orthorhombic) is considered metastable and apt to invert to pyrite over geologic time (Murowchick, 1992), but is probably more widespread than commonly acknowledged (Schieber, 2007). Presumed precursor minerals of sedimentary pyrite are mackinawite (FeS1−x), and greigite (Fe3S4), minerals that impart a black coloration to modern reducing sediments (Goldhaber and Kaplan, 1974). Both are metastable and transform over time into pyrite or a mixture of pyrite and pyrrhotite (Berner, 1967). Pyrrhotite, hexagonal FeS, is thermodynamically stable in sediments (Berner, 1967), but extremely rare in modern or ancient sediments (Kobayashi and Nomura, 1972) due to kinetic limitations (Canfield and Raiswell, 1991).

Mackinawite and greigite are also known as acid-volatile iron sulfides (AVS) because in contrast to pyrite and marcasite they are readily soluble in HCl. Mineralogically, greigite is the sulfur analog of magnetite and is indeed strongly ferromagnetic (Dekkers and Schoonen, 1996). It can be formed by magnetotactic bacteria and dominates the magnetic properties of some modern sediments and...
Pathways to sedimentary iron sulfides

The understanding of iron sulfide formation under Earth surface conditions has largely been informed by extensive laboratory studies (see summary by Morse et al., 1987). Multiple potential pathways for sedimentary iron sulfide (pyrite) formation have been recognized. As summarized by Goldhaber (2003), these include (1) direct precipitation of pyrite from polysulfide bearing solutions, (2) progressive conversion of solid iron monosulfides to pyrite (Figure 1) by polysulfides, (3) reaction of \( \text{H}_2\text{S} \) with a solid iron monosulfide to form pyrite (release of \( \text{H}_2 \text{gas} \)), and (4) reactions that involve iron loss from a solid iron monosulfide precursor through oxidation.

As indicated by this summation, the body of published literature lives by the assumption that iron monosulfides (generic for mackinawite and/or greigite) are necessary precursors for pyrite formation in sediments. Thus, the normal iron sulfide paragenesis in sediments is presumed to proceed from mackinawite over greigite to pyrite (e.g., Wilkin and Barnes, 1996; Benning et al., 2000). The tacit assumption is that sedimentary pyrite cannot form in the absence of acid-volatile iron monosulfides (AVS). Yet, direct evidence for this traditional view is scant. Rickard and Morse (2005) have argued strongly that in numerous studies of modern sedimentary pyrite formation abundant pyrite is produced in the absence of detectable AVS. They argue that many of the equations that describe sedimentary pyrite formation in the literature represent net mass balances and do not describe the actual reaction processes. Rickard and Morse (2005) point out that AVS are not required for reaction pathways that involve polysulfide or \( \text{H}_2\text{S} \) (numbers (2) and (3) from above), and that aqueous FeS clusters could be involved instead of solid monosulfide phases. They propose that the products of the diagenetic sulfide machinery, mackinawite, greigite, and pyrite, may form either simultaneously or in no particular order during sediment diagenesis.

The building blocks

Regardless of how sedimentary iron sulfides are ultimately produced, it is instructive to examine where the requisite iron and sulfur come from. Because iron is insoluble in the presence of oxygen, the iron content of sea water and freshwater is extremely low (Stumm and Morgan, 1996). It is, therefore, practical to assume that any iron that is available for iron sulfide formation has to be part of the particulate matter that makes up the sediment. Typical deltaic and marine muds have iron contents that range from 4% to 8% (Calvert, 1976; Chester and Morgan, 1996). It is, therefore, practical to assume that any iron that is available for iron sulfide formation has to be part of the particulate matter that makes up the sediment. Typical deltaic and marine muds have iron contents that range from 4% to 8% (Calvert, 1976; Chester and Asten, 1976; Aller et al., 1986), but not all of that iron is equally available for diagenetic iron sulfide formation. Although there may be plenty of iron in iron silicates (e.g., biotite, chlorite), release of that iron requires alteration/destuction of the containing silicate, a process that operates more efficiently under the higher temperatures of deep burial diagenesis (Figure 2). Thus, this iron fraction is not available during early diagenesis when the bulk of sedimentary iron sulfides is formed.
The iron fraction that is critical in early diagenesis is in the form of coatings of iron oxyhydroxides on detrital grains (especially on clays; Carroll, 1958), as well as in the form of particulate/colloidal iron oxyhydroxides (e.g., Allard et al., 2004). Of the 4–8% of total iron that we can expect in average muddy sediments, only some 10%, and under very favorable circumstances up to about half, is made available by reducing agents (Gibbs, 1977; Trefrey and Presley, 1982; Canfield, 1989) for the formation of early diagenetic iron sulfides.

Sulfur, of course, is readily available via seawater sulfate in marine sediments, and for surficial sediments the overlying seawater represents an essentially limitless reservoir. Only upon burial, once consumed sulfate can no longer be replenished by downward diffusion, does sulfur become a limiting commodity. In all sediments, the production of H₂S for sulfide formation is accomplished by sulfate reducing prokaryotes that utilize sulfate as an electron acceptor for energy producing chemical reactions. For carbohydrate oxidation this reaction may be written as

\[ \text{SO}_4^{2-} + 2\text{CH}_2\text{O} \rightarrow \text{H}_2\text{S} + 2\text{HCO}_3^- \]

Thus, organic matter that can be utilized by sulfate reducing microbes is essential for the operation of the sedimentary sulfide factory. Even in the presence of abundant sulfate, sulfide production will only occur if there is “food” for the microbes. Sulfate reduction equations proposed by Canfield and Raiswell (1991) suggest that it will require about 5 cm³ of microbially “digestible” organic matter (e.g., lactate, ethanol, acetate, sugar) to precipitate 1 cm³ of FeS₂. Thus, low organic matter contents in sediments will tend to limit sulfide production and the amount of iron that can be converted to pyrite.

Above considerations also have implications with regard to where we most likely will find large quantities of iron sulfides in sedimentary rocks. Because pure carbonates typically accumulate far from the influence of terrigenous clastics, the average carbonate rock tends to contain only minor quantities of iron and thus, sedimentary iron sulfides are generally low in abundance. Sandstones, even though they may contain appreciable quantities of iron in the form of iron bearing detrital silicates and as iron oxyhydroxide grain coatings, are comparatively poor in organic matter and thus lack the fuel to drive extensive early diagenetic sulfate reduction. When we do find substantial accumulations of iron sulfides in carbonates or sandstones, they are in the majority of cases due to late diagenetic processes that benefited from higher temperatures and constituent bearing formation waters. Exceptions occur when carbonates or sandstones are interbedded with shales and mudstones, because the latter carry the bulk of iron in the sedimentary mass balance (Taylor and McLennan, 1985). The lion’s share of sedimentary iron sulfides is therefore contained in fine grained terrigenous rocks (shales and mudstones).

Iron sulfide textures

Pyrite, the most commonly reported iron sulfide in sediments, is cubic in structure, and thus cubes and octahedra are common morphologies. In addition to these fundamental forms, iron sulfides are also patterned into a wide range of textural types (e.g., single grains, framboids, pore fill cements, concretions, pyrite cemented layers, and pyritic lags) that carry useful information about conditions during deposition and early diagenesis. From a geobiological perspective, concretionary iron sulfide accumulations are of particular interest because their formation was likely driven by decomposing organic matter. Their morphology varies widely, and many types have been described, such as pyritized burrow tubes, infilling and encrustation of fossils, replacement of fossil hard parts (carbonate shells, bones), and pyritization of soft tissues.

Single grains

As single grains, pyrite may occur as a disseminated component, ranging from less than 1 µm to tens of micrometer in size. Yet, while these may indeed be grains that record a single growth episode, etching (e.g., with HNO₃) may reveal internal textures that speak to multistage growth.

Pyrite framboids

Ubiquitous in modern and ancient sediments, pyrite framboids are typically near-spherical in shape, range in diameter from about 1 µm to tens of micrometers, and are themselves composed of tiny, discrete, and equigranular pyrite crystallites. They have been the subject of numerous studies, and early on there was an assumption that organic matter (Figure 3) somehow plays a role in their formation (Schneiderhöhn, 1923; Love,
Yet, because they can also be produced abiotically in the laboratory (Berner, 1969; Sweeney and Kaplan, 1973), the role of organic matter remains a matter of debate (e.g., Kalliokoski, 1974). The abundantly noted close association of pyrite frambooids and organic matter may well be coincidental because the reducing conditions that promote their formation are best developed in sediments with elevated contents of organic matter.

Most pyrite frambooids observed in sedimentary rocks consist of microcrysts (Figure 4a; typically cubes, octahedral, or pyritohedra) that show poorly defined to highly ordered packing into spherical aggregates (Wilkin and Barnes, 1997; Ohfuji et al., 2005). The size ratio of microcrysts to frambooid diameter is quite variable (Figure 4b). Directly adjacent frambooids may show vast differences in diameter/microcryst size ratios (Figure 4b), and also show contrasting microcryst morphology. These textural details probably carry some information about the pore water environment during formation, but at present their significance is poorly understood. Pyrite frambooids can survive intact into greenschist grade metamorphism, and can for example, identify slates that originated as marine shales (Figure 4d).

Iron Sulfide Formation, Figure 3 SEM photomicrograph of an etched pyrite frambooid in the Devonian Chattanooga Shale of Tennessee, USA. The etching with HNO₃ removed the pyrite crystallites and left behind a honeycomb of organic matter that filled the space between the pyrite grains. The organic matter has the same composition as the unstructured kerogen in the surrounding sample.

Iron Sulfide Formation, Figure 4 Pyrite frambooids in the rock record. All images are SEM photomicrographs (backscatter mode). (a) Moderately well-ordered frambooid from the late Devonian New Albany Shale (Indiana, USA). Note differential compaction of clays and organic matter around the frambooid, indicating frambooid formation prior to compaction when the sediment contained ~80% water. (b) Frambooids in modern sediments from the Santa Barbara Basin (offshore southern California, USA). Note the strong differences in diameter/microcryst size ratios and the contrast in microcryst morphology (pyritohedra vs. complex cubes). (c) Frambooid consisting mostly of variably size octahedra (Ordovician Powers Steps Formation, Newfoundland). (d) Pyrite frambooids that survived low-grade metamorphism and cleavage development (±horizontal) in the Ordovician Martinsburg Slate of New Jersey, USA.
The formation processes of pyrite framboids are discussed at length by Wilkin and Barnes (1997), and further perspectives are presented by Ohfuji et al. (2003) and Butler and Rickard (2000). Fundamentally, in order to form framboids within the pore spaces of a sediment, iron should be able to migrate to the site of formation and thus, the pore waters have to be nonsulfidic (anoxic-nonsulfidic; Berner, 1981). Furthermore, in order for iron oxyhydroxides to dissolve in the pore waters, they also need to be free of oxygen. In the idealized sequence of microbial decay reactions in sediments (Brett and Allison, 1998; Curtis et al., 2000), this is known as the “suboxic” zone, and is followed by the “anoxic” zone of sulfate reduction. The boundary between the suboxic and the anoxic zone is also referred to as the redox interface, and it is here where dissolved iron meets sulfide and iron sulfides can precipitate. Pyrite framboids form in that region. Yet, whereas these zones are thought to be layered atop each other in surficial sediments, in reality it is a bit more complex. Every single framboid implies that iron migrated from a surrounding sediment volume to form this localized accumulation, and by default this means that there was a localized source of sulfide that via iron sulfide precipitation maintained the underlying concentration gradient. The sulfide source was probably a colony of sulfate reducing bacteria that prospered around a lump of easily digested organic matter. Thus, rather than simply being a narrow bedding-parallel zone in which dissolved iron interacts with dissolved sulfide, the realm of framboid formation (the redox interface) is heterogeneous. It makes more sense to think of it as “islands” of microbial sulfide production in a “sea” of iron-bearing pore waters (Figure 5), the reducing microenvironments that have been mentioned elsewhere in the literature (e.g., Hudson, 1982).

Whereas in typical sediments the redox interface is situated somewhat below the sediment water interface, there are situations where the redox interface is actually located in the overlying water column, such as in the Black Sea. In euxinic basins, with the lower portion of the water column being anoxic and sulfidic, framboids can grow in the water column near the redox interface and then settle and become part of the accumulating sediments (Wilkin et al., 1996). Wilkin et al. (1997) report that framboids of that origin are rather small (mean framboid diameters <5 µm, narrow size distribution) when compared to those forming in sediments underlying oxic and dysoxic waters (mean framboid diameters 5–10 µm, broad size distribution). The implication of this is that one can use the framboid size distributions of sediments to determine whether they accumulated beneath an anoxic water column. Wignall and Newton (1998) applied this concept to ancient mudrocks and were able to correlate framboid size distributions with paleoecologically based reconstructions of bottom water oxygenation. However, framboid size in sediments may also be influenced by the availability of iron during early diagenesis. For example, a study of framboid size distributions in sediments from the Santa Barbara Basin (offshore southern California) indicates that framboid size distributions primarily reflect conditions within the immediate surface sediment (such as availability of readily soluble iron). Framboid size distributions in annual varves (1984–2004) from the Santa Barbara Basin suggest three euxinic interludes in the past 2 decades, even though seasonal bottom water surveys consistently recorded suboxic to dysoxic bottom water conditions (Schieber and Schimmelmann, 2006). It appears, therefore, that it may not be possible to draw conclusions about water column conditions from pyrite framboid size distributions.

Iron Sulfide Formation, Figure 5 SEM photomicrographs (backscatter mode) of localized pyrite framboid formation in reducing microenvironments of Santa Barbara Basin (offshore southern California) surface sediments. At left, a multichambered calcareous foraminifera with chambers filled by framboids. At right, a framboid filled diatom. Lower right half, imaged in secondary electron (SE) mode (framboids not visible). Upper left half, imaged in backscatter (BSE) mode, the framboids shine through the lower density opaline “lid.”
Polyframboids
Pyrite framboids may also occur as larger aggregates, so called polyframboids. These have been described from sediments of various ages (Schieber and Baird, 2001) and have a tendency to form in cavities of organic remains (Figures 5 and 6). Judging from differential compaction around polyframboids (Figure 6), they must form prior to any compaction, just like single pyrite framboids. They also imply iron migration from a surrounding sediment volume that was anoxic but not sulfidic. The difference between polyframboids and regular pyrite framboids may simply be a higher iron supply from the pore waters, or alternatively that the “islands” of microbial sulfate reduction are more widely spaced and thus, focus the available iron onto fewer sites of precipitation. Because most marine sediments start out with comparable amounts of reactive iron, the latter scenario is more likely.

Small-scale pyrite aggregates and clusters
Pyrite bodies that consist of coalescing, irregular, rounded clots of small pyrite grains have been described as aggregated pyrite by Hudson (1982). The cores of individual clots may be small pyrite crystals or pyrite framboids, and these cores are overgrown by coarser pyrite crystals (Figure 7). In places these overgrown grains show a radiating bladed habit. These overgrown grains tend to form somewhat later in diagenesis, at a point when downward diffusion of seawater sulfate is increasingly restricted (Strauss and Schieber, 1990). This type of pyrite aggregate ranges in size from tens of microns to several millimeters.

Concretions and pyritic layers
Iron sulfide concretions in sediments vary in size from a few millimeters to more than 10 cm. Like other occurrences of sedimentary iron sulfides they are most commonly encountered in mudstones and typically occur in distinct nodule bearing horizons (Figure 13). Although pyrite is usually reported as the sole iron sulfide mineral, careful examination reveals many examples of inverted or extant marcasite within these iron sulfide accumulations (Schieber, 2002b, 2007; Schieber and Riciputi,
Especially the larger concretions approximate prolate ellipsoids (Figure 8a) and show differential compaction of the surrounding mudstone (Figure 13). These features attest to growth prior to substantial compaction and growth within a stratiform chemical zone (anoxic and nonsulfidic; Berner, 1981). In order for iron to be directed to localized sites of precipitation within this zone, there need to be sites of abundant H₂S production, as, for example, provided by microbial decay of buried organisms (e.g., bivalve, fish). Thus, the interiors of iron sulfide concretions are good targets for finding well-preserved fossils. In order to allow the concretions to grow to large size, this chemical zone has to remain stationary for a long time, probably on the order of thousands of years (Canfield and Raiswell, 1991). Not uncommonly, the iron sulfide concretions in a given layer also add up to a substantial excess of iron relative to the background sediment, suggestive of prior concentration by mechanical and chemical reworking (see Section “Marcasite and its implications”). Thus, horizons with abundant iron sulfide concretions or abundant pore filling iron sulfides imply slow sedimentation at the time of nodule formation and should also alert us to the possibility of reworking and erosion of underlying sediments (Schieber and Ricciutti, 2005). Other options for the formation of iron sulfide-enriched horizons are, for example, to cover iron-oxide rich layers with an organic-rich anoxic sediment drape (Ingri and Ponter, 1986). Migration of a sulfide-rich reduction front into the iron-bearing layer would effectively convert the iron oxides to pyrite.

Iron sulfides and fossil preservation

Replacement and encrustation of calcareous shells

Encrustation and replacement of calcareous shells by iron sulfides are widely observed in the rock record. Typically, an assumption is made that the iron sulfide in question is pyrite, but a case can be made wherein marcasite was involved at least initially (see below). One way to think about calcite/aragonite replacement is that the saturation...
Iron sulfide formation, Figure 9  Fossil preservation by iron sulfides, SEM photomicrographs. (a) Chamber lining iron sulfides in an ammonite (secondary electron image), Lias delta (Amaltheenton) near Dotternhausen, Germany. The septa of the ammonite (white arrows) are now calcitic, and the chamber interior is filled with calcite as well. (b) Close-up of the iron sulfide (bright, backscatter image) encrusted septum. The encrusting sulfides are a mixture of pyrite (framboids) and marcasite (overgrowth). (c) Close-up of encrustation near septum (backscatter image). The marginal areas of the septum (marked between white arrows) have a different texture than the areas further away. The latter areas contain pyrite framboids (black arrow) in a coarser matrix of blocky to radiating crystals. (d) Close-up of framboids (white arrows) in coarser matrix grains. The latter are anisotropic in reflected light and are either still preserved as marcasite or are marcasite relics. (e) Details of the different textured replacement of the septum margin (between white arrows in (c)). The lamellar texture probably is a relict of the earlier lamellar structure of the septal aragonite.

Soft tissue preservation
The preservation of soft tissue by pyritization is another marvel of fossilization, with well known examples from the Hunsrück Slate (Stürmer, 1985), Beecher’s trilobite bed (Cisne, 1973; Briggs et al., 1991), and the Burgess Shale (Conway Morris, 1986). Ongoing research has been producing a stream of exceptional soft bodied fossils that are preserved in pyrite, such as worms (Gabbott et al., 2004; Farrell and Briggs, 2007), crinoids (Kammer and Ausich, 2007), and plant tissues (Grimes et al., 2001). Pyritization can occur by infilling of cellular cavities, by preferential replacement of more readily decomposed components, and by pyrite coatings on easily degraded soft parts (Canfield and Raiswell, 1991). The latter process may be aided by microbial coatings (Wuttke, 1983) on the decomposing tissues that help to stabilize chemical gradients around the decomposing material. Framboidal, clustered, and aggregated pyrites are the most commonly observed type in this style of preservation. Even microbial cells and textures can be preserved under the right circumstances (Figure 10). With regard to preservation fidelity, soft tissue preservation by pyrite does not preserve as much detail as preservation in a phosphate matrix.

Degree of pyritization (DOP)
A widely used proxy for paleo-oxygenation, DOP is defined (Raiswell et al., 1988; Raiswell and Canfield, 1998) as

\[
DOP = \frac{\text{pyrite Fe}}{\text{pyrite Fe} + \text{reactive iron}}
\]
An extraction with dithionite or 1 N HCl is supposed to mimic reactive iron dissolution in reducing sediments (Canfield, 1989; Leventhal and Taylor, 1990). The highly reactive iron fraction in sediments is defined as the sum of dithionite or 1 N HCl leachable iron, and iron in acid volatile sulfides (AVS). What DOP presumably measures is the completeness of the conversion of reactive iron into pyrite via microbial sulfate reduction during early diagenesis. The underlying premise is that DOP increases as the degree of environmental oxygenation decreases. Because of the need for sufficient quantities of organic matter and reactive iron, measurements of DOP typically focus on carbonaceous mudstones. DOP values below 0.45 are considered indicative of aerobic bottom waters, and those above 0.45 are thought to mark restricted bottom water conditions. Values above 0.75 are considered to indicate anoxic or even euxinic bottom waters (Raiswell et al., 1988). However, the DOP method should not be applied uncritically, because limitations of either metabolizable organic matter or of reactive iron can lead to DOP values that are not reflective of the actual environment (as calibrated by Raiswell et al., 1988). Several other constraints on DOP application, such as outcrop weathering, maturation, and age of rock are summarized by Raiswell et al. (1988).

DOP as an environmental indicator can also be invalidated as a consequence of sedimentary processes. Any reworking and winnowing of pyrite bearing sediments will increase the pyrite content of the sediment, without simultaneously raising the reactive iron content, and as a result the DOP can rise simply as a result of wave or current reworking. In mudstones, indications of reworking at the mm to cm scale will often go undetected (Figure 11), and can lead to inflated DOP values that are incompatible with the levels of oxygen restriction suggested by observations of frequent reworking and bioturbation (Schieber, 2001, 2003).

Marcasite and its implications

Background on marcasite

Reports of marcasite in terrigenous clastics are mainly associated with coal deposits (e.g., Nayar, 1946; Read and Cook, 1969; Wiese et al., 1987). Marcasite occurs either in the coal seams themselves, or in the roof shales, prompting Krumbein and Garrels (1952) to propose an acidic bog/peat association as the characteristic environment for marcasite formation. There have, however, been a sufficient number of diagenetic marcasite occurrences reported from normal marine sediments (e.g., Maynard and Laufenburger, 1978; Rykart, 1983; Jowett et al., 1991; Schieber, 2002b, 2007), as well examples of marcasite that has inverted to pyrite (e.g., Bannister, 1932; Van Horn and Van Horn, 1933), to suggest that it might be more widespread in the marine realm than commonly appreciated. If this is in fact the case, and if acidic conditions are indeed a basic requirement for marcasite formation, we have an obvious problem to reconcile these
basic realities with our current understanding of early diagenesis of marine sediments.

As pointed out above, the paragenetic relationships between sedimentary iron sulfide minerals are still poorly understood (Rickard and Morse, 2005). An essential guidepost for thinking about marcasite in sedimentary environments have been experiments conducted by Murowchick and Barnes (1986) that showed that marcasite is the dominant iron disulfide below pH 5. These experiments are consistent with results from much earlier work by Allen et al. (1914) and are also consistent with subsequent work by Schoonen and Barnes (1991a, b) and Benning et al. (2000). Thus, the relative abundance of pyrite versus marcasite in sediments should be a function of pH. Because marine waters have a slightly alkaline pH of approximately 8, it has been presumed that marcasite cannot form in marine sediments during early diagenesis (e.g., Rickard et al., 1995). Yet, regardless of this, early diagenetic marcasite unquestionably occurs in marine sedimentary rocks (Maynard and Lauffenburger, 1978; Siesser, 1978; Rykart, 1983; Jowett et al., 1991; Schieber, 2002b; Schieber and Ricuputi, 2005; Williams et al., 2003).

Detecting marcasite

Characteristic morphologies, such as “spearhead” twins and “cockscomb” crystals can be used to identify marcasite. However, morphology does not tell us whether the marcasite has inverted to pyrite and the approach does not work for dense masses of crystals. As long as crystallites are large enough, it is comparatively easy to differentiate marcasite from pyrite with a petrographic microscope in reflected light mode (Ramdohr, 1975), and it is also possible to detect marcasite that has inverted to pyrite (Murowchick, 1992). For micron scale grains a new technique, “electron backscatter diffraction” (EBSD), can be applied for identification with an electron microscope. EBSD allows phase identification of submicron mineral grains and is widely used in material sciences (Prior et al., 1999; Schwartz et al., 2000).

Marcasite in sedimentary rocks

Marcasite is probably much more widespread in marine clastics than commonly assumed. It occurs as a cement mineral in marine lag deposits associated with sequence boundaries, in condensed intervals, and even in shales. Lag deposits with marcasite occur, for example, in Late Devonian shale successions of the eastern USA (Schieber, 2007). The Late Devonian Chattanooga and New Albany Shale successions contain laterally continuous erosion surfaces that compartmentalize this black shale succession into a stack of depositional sequences (Schieber, 1998a, b; Schieber and Lazar, 2004). These erosion surfaces imply substantial erosion and winnowing of previously deposited black shale during low stands of sea level (Schieber and Ricuputi, 2004). The associated lag deposits contain
fish bone debris, conodonts, Lingula shells, quartz grains, and abundant pyrite in the form of reworked concretions and form conspicuous rust stained horizons in outcrop (Figure 12a).

In polished thin section pore filling iron sulfides show typical marcasite morphology (Figure 12b). Overall, these lags may contain 30–40% iron sulfides by volume. Loose packing of framework grains indicates precompaction cementation with marcasite. Detrital pyrite grains in these lags are often variably rounded due to mechanical reworking and show etch and corrosion pits on the surface of detrital pyrite grains that are in turn infilled and overgrown by marcasite (Schieber, 2007).

Terrigenous clastic sediments contain at best a few percent reactive iron in the form of iron oxyhydroxide coatings on terrigenous grains (Carroll, 1958; Berner, 1969). Thus, the high iron concentrations in lag deposits with up to 40% iron sulfide require a mechanism for iron enrichment, and in the Phanerozoic at least the iron was most likely reworked from underlying strata (see above). In essence, sedimentary pyrite grains need to be eroded and winnowed in order to add more iron to the surficial layer, a process favored by stratigraphic condensation when net sedimentation rates are negative (Schieber and Riciputi, 2005; Schieber, 2007).

After mechanical enrichment, however, a portion of the sulfide iron has to be remobilized to form the observed overgrowths and pore-fill cements. Preexisting grains are destroyed, either wholly or partially, in order to supply dissolved (Fe²⁺) or readily soluble iron (e.g., Fe(OH)₃) for the growth of new iron sulfides. Corrosion features on reworked iron sulfide grains (Schieber and Riciputi, 2005; Schieber, 2007) are evidence for iron sulfide destruction according to the following reaction:

\[ \text{FeS}_2 + 3.5\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + 2\text{SO}_4^{2-} + 2\text{H}^+ \]

This reaction produces acidity and dissolved ferrous iron. Further oxidation of iron (see following equation) produces iron hydroxide and further acidity.

\[ \text{Fe}^{2+} + 0.25\text{O}_2 + 2.5\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_3 + 2\text{H}^+ \]

The newly formed iron hydroxides are likely to coat surface sediment grains and would remain in the surficial sediment layer. The associated lowering of pore water pH also promotes large pore water concentrations of Fe²⁺ (Maynard, 1983). Such a setting favors reprecipitation of marcasite in the presence of H₂S influx from underlying sediments (Murowchick and Barnes, 1986; Schoonen and Barnes, 1991a, b; Benning et al., 2000).

For sandy lags the presence of marcasite and partial dissolution of reworked pyrite grains are probably a good indicator that a “reoxidation” model for marcasite formation is applicable (Schieber and Riciputi, 2005; Schieber, 2007). In shales with abundant pyrite a comparable sequence of events may be caused by downward oxidation of previously deposited muds. Potential examples are stratiform horizons of iron sulfide concretions in black shale successions (Figure 13). Where these nodule horizons occur, a gray shale bed is typically found just above them (Figure 13a), and the concretions have a flattened radial fibrous habit, a morphology suggestive of marcasite (Figure 13b).

Reoxidation of reducing organic and iron sulfide-rich sediments, also termed “burndown,” has been studied extensively in the context of Quaternary Mediterranean sapropels (e.g., Jung et al., 1997), and marcasite in shales can potentially be used as a mineral-based indicator of burndown events.

Although the generally finer grain size of diagenetic minerals in shales makes recognition of marcasite more difficult, EBSD analysis allows very tiny (micron-size)
Iron Sulfide Formation, Figure 13  Marcasite in late Devonian New Albany Shale, Kentucky, USA. (a) Stratiform horizon of marcasitic nodules (white arrows, black vs. gray shales marked by color bar at right). The nodules are located about 15 mm below the gray/black shale contact. The large amounts of iron sulfide accumulation at this level suggest that the redox interface stayed in that position for a long time, probably a reflection of zero to very low sedimentation. (b) Close-up of nodules that are broken through and show rims (white arrows) of radiating bladed marcasite (large arrow M). Note differential compaction.

Iron Sulfide Formation, Figure 14  SEM photomicrographs (backscatter mode) of marcasite in an iron sulfide-enriched lamina in the Cleveland Shale (Upper Devonian, NE Kentucky). (a) Clusters of bladed marcasite grains that form bedding parallel lamina. Note differential compaction around marcasite clusters, suggesting formation when the sediment still had water content in excess of 80%. It also suggests that marcasite in the Cleveland Shale formed within 10–20 cm of the sediment–water interface. (b) Close-up that shows the platy habit of marcasite crystals (marcasite identification with EBSD). The reoxidation that is implicit in this observation contradicts earlier interpretations of this shale as euxinic (e.g., Jaminski et al., 1998; Rimmer, 2004).
marcasite grains to be identified in situ. A preliminary application of this technique to shales has detected marcasite in carbonaceous shales that range from Ordovician to Cretaceous in age (Figures 14 and 15).

Unique fossil beds where preservation in pyrite and marcasite has been observed, such as Beecher’s trilobite bed (Cisne, 1973) and pyritized ammonoids (Hudson, 1982; Seilacher et al., 1985), are another phenomenon where our “burndown” model could find application. The large stratiform iron buildup that at least some of these deposits represent requires a similar level of stratigraphic condensation as seen in some of the examples above. Furthermore, iron needs to be mobile to form the observed mineralized and encrusted fossils. Thus, finding marcasite and dissolution features on reworked pyrite grains in pyritic Lagerstätten would support the view that such deposits may have formed during intermittent reoxygenation events.

The pH drop associated with pyrite oxidation during intermittent reoxygenation also explains textural features observed in association with iron sulfide replacement of calcareous shells. Examination of calcareous shell replacement in Carboniferous through Cretaceous mudstones showed marcasite as a common component,

Iron Sulfide Formation, Figure 15 Pyrite polyframboid cluster in Chattanooga Shale (Tennessee, USA) that formed within an algal cyst (dark rim). The infill was examined with EBSD. Marcasite (m) and quartz (Q) grew in the spaces between pyrite framboids (p). The open arrangement of framboids and the differential compaction around the cyst indicate that cementation happened prior to compaction. The marcasite observed here may well have been caused by intermittent pyrite oxidation in surface sediments. The relief in this image is an artifact, a consequence of oblique imaging (70° tilt) in EBSD mode.

Iron Sulfide Formation, Figure 16 SEM photomicrographs (backscatter) of marcasite replacing biogenic calcite. (a) Clam shell in Boquillas Formation (Cenomanian of West Texas). Skeletal marcasite crystals (marked m) grow as radiating clusters and replace calcite (ca). There is also partial replacement of the shell by quartz (marked qu). (b) Brachiopod shells in Barnett Shale (Mississippian of Texas). Shell in center is replaced by marcasite (marked m) that grows as bladed and sharply pointed crystals. Equant pyrite (py) formed toward end of iron sulfide deposition. Calcite shells marked ca, and replacing quartz marked qu.
intimately associated with calcite dissolution and precipitation of diagenetic quartz (Figure 16). This mineralogical triumvirate makes chemical sense because marcasite formation requires low pH, calcite dissolves at low pH, and dissolved silica (from opaline tests) precipitates under low pH conditions.

With regard to carbonate rocks, most published reports of marcasite are from chalks (e.g., Morgan-Jones, 1977; Kelts, 1976). This marcasite is typically in nodular form, measures up to 10 cm across, and shows crystals with marcasite morphology. Nonetheless, XRD analysis frequently shows that this marcasite has inverted to pyrite. There also seems to be an association with phosphate concretions, and thus with reworking, negative net sedimentation, and geochemical “reworking” of iron, just like that observed in marcasite occurrences in sandstones and shales.

Conclusions

The geobiological significance of sedimentary iron sulfides is far reaching. Microbial sulfate reduction as a metabolic pathway is of great antiquity and dates back to at least the early Archean (3.47-Ga; Shen and Buick, 2004). From that time onward, global cycling of sulfur and carbon has been essential for controlling the amount of oxygen in the Earth’s atmosphere (Berner, 2001), and some have even speculated that the emergence of life itself is tied to sulfur chemistry on the early Earth (e.g., Russell and Hall, 1997). It also appears that in itself oxygen buildup during the Precambrian led to the evolution of increasingly complex biochemical networks among microbes and eventually even to the evolution of complex life forms (Raymond and Segré, 2006). The iron sulfides that we find in the rock record are a substantial part of the raw data for understanding the complex interaction of life with the atmosphere and the oceans. Every bit of that record, be it in the form of isotope shifts (e.g., Goldhaber, 2003), proportions and distribution of different iron sulfides, sulfide textures, stratal distribution, enclosed and preserved micro- and macro-fossils, as well as subsequent alterations of these minerals, is valuable for understanding chemical changes that affected the biosphere on the local, global, and temporal scales.

For the rock record, the enduring product of all these processes is the iron sulfide pyrite (FeS₂). Yet, the dimorph of pyrite, marcasite, can still be recognized in sedimentary rocks as old as Proterozoic (personal observations) and probably played a much larger role than commonly appreciated. In the minds of many geologists, the presence of iron sulfides in sedimentary rocks is associated with anoxic environmental conditions. In reality, however, once iron sulfides begin to form localized concentrations, be it in the form of micron-size frambooids or fist-size concretions, one can imply that oxygen must have been close by. As elaborated repeatedly above, in order to form even microscopic features like pyrite frambooids, iron should be able to migrate through the pore waters, and those conditions only occur near the redox interface that “separates” deeper sulfidic from shallower oxygenated pore waters. Fundamentally, this holds true even in the most classical anoxic-euxinic locale of all, the Black Sea. There, pyrite frambooids do not form in the sulfidic bottom waters, but form again at the redox interface where these waters come in contact with oxygenated surface waters (Wilkin et al., 1996). The bulk of sedimentary iron sulfides in the rock records marks the interface between the anoxic and oxic worlds; they should not be taken as an indicator of environmentally pervasive anoxia.

Bibliography


**Cross-references**
- Acid Rock Drainage
- Anaerobic Transformation Processes, Microbiology
- Biofilms and Fossilization
- Black Shales
- Isotopes and Geobiology
- Microbial Degradation
- Pore Waters
- Pyrite Oxidation
- Pyritization
- Sediment Diagenesis – Biologically Controlled
- Shales
- Sulfate-Reducing Bacteria
- Sulfide Mineral Oxidation
- Sulfur Cycle

**ISOTOPE FRACTIONATION (METAL)**

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**Definition**

Variations in the abundances of the isotopes of metals, especially transition metals, arising from physical or chemical processes that discriminate among the isotopes of a given element.

**Overview**

Research into isotope fractionation of transition metals, alkaline earth metals, and metalloids is advancing rapidly as a result of analytical developments. As of this writing, fractionations have been reported for more than a dozen such elements (Figure 1). These investigations are heavily motivated by geobiological considerations. In particular, there is wide interest in the use of these new isotope systems as biosignatures and as proxies for the

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**Isotope Fractionation (Metal), Figure 1**

Observed range of isotopic variations in modern and ancient natural samples for a selection of metals and metalloids. All values are expressed in delta notation [parts per 1,000 (%o) per atomic mass unit], normalized to average Bulk Silicate Earth (indicated by the gray vertical line). Blue diamonds indicate the average isotope composition of modern seawater, where data are available. Data sources: Young and Galy (2004) (Mg); De La Rocha (2003); Robert and Chaussidon (2006) (Si); DePaolo (2004) (Ca); Ellis et al. (2002); Johnson and Bullen (2004); Schoenberg et al. (2008) (Cr); Dauphas and Rouxel (2000); Lacan et al. (2008); Johnson et al. (2000a) (Fe); Cameron et al. (2009) (Ni); Shields et al. (1965); Maréchal et al. (1999), Vance (2008) #1986 (Cu); Mason et al. (2005); Bermin et al. (2006); Giaöa et al. (2008) (Zn); Rouxel et al. (2006a); Siebert et al. (2006a) (Ge); Anbar (2004); Poulsen et al. (2006) (Mo); Wombacher et al. (2003); Ripperger and Rehkämper (2007); Schmitt et al. (2009) (Cd); Rouxel et al. (2003) (Sb); Foucher and Hintelmann (2006); Smith et al. (2008) (Hg); Rehkämper et al. (2002); Nielsen et al. (2006) (Tl); Stirling et al. (2007); Weyer et al. (2007, 2008)(U).
biogeochemical cycling of metals in modern and ancient settings. This article briefly reviews the analytical issues in historical context and the state of geobiological applications.

**Historical and analytical context**

Investigations into natural variations in the isotope compositions of transition metals and other metallic elements began in the middle of the twentieth century (Valley and Anderson, 1947; Shields et al., 1965), in parallel with research into isotope variations of non-metals (Urey, 1947; Bigeleisen, 1965). Most of this early metal isotope research focused on variations arising from radioactive decay for geochronology, i.e., “radiogenic isotope geochemistry,” whereas non-metal isotope research was dominated by investigations of the mechanisms and applications of mass-dependent fractionation (esp. of C, O, N, and S), which came to be called “stable isotope geochemistry.”

This bifurcation was largely a consequence of the different types of mass spectrometry (MS) most readily used for these two classes of elements. Because most metals do not readily form volatile compounds, analyses of their isotope compositions were conducted primarily using thermal ionization MS (TIMS), whereby purified samples are placed on metal filaments which are heated under vacuum to form thermal ions, which are then introduced into a mass analyzer (typically, a magnetic sector). This ionization method itself induces mass-dependent isotope fractionation of a magnitude comparable to natural variations. The effects of such fractionations can be precisely corrected by external or internal normalization schemes (Wasserburg et al., 1981), but these schemes also “correct” for any natural mass-dependent fractionation. As a result, TIMS is best suited for the analysis of isotope variations arising from non-mass-dependent processes, notably radioactive decay or nucleosynthetic anomalies. Precise measurements of mass dependent isotope fractionation using TIMS are possible, but require use of double spiking procedures (Russell et al., 1978; Albarède and Beard, 2004).

In contrast, isotope variations of non-metals were (and are) typically measured using gas source electron impact MS, taking advantage of the ease with which many of these elements are converted to gas phase compounds such as H₂O or CO₂. An electron beam can readily ionize these gases. This MS method lends itself to an analytical scheme in which gases derived from samples and standards are introduced in rapid alternation to the same instrument. Such “sample-standard bracketing,” not possible with TIMS, corrects for mass fractionation induced by the mass spectrometer without compromising the ability to detect preexisting natural isotope variations. Hence, stable isotope studies of mass-dependent effects flourished for the most abundant non-metal elements.

In the final years of the twentieth century and the first decade of the twenty-first, the development of inductively coupled plasma source MS coupled to multiple ion “collectors” (MC-ICP-MS) made it possible to easily ionize transition metals and other elements using procedures that allowed for precise determination of mass-dependent isotope variations (Walder and Freedman, 1992; Halliday et al., 1995; Maréchal et al., 1999). Specifically, in ICP-MS samples are introduced to the mass spectrometer by nebulization of aqueous solutions into a high-energy plasma (Houk and Thompson, 1988). This technique readily ionizes most elements, and is amenable to sample-standard bracketing analogous to that employed for traditional stable isotope analyses (as well as double spiking). The combination of ICP ionization, magnetic sector mass analyzers, and multiple ion collectors, greatly increased the precision of such analyses. High mass resolution (Weyer and Schwieters, 2003), collision cells (Turner et al., 1998), and specialized nebulizers (Belshaw et al., 2000) solved analytical problems specific to particular elements such as Fe. These developments made it practical to examine physical and chemical fractionations of the isotopes of many metals (reviewed in Johnson et al., 2004a; Anbar and Rouxel, 2007; Weiss et al., 2008). This research led to the discovery that such variations are ubiquitous in nature, easily produced in the lab, and relevant to geobiological applications.

Variations in isotope ratios of these elements are typically of order 0.1–1%, and so isotope compositions are usually reported as % deviations from a reference standard, using the classic “δ notation” (some studies report compositions as parts-per-ten-thousand deviations, using the analogous “ε notation”; e.g., Zhu et al., 2000; Rouxel et al., 2003; Rehkämper et al., 2004; Lacan et al., 2006; Stirling et al., 2007). Analytical precision is now routinely ±0.05% or better for most elements, although reproducibility of natural samples is often not as good as that of pure standards due to complications arising from impurities even in chemically processed samples.

**Biosignatures**

Much of the research into metal isotope fractionation is motivated by the possibility that Fe isotopes, in particular, could prove useful as biosignatures in ancient materials. This idea builds on an analogy with carbon, nitrogen, and sulfur isotopes. These elements are fractionated as a result of biologically mediated redox chemistry, which dominates the biogeochemical cycling of these elements (e.g., Rankama, 1948; Craig, 1953b; Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Wada and Hattori, 1976a, b; Altabet and Francois, 1994; Canfield, 2001; Horita, 2005). Fe oxidation and reduction at the Earth’s surface is likewise biologically catalyzed, by the actions of dissimilatory bacteria that derive metabolic energy from redox reactions involving Fe.

Fe isotope fractionation has been documented to occur during dissimilatory reduction of Fe(III) phases such as hydrous ferric oxide and hematite by bacteria including strains of the genuses *Shewanella* and *Geobacter* (Beard...
et al., 1999; Crosby et al., 2007). Fe isotopes are also fractionated during oxidation of dissolved Fe(II) and precipitation of Fe(III)-oxides mediated by Acidithiobacillus ferrooxidans (Balci et al., 2006) and an anoxicogenic photoautotroph of the genus Thiocytont (Croal et al., 2004). In all cases, Fe(II) is isotopically lighter by 1.3–3% relative to Fe(III)-oxide (here, Fe isotope variations are reported in terms of $^{56}\text{Fe}/^{54}\text{Fe}$). A dissolved Fe isotope profile from the Southern Ocean – the first data of its kind – shows small variations between $-0.13$ and $0.21\%$ that appear to be consistent with biological utilization and remineralization (Lacan et al., 2008).

The specific mechanisms governing these isotope effects are complex because a multitude of processes can cause Fe isotopes to fractionate, including adsorption of dissolved Fe(II) to Fe(III)-oxide surfaces (Icopini et al., 2004; Crosby et al., 2007; Jang et al., 2008), precipitation of Fe-bearing minerals (Bullen et al., 2001; Skulan et al., 2002; Wiesli et al., 2004; Butler et al., 2005), and isotope-exchange between different Fe-ligand species or ligand-promoted dissolution (Brantley et al., 2001; Schauble et al., 2001; Wiederhold et al., 2006; Dideriksen et al., 2008; Hill and Schauble, 2008; Domagal-Goldman et al., 2009), and even diffusion (Rodushkin et al., 2004). Many of these processes occur in even the simplest experiments involving dissimilatory Fe metabolizing bacteria. However, the largest effect is the equilibrium isotope fractionation between Fe(II) and Fe(III) species. Experiments (Johnson et al., 2002; Welch et al., 2003) and theory (Schauble et al., 2001; Anbar et al., 2005; Domagal-Goldman and Kubicki, 2008) indicate fractionation of $2.5-3\%$ favoring light Fe in the Fe(II) species when Fe(II) and Fe(III) aquo complexes equilibrate at $\sim 25^\circ\text{C}$. These issues and their consequences are reviewed in detail elsewhere (Johnson et al., 2004b; Dauphas and Rouxel, 2006; Johnson et al., 2008a).

A key consideration, however, is that, in contrast to microbial conversion of S or C, dissimilatory Fe reduction and oxidation are extracellular processes that take place at mineral surfaces, facilitated by electron shuttles. Therefore, isotope fractionation may result from isotope exchange between surface adsorbed Fe(II) and Fe(III) at the oxide surface (Crosby et al., 2005). This hypothesis may explain why the fractionation factors during dissimilatory Fe(III) reduction and Fe(II) oxidation are so similar, independent of the species of microbe or the substrate, and why they are also within error of the equivalent fractionation factor between dissolved Fe(II) and Fe(III)-oxide in abiotic systems (Crosby et al., 2007).

Other transition metals for which isotope fractionation during biological transformation has been demonstrated experimentally include Mo (Wasylkeni et al., 2007), Zn (John, 2007), Cr (Sikora et al., 2008), Ni (Cameron et al., 2009), and Hg (Bergquist and Blum, 2007; Kritee et al., 2007). Some organisms convert metal redox states to gain metabolic energy or for detoxification, or both. In such cases, isotope fractionation occurs during dissimilatory reduction or oxidation (e.g., Fe, Cr, and Hg). In other cases, metals are fractionated during assimilation (e.g., Zn, Mo, Fe, and Ni).

Redox changes, adsorption to inorganic and organic surfaces, precipitation, ligand exchange and diffusion can occur independent of biology in aqueous systems. Therefore, the same considerations lead to widespread nonbiological Fe isotope effects in experiments and nature (e.g., Anbar et al., 2000; Bullen et al., 2001; Johnson et al., 2002; Brandley et al., 2004; Icopini et al., 2004; Butler et al., 2005; Crosby et al., 2007; Dideriksen et al., 2008), making it difficult to differentiate biogenic and abiotic effects in natural systems. The same is true for other metals (e.g., Ellis et al., 2002; Rehkämper et al., 2002; Larson et al., 2003; Rouxel et al., 2003; Barling and Anbar, 2004; Mathur et al., 2005; Pokrovsky et al., 2005; Siebert et al., 2006a; Malinovsky et al., 2007; Stirling et al., 2007; Balistrieri et al., 2008; John et al., 2008; Vance et al., 2008). As more is learned, multi-element isotope fractionation patterns might be developed that constitute distinctively biological fingerprints. However, as of now no definitive metal isotope “magic bullet” biosignature has been identified.

In this regard the isotopes of Fe and other metals are no different than the C and S isotope systems. For these elements, too, abiotic effects are potentially significant (Ohmoto and Rye, 1979; Chang et al., 1983; Machel et al., 1995; Horita and Berndt, 1999; Pavlov et al., 2001; McCollom and Seewald, 2006). As a result, controversy erupts whenever isotope values alone, absent other context, are used as evidence of life (Rankama, 1950; Craig, 1953a; Schidlowski, 1988; Mojzsis et al., 1996; Schidlowski, 2001; van Zuilen et al., 2002). Nevertheless, these non-metal isotope systems are widely used to build logical, if not definitive, arguments about biological influence over C and S cycling in modern and ancient environments (Goldhaber and Kaplan, 1980; Canfield and Teske, 1996; Rosing, 1999; Shanks, 2001; Shen and Buick, 2004; Hinrichs et al., 2006). Similarly, for Fe and other metals. For example, it has been proposed that isotopically light Fe isotope values observed in anoxic sediment pore fluids are indicative of the importance of bacterial dissimilatory Fe reduction in such settings (Bergquist and Boyle, 2006; Severmann et al., 2006). Hence, $\delta^{13}$Fe measurements in rocks derived from ancient anoxic sediments may provide information about the evolution of this metabolism (Johnson et al., 2008a). Such applications are discussed below.

**Biogeochemistry and paleoenvironmental proxies**

In addition to their growing use in biosignature research, metal stable isotopes are evolving into powerful proxies of biogeochemical cycling and paleoenvironmental conditions (Anbar and Rouxel, 2007; Johnson et al., 2008a). Most of this research centers on paleoredox applications, particularly on understanding changes in ocean oxygenation in response to rising atmospheric $\text{O}_2$ before the Cenozoic era – a topic of intense interest to geobiologists.
Initial efforts have emphasized isotopes of Fe and Mo. These elements are both found at the Earth’s surface in two or more redox states, and the geochemical behaviors of both elements are strongly affected by the prevailing redox conditions encountered in the environment. For example, recent results from the Black Sea indicate that bulk sedimentary Fe isotope compositions are related to the availability of dissolved O2 and the presence of H2S in the water column (Severmann et al., 2008). Apparently, an isotopically distinct pool of Fe(II) is generated during early diagenetic processes, including dissimilatory Fe reduction of Fe(III) oxides, in sediment pore waters on basin shelves. If the water column is anoxic, this isotopically distinct Fe can migrate to the deeper basin, where it is immobilized in H2S-rich waters as pyrite. Isotopically light pyrite therefore can provide a paleorecord of basin anoxia and dissimilatory Fe metabolism. Similar system-atic Fe isotope variations have been observed in the sedi-ments of other modern (Fehr et al., 2008) and ancient (Czaja et al., 2010; Duan et al., 2010) anoxic depositional settings. Hence, under certain conditions the Fe isotope compositions in ancient sediments record evidence of ancient redox conditions.

This concept has been applied to the interpretation of systematic changes in sedimentary δ57Fe through time (Matthews et al., 2004; Rouxel et al., 2005; Yamaguchi et al., 2005; Archer and Vance, 2006; Jenkyns et al., 2007). In Archean black shales dating to at least 3 Ga, Fe isotope values of –1 to –3‰ relative to average igneous rocks are common (e.g., Rouxel et al., 2005; Yamaguchi et al., 2005; Archer and Vance, 2006); varia-tions among igneous rocks and minerals are much smaller; e.g., Beard and Johnson, 2004; Williams et al., 2005; Poitrasson, 2006; Weyer et al., 2007; Teng et al., 2008). After ~1.8 Ga, fractionation in such sediments is negligi-ble (Rouxel et al., 2005). These values may reflect the importance of dissimilatory Fe reduction in marine sediments as a source of dissolved Fe(II) to Archean ocean basins (Yamaguchi et al., 2005; Johnson et al., 2008b; Severmann et al., 2008; Czaja et al., 2010 but see also Rouxel et al., 2005). If so, they provide strong evidence that this metabolism was important in Archean oceans, consistent with the notion that the deep oceans at that time were anoxic and that Fe(III) was being produced in surface oceans and delivered to sediments (e.g., Walker, 1987; Kappler et al., 2005; Konhauser et al., 2005).

The production of Fe(III) may itself be biologically catalyzed (Walker, 1987; Kappler et al., 2005; Konhauser et al., 2005), and so it has been hypothesized that Precambrian Banded Iron Formations (BIFs) are manifestation of biogenic Fe(II) oxidation. So far it has not been possible to identify a unique organic biomarker for microbial Fe(II) oxidation in the rock record, which is why hopes are high for Fe isotopes to confirm a biogenic origin of BIFs (Yamaguchi et al., 2005; Johnson et al., 2008b). However, given the complexity of the often overlapping biotic and abiotic fractionations, it remains doubtful that Fe isotope evidence on its own will be able to deliver such a “smoking gun” (see discussion in Rouxel et al., 2006b; Yamaguchi and Ohmoto, 2006). Instead, Dauphas et al. (2004) have used Fe isotopes to make the case that the controversial early Archean Akilia Island rocks are indeed metamorphosed chemical precipitates (possibly biogenic) rather than a heavily metamorphosed igneous protolith (Fedo and Whitehouse, 2002).

Although, Mo isotopes, like Fe, presumably fractionate during Mo reduction or oxidation, this is paradoxically not the basis of the use of Mo isotopes as a redox proxy. Instead, Mo isotope paleoredox applications follow from the large isotope fractionation that occurs when Mo adsorbs to some oxide mineral surfaces. The best-studied effect, seen in natural samples and laboratory experiments, occurs on the surfaces of birnessite and other Mn oxyhydroxides. A fractionation of ~2.7‰ is observed in 98Mo/95Mo ratios, favoring adsorption of light Mo iso-topes (Barling and Anbar, 2004) that seems largely independent of temperature or solution composition (Wasylenki et al., 2008c). The origin of this surprisingly large effect remains under investigation (Tossell, 2005; Weeks et al., 2007; Wasylenki et al., 2008b). A fractionation approximately half as large is seen during adsorption to ferric oxyhydroxides (Canfield et al., 2008; Wasylenki et al., 2008a).

In contrast, Mo isotope fractionation is muted during removal into sediments accumulating under euxinic (H2S-rich) bottom waters. This finding probably reflects near-quantitative scavenging of Mo when the concentra-tion of H2S exceeds ~10 μM (Helz et al., 1996); sediments accumulating under conditions that are anoxic but not sulfidic exhibit modest isotope fractionation (Poulson et al., 2006; Neubert et al., 2008), as do suboxic sediments (McManus et al., 2002; Siebert et al., 2006b). In both cases, light isotopes are preferentially removed.

The net result of these processes is that δ98/95Mo of sea-water – in which the residence time of Mo today exceeds 500,000 year – reflects the balance between oxic and reducing sedimentary sinks. Today, most Mo is removed to oxic and suboxic sedimentary sinks, shifting the dissolved Mo pool in the oceans to heavy values as com-pared to riverine and other inputs (Barling et al., 2001; Siebert et al., 2003); δ98/95Mo of the modern oceans is ~2.4‰ vs. an assumed continental crust value of ~0‰ and rivers carrying Mo with an average isotopic composition of ~0.7‰ (Archer and Vance, 2008). The balance of fluxes in ancient, less oxygenated oceans would have been shifted toward euxinic and suboxic sinks that cause less fractionation than Mn oxides. Hence, Mo in such oceans would be lighter than today. Ancient ocean δ98/95Mo can be reconstructed from measurements in black shales (Barling et al., 2001; Siebert et al., 2003), so long as care is taken to ensure that conditions were locally euxinic during deposition (Gordon et al., 2008; Neubert et al., 2008). This concept has been used to examine the extent of ocean anoxia in the Archean and Paleoproterozoic (Siebert et al., 2005; Wille et al., 2007).
Summary

Technological advances in MS have led to the emergence of a new subdiscipline in the field of stable isotope geochemistry over the past decade: natural variations in isotope compositions have now been shown to occur for many metals that were previously very difficult or impossible to measure with sufficient precision and accuracy. Isotope fractionations have been linked to a wide range of processes, including biological utilization (dissimilatory and assimilatory), surface adsorption, redox exchange, mineral precipitation, ligand exchange, or diffusion. A clear distinction between biological and abiotic processes based on metal isotope variations alone is often not possible, because both processes may result in isotope fractionations of similar magnitude, and may occur simultaneously. This apparent complication can be resolved by integrating these novel proxies with more traditional proxies that can provide context for their interpretation. The two isotope systems that have so far received the most attention are Fe and Mo, and this contribution briefly reviews examples of their application in biogeochemical or paleoenvironmental research.

Bibliography


ISOTOPES AND GEOBIOLOGY

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Definition
It has long been recognized (e.g., Thode et al. (1949), Craig (1953) and Wellman et al. (1968)) that biological processes significantly fractionate the isotopes of C, N, and S, leading to characteristic biosignatures in sedimentary rocks that will be more or less preserved in the geological record. In the following, a brief overview is given of the major isotope fractionation processes in the biosphere and the geosphere. Special attention will be paid to secondary bacterial activity after deposition of the organic matter. Questions of general interest are isotopic indicators of early life on Earth and on Mars. A note of caution, however, is given whether abiogenic processes may lead to isotope compositions similar to that of biological activity.

Carbon isotope fractionation during photosynthesis
Early reviews by O’Leary (1981) and Farquhar et al. (1989) have provided the biochemical background of carbon isotope fractionations during photosynthesis, with more recent accounts by Hayes (2001) and Freeman (2001). The main isotope-discriminating steps during biological carbon fixation are (1) the uptake and intracellular diffusion of CO2 and (2) the biosynthesis of cellular components. Such a two-step model was first proposed by Park and Epstein (1960):

\[
\text{CO}_2^{\text{external}} \leftrightarrow \text{CO}_2^{\text{internal}} \rightarrow \text{organic molecule}
\]

From this simplified scheme, it follows that the diffusional process is reversible, whereas the enzymatic carbon fixation is irreversible. The two-step model of carbon fixation clearly suggests that isotope fractionation is dependent on the partial pressure of CO2, i.e., pCO2 of the system. With an unlimited amount of CO2 available to a plant, the enzymatic fractionation will determine the isotopic difference between the inorganic carbon source and the final bioprocess. Under these conditions, 13C fractionations may vary from −17 to −40‰ (O’Leary, 1981). When the concentration of CO2 is the limiting factor, the diffusion of CO2 into the plant is the slow step in the reaction and carbon isotope fractionation of the plant decreases.

ISOTOPES (METHODS)

Please refer to entries “Isotope Fractionation (Metal), “Geochronology” and “Biomarkers (Organic, Compound-Specific Isotopes).”
CO₂ is directly converted by the enzyme ribulose biphosphate carboxylase/oxygenase ("Rubisco") to a 6-carbon molecule that is then cleaved into two molecules of phosphoglycerate (PGA), each with three carbon atoms (plants using this photosynthetic pathway are, therefore, called C₃ plants). Most PGA is recycled to make ribulose biphosphate, but some are used to make carbohydrates. Free exchange between external and mesophyll CO₂ makes the carbon fixation process less efficient, which causes the observed large ¹³C depletions of C₃ plants.

C₄ plants incorporate CO₂ by the carboxylation of phosphoenolpyruvate (PEP) via the enzyme PEP carboxylase to make the molecule oxaloacetate, which has four carbon atoms (hence C₄). The carboxylation product is transported from the outer layer of mesophyll cells to the inner layer of bundle sheath cells, which are able to concentrate CO₂, so that most of the CO₂ is fixed with relatively little carbon fractionation. Because mesophyll cells are permeable and bundle sheath cells are less permeable, C₃ versus C₄ plants have ¹³C depletions of −18‰ versus −4‰ relative to atmospheric CO₂. Even more complex is C-isotope fractionation in aquatic plants. Factors that control the ¹³C of phytoplankton include temperature, availability of CO₂(aq), light intensity, nutrient availability, pH, and physiological factors such as cell size and growth rate.

Since the pioneering work of Park and Epstein (1960) and Abelson and Hoering (1961) it is well known that ¹³C is not uniformly distributed among the total organic matter of plant material, but varies between carbohydrates, proteins, and lipids. The latter class of compounds is considerably depleted in ¹³C relative to the other products of biosynthesis. Although the causes of these ¹³C differences are not entirely clear, kinetic isotope effects seem to be more plausible than thermodynamic equilibrium effects.

Organic matter in the geosphere is a complex mixture of source organisms having variable biosynthetic pathways and detrital remains. The determination of ¹³C values of bulk organic matter is thus unable to distinguish among the different carbon sources. Immediately after burial of the biological organic material into sediments, complex diagenetic changes occur in the organic matter. Two processes have been proposed to explain the observed changes in carbon isotope composition: (1) preferential degradation of organic compounds which have different isotope composition compared to the preserved organic compounds. Since easily degradable organic compounds such as amino acids are enriched in ¹³C compared to the more resistant compounds such as lipids, degradation causes a shift to slightly more negative δ values. (2) Isotope fractionations due to metabolism of microorganisms produce new compounds having different isotopic compositions than the original source material. A classic example has been presented by Freeman et al. (1990) analyzing hydrocarbons from the Messel shale in Germany (see Table 1). While the major portion of the analyzed hydrocarbons reflects the primary biological source material, some hydrocarbons having low concentrations are extremely ¹³C depleted indicating their secondary microbial origin in a methane-rich environment. Later studies, summarized by Peckmann and Thiel (2004), have documented even larger ¹³C depletions (δ¹³C values as low as −120‰) in various biomarkers that have been formed by diverse methanotrophic taxa.

### C-isotope evidence for earliest signs of life on Earth

The idea of using C-isotope compositions of organic matter for the detection of early life dates back to the classic paper of Craig (1953). Already, at that time, it was discussed whether graphitic carbon in old rocks could be identified as being of biogenic origin (Craig, 1954; Rankama, 1954). This controversy about the meaning of graphite δ¹³C values from early Archean (3.5–3.8 Ga) rocks has continued till today (e.g., Bolhar et al., 2004). The validity of using carbon isotopes in the search for early life hinges on the assumption that early organic metabolism produced a C-isotope fractionation effect similar to that observed today. In addition, the thermal history of the metasediments has to be considered. In early Archean metasedimentary rocks from Greenland, δ¹³C values range from −22 to −50‰, which could be interpreted as biogenic (Mojzsis et al., 1996). Very negative values were measured for tiny particles of reduced carbon within apatite grains, which apparently had been shielded from metamorphic alteration. However, a case also can be made that graphite from Archean rocks may be of abiotic, hydrothermal origin. In recent years, more experimental and natural evidence has been presented that Fischer–Tropsch type reactions may produce abiogenic organic matter with δ¹³C values similar to biogenic organic matter (Taran et al., 2007).
Nitrogen

Microorganisms are responsible for all major conversions in the biological nitrogen cycle, which generally is divided into nitrogen fixation, nitrification, and denitrification. Other bacteria return nitrogen to the atmosphere as N₂. 

**Fixation** describes the conversion of unreactive atmospheric N₂ into reactive nitrogen such as ammonium, usually involving bacteria. Fixation commonly produces organic materials with δ¹⁵N values slightly less than 0‰, ranging from -3 to +1‰ (Fogel and Cifuentes, 1993) and is performed in the roots of plants by many bacteria. The large amount of energy needed to break the molecular nitrogen bond makes nitrogen fixation a very inefficient process with little associated N-isotope fractionation.

**Nitrification** is a multistep oxidation process mediated by several different autotrophic organisms. Nitrate is not the only product of nitrification, different reactions produce various nitrogen oxides as intermediate species. Nitrification can be described as two partial oxidation reactions, each of which proceeds separately. Oxidation by *Nitrosomonas* (NH₄ → NO₂) followed by oxidation by *Nitrobacter* (NO₂ → NO₃). Because the oxidation of nitrite to nitrate is generally rapid, most of the N-isotope fractionation is caused by the slow oxidation of ammonium by *Nitrosomonas*. In N-limited systems, fractionation is minimal.

**Denitrification** (reduction of more oxidized forms to more reduced forms of nitrogen) is a multistep process with various nitrogen oxides as intermediate compounds resulting from biologically mediated reduction of nitrate. Denitrification takes place in poorly aerated soil and in stratified anaerobic water bodies. Denitrification causes the δ¹⁵N values of the residual nitrate to increase exponentially as nitrate concentrations decrease. Experimental investigations have demonstrated that fractionation factors may change from 10‰ to 30‰ with the largest values obtained under lowest reduction rates. Table 2, which gives a summary of observed N-isotope fractionations, clearly indicates the dependence of fractionation factors on nitrogen concentrations and demonstrates that at low nitrogen concentrations, fractionation is nearly zero because virtually all the nitrogen is used.

### Isotopes and Geobiology, Table 2

<table>
<thead>
<tr>
<th>Reaction</th>
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<tr>
<td>N₂ fixation</td>
<td>-3 to +1‰</td>
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<tr>
<td>NH₄⁺ assimilation</td>
<td></td>
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<tr>
<td>Cultures</td>
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<tr>
<td>Millimolar conc.</td>
<td>0 to -15‰</td>
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<tr>
<td>Micromolar conc.</td>
<td>-3 to -27‰</td>
</tr>
<tr>
<td>Field observations</td>
<td></td>
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<tr>
<td>Micromolar conc.</td>
<td>-10‰</td>
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<tr>
<td>NO₃⁻ assimilation</td>
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<td>Cultures</td>
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<td>Micromolar conc.</td>
<td>-4 to -5‰</td>
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**Sulfur-dissimilatory sulfate reduction**

Large δ³⁴S-isotope fractionations are induced during bacterial sulfate reduction. Since the early work with living cultures (Harrison and Thode, 1957a, b) it is well known that sulfate-reducing bacteria produce sulfide depleted in ³⁴S by 4–49‰. In general, the rate-limiting step is the breaking of the first S–O bond, namely the reduction of sulfate to sulfite (Goldhaber and Kaplan, 1974; Brunner et al., 2005).

Despite decades of intense research, the factors that determine the magnitude of sulfur isotope fractionation during bacterial sulfate reduction are still under debate. The magnitude of isotope fractionation depends on the rate of sulfate reduction with the highest fractionation at low rates and the lowest fractionation at high rates. One parameter which remains unclear is sulfate concentration.

Naturally occurring sulfides in sediments and euxinic waters are commonly depleted in ³⁴S by up to 70‰, far beyond the apparent capabilities of sulfate-reducing bacteria in the laboratory. Much of the sulfide produced by sulfate reduction in sediments is reoxidized, often via compounds in which sulfur has intermediate oxidation states that do not accumulate, but are readily transformed and can be further disproportionated by bacteria.

Another factor that is of great importance for the observed sulfur isotope variations of natural sulfides is whether sulfate reduction takes place in an open or a closed system. An “open” system has an infinite reservoir of sulfate in which continuous removal from the
source produces no detectable loss of material. Typical examples are the Black Sea and local oceanic deeps. In such cases, H$_2$S is extremely depleted in $^{34}$S while consumption and change in $^{34}$S remain negligible for the sulfate. In a “closed” system, the preferential loss of the lighter isotope $^{32}$S from the reservoir has a feedback on the isotopic composition of the unreacted source material. The changes in the $^{34}$S content of residual sulfate and of the H$_2$S are modeled in Figure 1, which shows that $\delta^{34}$S values of the residual sulfate steadily increase with sulfate consumption (a linear relationship on a log-normal plot). The curve for the derivative H$_2$S is parallel to the sulfate curve at a distance which depends on the magnitude of the fractionation factor. As shown in Figure 1, H$_2$S may become isotopically heavier than the original sulfate when about two third of the reservoir has been consumed. The $\delta^{34}$S curve for “total” sulfide asymptotically approaches the initial value of the original sulfate. It should be noted, however, that apparent “closed-system” behavior of covarying sulfate and sulfide $\delta^{34}$S values might be also explained by “open-system” differential diffusion of the different sulfur isotope species (Jørgensen et al., 2004).

Another characteristic feature of microbial sulfate reduction is significant small-scale spatial variability in S-isotope composition of pyrite. Up to 105‰ variations in $^{33}$S/$^{32}$S ratios within strongly zoned pyrite was observed by McKibben and Riciputi (1998). This zonation arises from progressive precipitation of sulfide from pore water sulfate enriched in $^{34}$S providing more $^{34}$S-enriched sulfide.

New insights on sulfur isotope fractionation mechanisms have been obtained from the analysis of the minor isotopes $^{33}$S and $^{34}$S (Farquhar et al., 2003; Johnston et al., 2005; Ono et al., 2006). By studying all sulfur isotopes with very high precision, these authors could demonstrate that bacterial sulfate reduction follows a mass-dependent relationship that is slightly different from that expected by equilibrium fractionations. As a result, samples with the same $\delta^{34}$S value can have different $\Delta^{32}$S and $\Delta^{34}$S values. This opens the possibility to distinguish between different fractionation mechanisms and biosynthetic pathways (Ono et al., 2006). For instance, bacterial sulfate reduction shows slightly different fractionation relationships compared to sulfur disproportionation reactions (Johnston et al., 2005).

When did bacterial sulfate reduction start on Earth?

Considering a typical difference in $\delta^{34}$S values of 20–60‰ between marine sulfate and bacteriogenic sulfide in present-day sedimentary environments, similar fractionations in ancient sedimentary rocks may be interpreted as an evidence for the activity of sulfate-reducing bacteria. The presence or absence of such fractionations in sedimentary rocks thus may constrain the time of emergence of sulfate-reducing bacteria. Sedimentary pyrite depleted in $^{34}$S has been observed as far back in geological time as the Precambrian. There is, however, debate about the dating of first onset of bacterial reduction in the geological record.

In early Archean sedimentary rocks, most sulfides and the rare sulfates have $\delta^{34}$S values near 0‰ (Monster et al., 1979; Cameron, 1982), which has been interpreted as indicating an absence of bacterial reduction in the Archean. Shen et al. (2001) and Shen and Buick (2004) argued that the large spread in $\delta^{34}$S values of microscopic pyrites aligned along growth faces of former gypsum in the 3.47 Ga North Pole barite deposit; Australia represents the oldest evidence for microbial sulfate reduction. By measuring all four stable isotopes $^{32}$S, $^{33}$S, $^{34}$S, and $^{36}$S, additional evidence can be gained to decouple hydrothermal versus biological processes (Ono et al., 2006) even when $\delta^{34}$S values are inconclusive.

Mars

McKay et al. (1996) have claimed that Martian meteorite ALH 84001 – found in Antarctica – contains evidence of post-Martian life. Various kinds of apparent biosignatures with characteristic isotope compositions have been suggested: organic matter, carbonate minerals, magnetite grains, and sulfide minerals. After intensive investigations during the last years, none of these proposed biosignatures have survived as such.

Carbonates in Martian meteorites have been especially well studied because understanding the formation conditions of the carbonates is crucial in this connection. Despite extensive chemical and mineralogical studies, the environment of carbonate formation has remained unclear. For instance, in situ C-isotope analysis by Niles et al. (2005) gave highly zoned $\delta^{13}$C values from +30‰ to +60‰ consistent with a derivation from the Martian atmosphere and suggesting abiogenic formation.

Further evidence about a nonbiogenic origin of Martian carbonates (and even less abundant sulfates) has been presented by Farquhar et al. (1989). By measuring $\delta^{17}$O and $\delta^{18}$O values, these authors observed an $^{17}$O anomaly in the carbonates relative to the silicates which they interpreted as being produced by the photochemical decomposition of ozone just as in the Earth’s stratosphere. This finding suggests that carbonates (and sulfates) are derived from atmosphere–regolith interactions on Mars and not from biological activities.

Fe isotopes as biosignatures?

Virtually all reduction from Fe$^{3+}$ to Fe$^{2+}$ at the Earth’s surface is mediated by the metabolism of dissimilatory bacteria. Biological processes, therefore, may produce measurable Fe-isotopic fractionations because the metabolic processing of Fe involves a number of steps such as transport across membranes that may fractionate Fe isotopes. Experiments with dissimilatory Fe-reducing bacteria of the genus *Shewanella* indicate that iron isotope fractionations are a function of Fe(III) reduction rates: at low rates the produced Fe$^{2+}$ is isotopically depleted by 1.3‰, whereas the depletion is up to 3‰ at high rates (Johnson et al., 2005).
Iron isotope fractionation has also been observed during bacterial Fe oxidation (Croal et al., 2004). Fe(II) oxidizing phototrophs may produce under anaerobic conditions a ferrihydrite precipitate that is 1.5% higher than the aqueous Fe(II) source. Controversy still exists whether the iron isotope variations observed in microbial experiments are primarily controlled by kinetic or nonbiological equilibrium factors. Since abiotic iron reduction/oxidation reactions may reveal fractionations similar in direction and magnitude to microbial reactions (Johnson et al., 2002; Skulan et al., 2002), the presence of Fe isotope variations is not in itself conclusive evidence of biotic activity. This complicates the ability to use iron isotopes to identify microbiological processing in the rock record (Balci et al., 2006).

Conclusions

“Biological processes fractionate the stable isotopes of C, N, S, and Fe in a characteristic way leading to biosignatures in the geosphere. Such isotope fingerprints have been used as indicators of early life on Earth and Mars. Since abiological processes sometimes may lead to isotope compositions comparable to biological processes, isotope biosignatures have to be applied with care.”

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topes (mother nuclides) are spontaneously transferred via radioactive decay. Radioactive decay means that unstable isotope system as a new tracer of sulphur biogeochemical cycles. Geochimica et Cosmochimica Acta, 70, 2238–2252.


Cross-references

Anaerobic Oxidation of Methane with Sulfate
Astrobiology
Biomarkers (Organic, Compound-Specific Isotopes)
Cold Seeps
Critical Intervals in Earth History
Fe(II)-Oxidizing Prokaryotes
Fe(III)-Reducing Prokaryotes
Iron Sulfide Formation
Isotope Fractionation (Metal)
Nitrogen
Methane, Origin
Photosynthesis
Sulfate-Reducing Bacteria
Sulfur Isotopes

ISOTOPES, RADIOGENIC

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Definition

Radiogenic nuclides (more commonly referred to as “radiogenic isotopes”) are produced by a process of radioactive decay. Radioactive decay means that unstable isotopes (mother nuclides) are spontaneously transferred into stable isotopes (daughter nuclides) by emission of particles and loss of energy (radioactive radiation).

Introduction

The radioactive decay provides an accurate method of measuring the ages of rocks and minerals. This possibility was recognized both by Rutherford (1906) and Boltwood (1907) at the beginning of the twentieth century. However, the measurement and interpretation of variations in the isotopic composition of certain elements in natural materials were not possible until modern mass spectrometers were developed based on the design by Nier (1940). After the World War II, isotope determinations became an important tool in modern geology, geochemistry, and geobiology not only for dating geological processes, but also for investigations of climate change, environmental, and biological parameters. The most frequently used systems are Rb/Sr, Sr/Sr, Sm/Nd, Nd/Nd, U/Pb, and Pb/Pb. In the following, typical examples of the most common radiogenic isotope applications are presented.

Strontium isotopes

There are four stable isotopes of Sr: 88Sr, 87Sr, 86Sr, and 84Sr. All but 87Sr are nonradiogenic (that is, not the products of radioactive decay), whereas 87Sr is produced by radioactive decay of 86Rb (half-life 0.488 × 10^9 years). The 87Rb → 87Sr radioactive decay pair has therefore produced different 87Sr/86Sr ratios depending on the age and the amount of Rubidium in the material investigated. For more than half of the century, the Rb/Sr system is one of the most frequently used decay systems for dating geological events.

The isotopic composition of strontium is not only used for dating purposes, but also has turned out to be a universal tool for describing a variety of geological, biological, and environmental processes.

Groundwater and basement brines

Strontium is easily dissolved in water. The advantage of the strontium isotopic system is that the isotopic composition assumed till recently will not be changed by fractionation processes; it means that strontium in circulation depends on the 87Sr/86Sr ratios of the rocks and minerals that interact with water at or near the surface of the Earth. The Sr isotopic composition of groundwater aquifers is constant over years, independent of the amount of rainfall, as demonstrated in a recent study by Klaus et al. (2007). Investigations in the Bangkok Metropolitan Area showed that the 87Sr/86Sr ratios are useful tools to characterize groundwater flow and related biological and environmental processes.

Strontium isotope variations also play an important role in evaluating the suitability of crystalline rocks for geological disposal of nuclear waste. At the Hard Rock Laboratory at Åspö in southern Sweden, Sr isotope composition of groundwater has been investigated by Peterman and Walin (1999) down to a depth of 850 m from boreholes. Microbial
biofilms found in the deeper part of the tunnel are controlled in their Sr composition by highly saline brines (58 mg/l and $\delta^{87}$Sr values as large as +13.9%). Mixing with Baltic seawater from the surface (1.5 mg/l and $\delta^{87}$Sr values of +0.3%) is negligible; nevertheless, the method is sensitive enough to show the very small input of Baltic seawater even in the deepest parts of the Åspö tunnel.

Sr isotope investigations on high saline brines within the German Continental Deep Drilling Program (KTB) showed that the Sr isotopic compositions of paleofluids and recent fluids in the upper continental crust can be distinguished by means of $^{87}$Sr/$^{86}$Sr versus 1/Sr mixture curves (Möller et al., 1997). Similar to saline brines, mesophilic and (hyper) thermophilic microorganisms living in shallow or deeper crust showed different Sr isotope compositions. Hence, Sr isotopic compositions may comprise a promising tool for the correlation of deep biosphere systems with depth and temperature regimes.

**Strontium in animal and human skeletons**

As the ionic radius of Sr (1.13Å) is similar to that of Ca (0.99Å), strontium replaces calcium in organic matters and can therefore also be used as a tracer for animal and human mobility. Different geographic (geological) regions yield different Sr isotopic compositions and as natural variations are not changed through the food chain, the strontium composition measured in skeletal elements can be used to identify the region where an animal or a human being lived (Brian and Johnson, 2000). The $^{87}$Sr/$^{86}$Sr ratios reflect the Sr that was ingested while a specific skeletal element grew. Because different skeletal elements grow and exchange Sr at different stages during the lifetime, Sr isotopic analyses of different skeletal parts can be used to infer changes in geographic location at different stages in the life of an organism. Regarding a human being, this means that the Sr isotopic composition of the enamel in the teeth will reflect the composition as a child, due to the immobile nature of Sr and Ca in teeth, whereas the Sr composition in a bone (e.g., femur) will reflect the average composition over the last 10 years of life, due to continuous processing of Sr and Ca in bone. These conclusions, however, are only valid for skeletons which are of “High Middle Age” or older. In the time of globalization, no relationship between living place and geogenic Sr input in the food chain can be reconstructed anymore.

**Strontium in seawater**

Biogenic carbonates are fairly resistant to diagenetic alteration. As they are secreted directly from seawater by the organisms, they contain no detrital material, and therefore reflect the isotopic composition of the seawater at the time of deposition. Biogenic carbonates are nearly free of Rb and can therefore not be dated directly. However, the $^{87}$Sr/$^{86}$Sr ratios of these carbonates can be used to define the evolution of the seawater isotopic composition by known stratigraphic age deduced from fossils. Fundamental studies on the Sr seawater evolution curve were published between 1970 (Peterman et al., 1970) and 1982 (Burke et al., 1982). The obtained curves were all based on Sr isotope data from Phanerozoic carbonates. The seawater Sr data set was later extended back to the Late Proterozoic (Derry et al., 1989; Asmerom et al., 1991; Kaufman et al., 1993). In the absence of fossils, these studies were carried out on leached whole-rock carbonate samples to avoid contamination by detrital material (Figure 1).

The seawater evolution curve can not only be used as an indirect dating tool for biogenic precipitations but may also be used as a geobiological tool, e.g., for indicating the setting of carbonate precipitations accompanying methane seeps. Investigations on methane-derived carbonates from the northwestern Black Sea showed $^{87}$Sr/$^{86}$Sr ratios of microcrystalline carbonates of 0.70927 and aragonitic cement of 0.70918. Both ratios are indistinguishable from ambient seawater (0.70917), and therefore indicate a shallow Sr source (Peckmann et al., 2001). The higher ratio of 0.71005 in the background sediments is related to detrital mica.

**Paleotemperatures**

Corals are primarily known as archives not only for changes in the sea surface temperature, but also for the variability in salinity and productivity. The latter are reflected in the common proxies such as $\delta^{18}$O and $\delta^{13}$C. However, the temperature equations are only valid for shallow-water corals. Further, proxies such as Sr/Ca, Mg/Ca, and U/Ca are dependent on growth parameters which are linked to specific coral species. As mentioned above, up to now it is assumed that geological processes do not cause any fractionation of the Sr isotopes. Recently, new techniques (Fietzke and Eisenhauer, 2006) have shown that this may not always hold true for calcium carbonate precipitation, including inorganic aragonite precipitation. Temperature-controlled precipitation of aragonite shows only minor variations in the Sr isotope fractionation (0.005%ooC in the $^{88}$Sr/$^{86}$Sr ratios, whereas a natural coral sample (Pavona clavus) shows a fractionation nearly ten times higher (0.033%ooC) in the $^{88}$Sr/$^{86}$Sr ratios. This means that the strontium isotope system can also be used as a paleothermometer for the identification of different fluid sources involved in calcium carbonate precipitation at low temperatures and to characterize processes of biomineralization. The consequence of these findings is that the seawater evolution curve discussed above also has to be corrected. The high potential of this method for studying climate-dependent variables especially for cold-water corals is demonstrated in a recent paper by Rüggeberg et al. (2008).

**Neodymium isotopes**

There are seven stable isotopes of Nd: $^{150}$Nd, $^{148}$Nd, $^{146}$Nd, $^{144}$Nd, $^{142}$Nd, and $^{140}$Nd. All but $^{148}$Nd and $^{144}$Nd are nonradiogenic (that is, not the products of
radioactive decay). $^{143}$Nd is produced by radioactive decay of $^{147}$Sm (half-life $10.6 \times 10^{10}$ years). The $^{147}$Sm$\rightarrow^{143}$Nd radioactive decay pair has therefore produced different $^{143}$Nd/$^{144}$Nd ratios in the investigated materials, depending on the age and the amount of Sm. Due to the short half-life ($1.0 \times 10^8$ years) of $^{146}$Sm$\rightarrow^{142}$Nd, this system so far has played no significant role in interpreting geological processes.

Neodymium is, like strontium, released by selective chemical weathering of polymineralic rocks, and therefore dissolved Nd does not have the same isotopic composition as in the weathered rock. However, particulate matter transported into the sea is a characteristic product of eroded rocks in a given source area on the continent. Bock et al. (2005) showed that young sediments (4300 and 1000 year BP) in the Baltic Sea reflect a substantial change of Nd isotopic composition. This can be related either to changing inputs from the source areas or as different inflow of North Sea water. Variations of both, erosive continental input and North Sea inflow indicate a direct response of the Nd isotope signal in the Baltic Sea to climate change. The authors show that the Nd signal can be related to the cyclic shifts in the atmospheric circulation as well as demographic changes affecting agricultural activities. The reduction of farmland would also cause a reduction of soil erosion that changes the amount and composition of river-transported sediments into the Baltic Sea. This example shows that neodymium isotopic composition of sediment cores is a valuable tool for tracing climate changes.

Glacial-interglacial cycles are reflected in both Sr and Nd isotopic compositions (Tütken et al., 2002). The investigation of Arctic marine sediment cores revealed that it is possible to correlate ice rafted sediments with their source rocks, and therefore the glacial distribution as a function of time. The fact that Sr isotope composition is anticorrelated to the Nd isotopic composition makes such combined isotopic studies very sensitive to climate changes, and therefore also contributes to elucidate biological processes.

**Lead isotopes**

There are four stable isotopes of lead: $^{208}$Pb, $^{207}$Pb, $^{206}$Pb, and $^{204}$Pb. Only $^{204}$Pb is not a product of radioactive decay, whereas $^{208}$Pb, $^{207}$Pb, and $^{206}$Pb are produced by radioactive decay of $^{232}$Th (half-life $14.010 \times 10^9$ years), $^{235}$U (half-life $0.7038 \times 10^9$ years), and $^{238}$U (half-life $4.468 \times 10^9$ years), respectively.

The so-called “common lead method” is used for dating minerals whose U/Pb and Th/Pb ratios are so low that their isotopic compositions do not change with time. The obtained ages, however, may vary with the evolution models used for the common lead method. One of the models, “the single-stage model” assumes that the primordial lead isotope ratios evolved in different reservoirs with different U/Pb ratios. This model is named the “Holmes–Hautermans Model” (Holmes, 1946; Hautermans, 1946). The measured isotopic compositions of the lead often resulted in ages which were to be obtained in the future.

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**Isotopes, Radiogenic, Figure 1** Time-dependent variation of the $^{87}$Sr/$^{86}$Sr ratio of seawater. T = Tertiary, K = Cretaceous, J = Jurassic, TR = Triassic, P = Permian, C = Carboniferous, D = Devonian, S = Silurian, O = Ordovician, Ė = Cambrian, V = Vendian, Stu = Sturtian. (Data compiled after McArthur et al., 2001 and Kaufmann et al., 1993.)
if the single-stage model was applied. Therefore, the “two- stage” Pb evolution mod was proposed by Stacey and Kramers (1975). In this model, Pb evolves from primordial isotope ratios between 4.57 Ga and 3.7 Ga in a reservoir with $^{238}\text{U}/^{204}\text{Pb}$ of 7.192. At 3.7 Ga, the $^{238}\text{U}/^{204}\text{Pb}$ ratio of the reservoir changed by chemical differentiation to 9.735. Thereafter, lead evolution continued without change until present. A line connecting measured isotope ratios ($^{207}\text{Pb}/^{204}\text{Pb}$ vs. $^{206}\text{Pb}/^{204}\text{Pb}$) and the corresponding ratios at 3.7 Ga will intersect with the evolution line; these lines are called isochrons. The slope of these isochrons are related to the time elapsed since a lead sample was extracted from the reservoir, and therefore the age since separation can be calculated. Even if this model gives more geological relevant ages, it is still insufficient for geological dating. Nonetheless, lead isotopes may reveal excellent tracers for environmental investigations and, as lead is a toxic element, for characterizing biological processes. Lead isotope compositions do not change during industrial and environmental processes, and therefore reflect the lead source, thus allowing to distinguish between natural and anthropogenic origins (e.g., Cicchella et al., 2008). The anthropogenic lead has until recently mainly been contributed by leaded gasoline, with additional inputs caused by the combustion of coal. The lead isotopic signature of coal is more radiogenic, as coal is found in sedimentary rocks. Lead from basement rocks, which are the sources of ore deposits, show a different (lower) isotopic signature. Isotope analyses of, e.g., snow and surface waters can therefore contribute to unravel the sources and dynamics of lead in the oceans and the atmosphere and contribute to the database concerning climate change (Hamelin et al., 1997).

Exposure to lead as well as other heavy metals in the environment is a matter of public health concern. The lead isotopes play an important role in the development of biomarkers. Lead transfer across the placental barrier and injections of marked lead ($^{206}\text{Pb}$ and $^{204}\text{Pb}$, respectively) result in deposition in the enamel. Analyses of the enamels can be used to observe biological removal and assimilation of prenatals and postnatal tracers, respectively. This technique can be used to demonstrate the toxicokinetics of incorporating lead into fetal and neonatal steady-state system processes (Rinderknecht et al., 2005). The accumulation of heavy metals in aquatic organisms through the food chain may lead to serious health problems. Investigations of lead isotopes showed that the bioaccumulation of Pb in fish is due to a wide variety of food sources and/or exposure pathways, particularly anthropogenic inputs (Ip et al., 2005). These are only a few examples of the usefulness of lead isotopes in modern environmental research which becomes more important with increasing pollution.

Conclusions

Summarizing the applicability of selected radiogenic isotope systems, it can be concluded that a very wide field of applications can be covered, not only traditional dating of geological events but also numerous applications in geological, (geo)biological, environmental, archeological, and forensic processes can be answered by means of radiogenic isotope investigations (further application references can be found in Hansen, 2005).

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Cross-references

- Biomarkers (Organic, Compound-Specific Isotopes)
- Geochronology
- Isotope Fractionation (Metal)
- Isotopes and Geobiology
Karst Ecosystems
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Definition
Karst. A landscape in and on the Earth’s surface produced by the natural processes of solution and leaching of soluble rocks, generally carbonate rocks (e.g., limestone), in which the ensuing topography is mainly characterized by sinkholes, sinking streams, underground drainage networks, and caves.

Introduction
Karst landscapes comprise ~15–20% of the Earth’s ice-free land surface. Because karst forms in soluble rocks, the global occurrence coincides roughly with the distribution of carbonate sedimentary rocks (e.g., Ford and Williams, 2007) (see Chapters Carbonates and Carbonate Environments). Karst landscapes link the Earth’s surface to the subsurface, being characterized by features such as sinking streams, sinkholes, caves, and extensive underground water flow systems. Caves, which can extend up to 100 m into the subsurface, are solutionally- or collapse-enlarged discontinuous openings in rock. Surface karst features neither have to be extensively developed, nor present, for subsurface karst development and aquifer processes to be operative. Approximately, 25% of the global population depends upon the health of karst terrains and karst aquifers for its water supply (Ford and Williams, 2007).

Air, water, and rock within karst landscapes offer reactive interfaces for microbial activity. Considering the extent to which carbonate rocks comprise the rock record, and the depths to which subsurface carbonates can be karstified, the microbial biomass within karst settings and at karst interfaces is potentially tremendous. Hence, microbial diversity and microbial processes are central to all of the karst-related sciences. Moreover, as some karst landscapes have remained relatively unchanged for thousands, if not millions, of years (e.g., Gale, 1992), the longevity of the karst habitat makes karst a potentially long-term reservoir for microbial communities and subsurface ecosystems (see Chapter Deep Biosphere of Continental Rocks). The goals of this review are two-fold: (1) to summarize the geobiology of karst ecosystems by describing the range of karst habitats that are important to maintain biological (and microbiological) diversity, and (2) to examine the range of geochemical, mineralogic, and ecological processes that are influenced by microbes in karst.

Types of caves and karst
Karst development, and subsequently cave formation (speleogenesis), is primarily due to rock dissolution, mechanical weathering, volcanic activity, or the melting of glacial ice (Figure 1). Karst can be classified in a number of ways, including from determining the solid that a feature has developed within (e.g., limestone, dolomite, gypsum, basalt, granite), the proximity to the groundwater table (e.g., above, at, or below it), the origin of the feature, and the morphology of the feature (e.g., cave passage shape, cave length, passage arrangement, passage levels). As Figure 1 demonstrates, there are a number of karst, pseudokarst, and cave types. One of the dominant karst-forming processes is the dissolution of soluble rock by the action of water, forming solution, or dissolution caves (White and Culver, 2000; Palmer, 2007); consequently, most caves are dissolutional in origin. Erosion caves also develop by moving water, but from mechanical weathering (e.g., scouring or wave active) rather than from dissolution. Dissolution caves can transit into erosion caves through time. Sea caves form...
as erosional caves along sea coasts in carbonate or basaltic rock, but anchialine caves typically form from dissolution along the sea coasts (see Chapter Caves, Submarine). Volcanic caves, or lava tubes, are starkly different than dissolution caves, resulting from a crust that formed at the surface of flowing lava while the remaining fluid (e.g., lava) continued to flow beneath the crust. The reader should review Palmer (2007) and Ford and Williams (2007) for more information about cave and karst types and speleogenetic processes. Some karst features are rare (e.g., White and Culver, 2000; Palmer, 2007), and few geobiological studies have been conducted in those settings. Therefore, this chapter focuses on dissolution caves, but briefly describes some research from lava tubes.

For dissolution caves, the proximity to the groundwater table (i.e., whether within the phreatic or vadose zone) and hydrologic conditions (i.e., confined or unconfined aquifer) are critical to the development of the karst system. When formed from dissolution at or above the water table, perhaps due to underground stream flow and/or the gravity-driven movement of meteoric waters, these features are termed epigenic. Mammoth Cave in Kentucky (USA), at over 580 km of mapped cave passages, is the longest cave currently known and epitomizes epigenic systems worldwide (Palmer, 2007).

Epigenic development proceeds due to carbonic acid ($\text{H}_2\text{CO}_3^0$) promoted dissolution of carbonate rocks, such as those consisting of calcite ($\text{CaCO}_3$), whereby $\text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{H}_2\text{CO}_3^0$, and so

$$\text{CaCO}_3(s) + \text{CO}_2(g) + \text{H}_2\text{O} \rightarrow \text{Ca}^{2+} + 2\text{HCO}_3^- \quad (1)$$

These reactions are generally considered fast, and are usually modeled as being at equilibrium (e.g., White, 1997). Through time, epigenic karst development is controlled by the hydrological connectivity to the surface, as well as the complexity of flow path and storativity in the subsurface, rate of water movement, antecedent geochemical conditions, as well as the composition, function, and activities of microbes within the groundwater system. The location and arrangement of epigenic cave passages relative to overall landscape and surface drainage basin evolution...
Hypogenic development is usually not influenced by base-level conditions or fluid geochemistry (Figure 1). In contrast, hypogenic caves and karst form at or below the water table due to the action of rising fluids (water or gases), with little or no relationship to surface karst features or processes (Klimchouk, 2007). Various types of hypogenic caves can develop (Figure 1), with one of the most dramatic being due to the activity of sulfuric acid. At least 10% of carbonate caves worldwide have been attributed to this process (Palmer, 1991; Engel, 2007), including some ancient caves such as Carlsbad Cavern, New Mexico (USA) (Hill, 1996; Polyak et al., 1998). Egemeier (1981) was one of the first to examine in detail the sulfuric acid process. By observing hydrogen sulfide (H₂S)-bearing thermal springs in Lower Kane Cave in Wyoming (USA), extensive gypsum (CaSO₄·2H₂O) deposits, and gypsum-replaced carbonate rock walls, he hypothesized that volatilization of H₂S from the sulfidic groundwater to the cave atmosphere, and H₂S autooxidation to sulfuric acid on the moist cave walls, caused the carbonate rock to be replaced with gypsum through the dissolution-replacement mechanism,

\[ \text{H}_2\text{SO}_4(\text{aq}) + \text{CaCO}_3(\text{s}) + \text{H}_2\text{O} \rightarrow \text{CaSO}_4 \cdot 2\text{H}_2\text{O}(\text{s}) + \text{CO}_2 \]  

(2)

Because most natural low-temperature waters are undersaturated with respect to gypsum, the net result is mass removal and an increase in void volume. Nearly all of the subsequent studies regarding sulfuric acid speleogenesis assumed that H₂S was oxidized and consequently the sulfuric acid was produced by strictly abiotic processes. However, sulfur-oxidizing bacteria (see Chapter Thiotrophic Bacteria), which can generate sulfuric acid as a by-product of their metabolism, play an important role in carbonate dissolution (Engel et al., 2004b). Based on work from several sulfidic caves and the Edwards Aquifer in Central Texas (USA), it has been demonstrated that microbes focus acidity at the site of colonization and locally dissolve carbonates to a greater extent than the bulk karst fluids are capable of, because they are usually supersaturated with respect to carbonate phases (Engel, 2007).

For the most part, however, recognizing whether a cave has formed from hypogenic processes or not is still a matter of debate (Klimchouk, 2007). Hypogenic caves are considered rare, even if hypogenic processes are perhaps important to nascent karst development (e.g., Palmer, 1991; Palmer, 1995; Klimchouk, 2007). The debate is perpetuated because most caves and karst terrains have polyphasic speleogenetic histories; this means that inception may have been due to hypogenic processes, but that tectonic activities or climate change may force a shift to epigenic development due to evolving base-level conditions or fluid geochemistry (Figure 1). Hypogenic development is usually not influenced by surface hydrologic processes, as passages do not reflect contemporaneous surface drainage basin patterns. But, in some instances, polyphasic development, and epigenic processes specifically (e.g., sediment deposition due to surface-subsurface drainage, speleothem formation, etc.), may completely overprint evidence pointing to past hypogenic development. From a geobiological perspective, the role of microbes in both epigenic and hypogenic processes also confounds interpretation. Moreover, changes from one development type to another may impact how microbes come to colonize and become distributed within the subsurface. Microbial populations could potentially become isolated from each other during habitat modification. Similarly, changes in geochemical regime will impact the types of organisms that can grow, or tolerate, the new conditions, such as being thrust from oxygenated conditions to sulfidic and reducing conditions, or from neutral pH to acidic conditions (see Chapter Extreme Environments).

The cave habitat
The cave habitat is divided into three major zones based on light intensity: entrance, twilight, and dark zone (Figure 2). Each habitat zone has a certain set of physicochemical and nutrient conditions that control the types of organisms present in that zone. Cave and karst habitats have nearly constant temperatures and stable geochemical conditions, although some sections of a cave may change rapidly, such as in a stream due to seasonal or periodic flooding, or even within the entrance zone from daily changes in light level. But, it is the subtle physicochemical variability, availability, and speciation of redox-sensitive elements from one site to another within a cave, and the types and loading of carbon and other nutrients that can influence the diversity and metabolism of (micro) organisms that will colonize karst. Within the different zones, aquatic and terrestrial (or subaerial) habitat conditions are distinctive, and the stresses exerted on organisms will be specific. There is potentially less noticeable stress for aquatic organisms compared to the desiccation or nutritional stresses for organism living on dry cave-wall surfaces (e.g., Barton et al., 2007), although it was the juxtaposition of perceived stable physicochemical conditions to possibly extreme environmental circumstances that originally attracted biologists to study cave and karst organisms.

Consequently, speleologists who first began investigating the life of caves were predominately interested in the strange and exotic animals that they found. Troglobites are cave-adapted terrestrial animals, and stygobites are groundwater-adapted animals. Cave-adapted animals have evolved unique characteristics suitable for the subsurface habitat. Some of the organisms that live in caves are able to move freely in and out of a cave (e.g., trogloloxenes, such as bats and cave crickets), whereas other organisms (e.g., microbes) are more likely to be dependent on transport or translocation to move them into or
throughout a cave. Unlike the obvious transport mechanism from surface-derived drip waters, however, the extent to which air currents and groundwaters bring microbes into the cave environment is unknown. But, because microbes can also colonize virtually every surface and interface in all zones from a cave system (Figure 2), it is clear that understanding their diversity and their activities are central issues to all of the karst-related sciences (e.g., Rusterholtz and Mallory, 1994; Mahler et al., 2000; Jones, 2001; Northup and Lavoie, 2001; Simon et al., 2003; Cacchio et al., 2004; Farnleitner et al., 2005; Barton, 2006; Goldscheider et al., 2006; Ikner et al., 2007).

As photosynthesis is not possible in the dark zone, most cave and karst ecosystems are assumed to be dependent on allochthonous material brought into the system by wind, speleothem drip waters, stream drainage, as guano, and by the processing of the organic matter by microorganisms. Karst ecosystem energy flow has been poorly investigated (e.g., Simon, 2000; Simon et al., 2003), summarized in Figure 3, where cave ecosystem energy flow is compared to open surface stream energy flow. The most common type of allochthonous material is as dissolved and particulate (coarse and fine) organic matter (DOM, CPOM, and FPOM) (Figure 3), although it is possible that a wide size range can be found in caves, depending on the complexity of passages (e.g., when cave entrances and passages are sufficiently large, catastrophic flooding can bring tree trunks and automobiles deep inside caves). In some shallow settings, including in lava tubes, plant roots (rootsicles) may penetrate cave passages or aquifers and rich biota have been described from these settings (e.g., Howarth, 1973; Jasinska et al., 1996).

Some researchers have suggested that depauperate allochthonous food sources have influenced the evolution of organisms within the subsurface and could be one reason for the diversity of organisms in epigenic caves. In general, troglobitic and stygobitic diversity is generally low compared to the diversity of surface organisms; in fairness, however, other reasons could include reduced habitat diversity (e.g., Sket, 1999), as well as fragmentation of the habitat and restricted opportunities for dispersal (Culver, 1970; Gibert, 1986; Culver and Sket, 2000). Similarly, trophic structure diversity may also be impacted by the reduced energy within the ecosystem, demonstrated by the elimination of shredders from food webs with increasing isolation from the surface and allochthonous energy input (Figure 3). Unfortunately, very little research has been conducted that addresses the mechanisms that modulate trophic structure in karst ecosystems, or that characterizes organism behavior to understand trophic functional roles.

Based on the research that has been done from caves and karst, however, the “world is green” view is beginning to change. Microbes are not just translocated soil heterotrophic, chemoorganotrophic, or fecal coliform communities that are food sources for higher trophic level organisms (Figure 3). In the absence of sunlight, groundwater rich in redox-sensitive compounds and reactive mineral surfaces (e.g., methane, hydrogen sulfide, or other reduced substances such as iron or manganese) can be used as energy sources by chemolithoautotrophs (Figure 3) (see Chapter Chemolithotrophy). In 1986, it was the discovery of the chemolithoautotrophically based cave ecosystem from Movile Cave in southeastern Romania that started to change our perceptions of what types of microbial activities could occur in the subsurface, as well as what types of ecosystem could be expected from such activities (Sarbu et al., 1996). Movile Cave is an access point to a large sulfidic aquifer and currently,
there have been 33 new cave-adapted taxa identified from 30 terrestrial species (24 endemic troglobites) and 18 aquatic species (nine endemic stygobites). Chemolithoautotrophic primary productivity estimated for Movile Cave was as high as 281 g C m⁻² year⁻¹, being comparable to rates estimated from other aquatic surface systems (Porter, 1999). Based on the carbon to nitrogen ratios, the microbial mats in these sulfidic caves are also a high-quality food source (Porter, 1999; Engel et al., 2004a), although there have been relatively few studies that explore the
assimilation efficiency of higher trophic level organisms who feed on the mats in the sulfidic caves. The diversity and endemicity of stygobites and troglobites from sulfidic ecosystems, compared to epigenic cave ecosystems dependent on allochthonous energy, may be related to the rates of autotrophic primary productivity and the quality and quantity of the food source (Engel, 2007).

Most of the research describing chemolithoautotrophy, in caves and karst has been from hypogenic systems and anchialine caves (e.g., Sarbu et al., 1996; Humphreys, 1999a; Opsahl and Chanton, 2006; Engel, 2007). For example, the sulfur-based ecosystems from the Frasassi Caves (Italy) (e.g., Vlasceanu et al., 2000; Macalady et al., 2006, p 59), the Cueva de Villa Luz (Mexico) (Hose et al., 2000), and some stratified sinkholes around the world (e.g., Stoessell et al., 1993; Pohlman et al., 1997), are also based in chemolithoautotrophy. Similarly, microbes have been implicated in sustaining the ecosystems within the Edwards Aquifer in Central Texas (USA) (e.g., Longley, 1981) and the Cape Range or Pilbara aquifers in Australia (e.g., Humphreys, 1999b; Eberhard et al., 2005), although the role of autotrophy is currently not known. The recent discovery of the Ayalon Cave ecosystem in Israel, with possibly new chemosynthetically based ecosystems and eight invertebrates new to science, is testimony that substantial and unknown subterranean diversity still exists (Por, 2007). Evidence that microbial autotrophy from methane cycling serves as a base for the Upper Floridan Aquifer (USA) ecosystem trophic structure has been suggested from isotope systematics (Opsahl and Chanton, 2006). Recognition of methane-based cave and karst ecosystems can transform what we thought we knew of ecosystem function and the geobiology of karst. Lastly, some work on microbial autotrophy has also been done from epigenic systems, finding that sediments and biofilms on cave walls and in streams also contain chemolithoautotrophic microbial communities (e.g., Simon et al., 2003; Famleitner et al., 2005) and that karst waters can have high microbial cell abundances and DOM is associated with autochthonous microbial activities rather than plant- or soil-derived humic substance (e.g., Goldscheider et al., 2006). This research direction will impact our understanding of contaminant transport in karst (e.g., Mahler et al., 2000; van Beynen and Townsend, 2005).

Geobiology and microbial activities

Despite knowing little about microeukaryotes and viruses from karst, we do know that all three domains of life and viruses occur in caves and karst. Figure 4 summarizes the variety of cave habitats with microbial communities, and associated microbial processes that have been studied to date. Details regarding the geomicrobiology of caves and karst have been reviewed in a variety of venues (e.g., Jones, 2001; Northup and Lavoie, 2001; Barton, 2006; Barton and Northup, 2007), so the reader should refer to these papers for more information. Here, discussion begins with lava tubes because comparatively little is known about the microbial diversity, activities, and ecosystem parameters. Some of the most recent research using molecular techniques that have elucidated microbial activities and diversity of hypogenic and epigenic caves and karst, in the context of key processes, is also described (see Chapters Geomicrobiology, Molecular Aspects, Molecular Geomicrobiology, and Phylogenetics). The chapter is concluded with future perspectives on cave and karst geobiological studies.

Lava tubes and basaltic caves

There are few studies of microbes from basaltic lava tube or sea caves. Several investigations focus on humid lava tubes, rather than dry basaltic systems (e.g., Northup and Welbourn, 1997, in press), and on rootcicles from lava tubes that support complex fungi and arthropod diversity (e.g., Howarth, 1973, 1981) (Figure 4). From 16S rRNA gene surveys and culture-based growth studies, humid lava tubes contain a variety of slime deposits, consisting of actinomycetes and members within the Chloroflexi, Verrucomicrobia, and Proteobacteria (Ashmole et al., 1992; Northup and Welbourn, 1997). Somewhat related has been the geomicrobiological and mineralogical work on microbial biofilms in the photic zone of basaltic sea caves in Hawaii (USA) (Levelle et al., 2000).

Hypogenic caves

Early research was based on microscopy and culturing (e.g., Hubbard et al., 1986; Olson and Thompson, 1988; Stoessell et al., 1993; Brigmon et al., 1994), but more recently the known microbial diversity from these systems has been expanded by molecular genetics, and specifically from 16S rRNA gene sequence surveys. The biodiversity and geomicrobiology of active sulfidic caves have been more extensively studied than from other types of hypogenic karst, and the reader should refer to these publications for specifics (e.g., Brigmon et al., 1994; Angert et al., 1998; Hose et al., 2000; Barton, 2006; Macalady et al., 2006; Barton and Northup, 2007; Engel, 2007). Microbes in hypogenic systems occur in microbial mats in streams and pools, sediments, elemental sulfur and other mineral deposits, and as snotties or microbial draperies on cave-wall surfaces (e.g., Hose et al., 2000; Macalady et al., 2007), as well as in corrosion residues (e.g., Northup et al., 2000, 2003) (Figure 4). The role of microbes in sulfuric acid speleogenesis was described from Lower Kane Cave (Engel et al., 2004b), and microbial communities have also been shown to be important to sulfidic karst aquifer development (Engel, 2007). From 16S rRNA gene surveys, Proteobacteria are one of the most prevalent groups studied to date from hypogenic caves and karst. Most of the focus has also been on sulfur-oxidizing bacteria belonging to the classes Epsilonproteobacteria, Gammaproteobacteria, and Betaproteobacteria (see Chapters Thiotrophic Bacteria...
Karst Ecosystems, Figure 4  Schematic representation of the three common cave types discussed in this article, including lava tubes, hypogenic caves, and epigenic caves. For each cave type, specific habitats are associated with microbial activities and deposits are produced because of these activities (e.g., corrosion residues in hypogenic systems).
and *Sulfur Cycle*). Stable isotope ratio systematics and radiolabeled isotope experiments have unveiled possible ecosystem function for some of the microbes in these systems (e.g., Sarbu et al., 1996; Pohlman et al., 1997; Vlasceanu et al., 1997; Humphreys, 1999a; Porter, 1999; Engel et al., 2001; Hutchens et al., 2004; Herbert et al., 2005), but more research is needed.

One of the most interesting habitats for microbes in active sulfidic caves is on cave-wall surfaces. These habitats are acidic, due in part to gypsum mineralization and microbial activity, with pH of condensation droplets on biofilms and snottites being as low as 0 (range pH 0–4) (Hose et al., 2000; Vlasceanu et al., 2000; Macalady et al., 2007) (see Chapter *Acidophiles*). Interestingly, the phylogenetic diversity of these biofilms is similar, consisting of extremophilic groups belonging to the *Proteobacteria*, actinomycetes, the bacterial candidate lineage TM6, as well filamentous fungi and protists. The biofilms from the Frasassi and Lower Cave caves have δ13C values that range between −36% and −39‰, suggesting that carbon within the biofilms has been produced from chemolithothotrophy (Vlasceanu et al., 2000; Engel, 2007).

The role of microbes in iron and manganese cycling has been studied from both hypogenic and epigenic systems (e.g., Peck, 1986), although most of the current research has been done from caves that had formed from hypogenic processes but are currently under epigenic, if any, modification processes. Specifically, a diverse, metabolically active microbial communities, some of which were related to known manganese- and iron-oxidizing bacteria, are present in corrosion residues on cave-wall surfaces (Figure 4) underlying ferromanganese deposits from Lechuguilla Cave and other caves in the Guadalupe Mountains of West Texas and New Mexico (Northup et al., 2003). Among the diverse bacterial groups identified, clones belonging to both the Crenarchaeota and Euryarchaeota have been retrieved from corrosion resides (Northup et al., 2003). Similar archaeal groups have been retrieved from paleofill sediment deposits in Wind Cave in South Dakota (USA) (Chelius and Moore, 2004). Several research groups have interpreted a variety of poorly crystalline manganese oxide and hydroxide minerals in caves to be microbially induced (e.g., Northup et al., 2000).

Most studies from epigenic systems can be generally categorized into three groups: (1) those that attempt to understand the abundance, diversity, and activities of microbial communities; (2) those that attempt to relate microbial diversity to ecosystem function; and (3) those that attempt to use microbes to explain mineralogical phenomena such as precipitation and dissolution of minerals or deposits found in caves. The majority of cave and karst geomicrobial studies can be summarized as fitting into the first category.

The health of cave and karst ecosystems can be better understood by recognizing that there is an intimate hydrologic and ecologic connection of dissolution caves between the surface and subsurface. In particular, because the geochemistry and hydrology of most karst systems respond dramatically over short periods of time following disturbances, changes in the major taxonomic and metabolic potential of a system can alert to possible water quality or sustainability issues (van Beynen and Townsend, 2005). As such, considerable effort has been made to characterize microbial communities, including heterotrophic groups, within epigenic caves and from karst aquifers (e.g., Rusterholtz and Mallory, 1994; Mahler et al., 2000; Bates et al., 2006; Goldscheider et al., 2006; Pronk et al., 2006). Results have shown that pulses of different microbial groups over time, or even from episodic inoculation events, may disturb overall ecosystem balance (e.g., Mahler et al., 2000; van Beynen and Townsend, 2005; Goldscheider et al., 2006).

Similarly, identifying the microbes in vadose zone cave habitats, such as in river sediments and from exposed cave-wall surfaces, has been done to compare pristine communities to those that may be influenced by human impact, such as from tourism (Chelius and Moore, 2004; Ikner et al., 2007). Some of the microbes found from high impact zones in tour caves are comparable to communities found in association with Paleolithic cave paintings (e.g., Schabereiter-Gurtner et al., 2002a, b, 2004; Zimmermann et al., 2005). The microbial diversity and physiological types associated with higher impact areas may be related to the increased organic matter of anthropogenic origins (from paint to lint) (Ikner et al., 2007). One of the issues pertaining to the diversity of microbes from human impacted zones is whether or not these microbes have any influence on mineral precipitation or dissolution. These microbes could negatively impact any of the natural cave processes, or even destroy cave paintings by ancient man (e.g., Schabereiter-Gurtner et al., 2004).

The mineralogy and nutrient content of rock has been suggested as the important drivers in the microbial groups that are able to colonize cave surfaces (Barton et al., 2007). Because the geomicrobiology of cave minerals, including carbonates, iron oxides, and nitrates, has been extensively studied and the studies reviewed (e.g., Jones, 2001; Northup and Lavoie, 2001; Barton, 2006; Barton and Northup, 2007), only exemplar investigations are provided here (see Chapter *Microbial Biomineralization*). Carbonate
mineralization is known to be influenced by microbes, as enrichment culturing from carbonate speleothems in Cervo Cave in Italy demonstrated that Kocuria spp. were precipitating carbonate at unusually high rates compared to microbes from noncave environments (Cacchio et al., 2004). Similarly, filamentous cells caused calcite to precipitate after colonizing rock surfaces (e.g., Cañaveras et al., 2006). According to Caumartin (1963), most cave sediment contains iron bacteria, and indeed, microscopy and culturing studies revealed that chemolithoautotrophic Gallionella spp. and heterotrophic Leptothrix and Crenothrix spp. are common (see Chapters Leptothrix and Gallionella). Encrusted sheaths and stalks of bacteria resembling modern iron-oxidizing bacterial cells have been seen from stalactites, sediments, and corrosion crusts from numerous caves (Caumartin, 1963; Peck, 1986; Onac et al., 1997; Northup et al., 2003). Despite the significance of cave nitrates in the early history of caves (i.e., for salt peter deposits), there has been little work to advance our understanding of the role of microbes in cave nitrate formation (see Chapter Nitrogen). Researchers still deliberate over the degree to which bacteria, such as Nitrosomonas spp. and Nitrobacter spp., participate in the creation of the cave salt peter deposits, although Nitrobacter spp. had been cultured from cave sediments in Mammoth Cave (Fliermans et al., 1974).

### Karst ecosystem studies in the future

Like the discovery of life at the deep-sea hydrothermal vents more than 25 years ago (see Chapter Hydrothermal Environments, Marine), ongoing research in karst habitats has revealed a rich and diverse microbiota. Some of these cave and karst ecosystems are supported by chemolithoautotrophic primary productivity, thereby changing our perception that all life on Earth is dependent on photosynthesis. Caves and karst are relatively accessible habitats to study from the perspective of carbon and nutrient cycling, and discoveries are being made that relate to rock cycling (from mineral dissolution to precipitation). But, we need to move beyond observational investigations that focus on categorizing microbial communities, and instead to make important advances in understanding the geomicrobial- and biogeochemical roles of microbes in cave and karst to address the rates and mechanisms controlling microbial ecophysiology and ecosystem functioning. This methodology exists and so we should expect that exciting new discoveries will be made with continued technological advances. One outcome from these efforts will be an increase in our knowledge of cave and karst biodiversity to better protect and conserve these novel geological and ecological systems.

### Bibliography


**Cross-references**

Acidophiles  
Carbonate Environments  
Carbonates  
Chemolithotrophy  
Extreme Environments  
Gallionella  
Hydrothermal Environments, Terrestrial  
Leptothrix  
Microbial Biomineralization  
Microbial Ecology of Submarine Caves  
Microbial Mats  
Moonmilk  
Nitrogen  
Speleothems  
Sulfur Cycle  
Terrestrial Deep Biosphere  
Thiotrophic Bacteria
LATERAL GENE TRANSFER

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Synonyms
Horizontal gene transfer

Definition
The use of the term in the literature is not entirely unambiguous. For the purpose of this entry, lateral gene transfer (LGT) is defined as the stable transfer of genetic material (DNA, possibly RNA) from one organism to another where the two organisms belong to lineages which are genetically too distant to allow a mutual act of homologous recombination.

Introduction
Vegetative proliferation is characterized by a vertical line of genetic transfer from one cell to two daughter cells. Upon zygote formation, in contrast, sexual reproduction mixes the genetic outfit of two parent organisms. Fertile offspring, however, is only produced if the two parents are genetically close enough to allow for gamete formation (i.e., meiosis with mandatory homologous recombination) in the offspring. This limit defines a breeding pool which, in turn, can serve as a useful approach to the term “species.”

In the bacterial and the archaean domains of life, the species concept is much more contentious – for reasons partly illustrated by this article. Nevertheless, the homologous recombination criterion can be upheld at least in cases like Escherichia coli, in which genetic traits are transferred by conjugation between two pre-existing cells. Conjugation proceeds by transient cell fusion between an Hfr+ and an Hfr− cell, followed by physical transfer of DNA from the former to the latter, followed by homologous recombination. Natural transformation in Streptococcus pneumoniae provides is a similar example.

Against this backdrop, LGT can be viewed as any transfer of genetic material between two organisms that are separated by the homologous recombination barrier. This criterion separates, for example, E. coli from the closely related Salmonella typhimurium; in cases with no known system of homologous recombination, the criterion has to be replaced by corresponding and somewhat arbitrary measures such as sequence distance. There are two hallmarks of LGT which may be detected simultaneously or alternatively in any particular single case.

1. Presence of genes in a genome that are not present in genomes of otherwise closely related organisms.
2. Topologically different branching patterns obtained in attempts to delineate phylogenetic relationships between a host of lineages, based on sequence comparisons within more than one set of (seemingly) orthologous genes.

Other, less clear-cut indicators of LGT include G/C content and codon usage that deviate from those of the bulk genome and also physical gene linkages that differ from those observed with close relatives.

These statements together make it immediately understandable why the intensity of the debate of LGT developed in parallel with the unfolding of the genome analysis era that followed the publication of the Haemophilus influenzae sequence in 1995 (Fleischmann et al., 1995). To date, several hundred genomic sequences have been determined – mostly of bacteria and archaea. By far most of the knowledge we have about LGT concerns the bacterial and archaean domains (including trans-domain transfer) and it is not entirely clear to which extent this fact reflects (non)availability of the necessary mechanisms in the different domains and to which simple numerical preponderance of sequenced prokaryotic
genomes. The following discussion focuses on LGT in bacteria and archaea (and between bacteria and archaea). Establishment of endosymbiosis can be regarded as an extreme case of LGT; because it is such an exception with respect to mechanism and frequency of occurrence, it is not covered here.

Mechanisms
Mechanistically, LGT can be divided into two consecutive steps:

1. Introduction of foreign DNA into the recipient cell. (In the following, the alternative option of RNA being introduced is no longer explicitly mentioned.)
2. Stable establishment of the transferred DNA as part of the genetic outfit of that cell.

**Step 1:** DNA can arrive in a recipient cell as the result of conjugation (i.e., as a plasmid or genome fragment) of phage infection (i.e., as a phage genome, in particular of a transducing phage carrying genes of a former host) or by physical uptake (i.e., any piece of naked DNA).

**Step 2:** The newly arrived DNA can either be degraded or stably established in the cell in parts or in toto by any of the following mechanisms: (i) independent replication as an episome (plasmids, certain kinds of lysogenic phage such as *E. coli* P1); (ii) transposition (transposon, IS-elements or the like) from the entering DNA molecule to the recipient’s chromosome (or other residing replicon); (iii) site-specific recombination as seen in lysogenic phage such as phage Lambda; (iv) illegitimate recombination (a term collectively used for a number of mechanisms that require no or only very short stretches of high sequence similarity between the two recombining molecules).

Note that in order for any combination of Steps 1 and 2 to qualify as LGT, the criterion of the homologous recombination barrier has to apply for the lineage of the donor and the receptor of the DNA (compare above: Definition).

Plausibly, the acquisition of genetic traits through LGT is limited by incompatibilities of gene expression signals present in the transferred DNA with cognate proteins (RNA polymerases, repressors, gene activators) or other macromolecular components of the gene expression apparatus of the recipient cell (e.g., rRNA).

Some selected cases
**Extent of LGT**
Porwollik and McClelland (2003) have used comparative genomics and microarray analysis to determine the extent to which LGT participated in shaping the genomes of different *Salmonella enterica* serovars. They come to the conclusion that almost one quarter of the entire *S. enterica* sv. *Typhimurium* genome may have been introduced by LGT.

**Trans-domain LGT**
In bacteria and eukarya, the repair of DNA-uracil residues (pre-mutagenic DNA lesions resulting from spontaneous hydrolytic deamination of DNA cytosine residues) is almost invariably initiated by a family 1 UDG enzyme (UDG: Uracil DNA Glycosylase). On the other hand, no family 1 UDG gene has been discovered to date in any archaeal genome. For archaea, UDGs of families 4 and 5 seem to be typical. The genome of *Thermus thermophilus*, a Gram-negative, extremely thermophilic bacterium, almost uniquely for a bacterium, contains no gene for a family 1 UDG, but one gene each for a UDG enzyme of family 4 and 5 (Starkuviene and Fritz, 2002; Henne et al., 2004). This highly unusual finding is most simply explained by lateral transfer of the family 4 and family 5 genes into *T. thermophilus* (or an ancestor thereof) across the domain barrier, followed by loss of the formerly residing family 1 gene whose activity had been made redundant by the LTB event.

**Consequences and summary**
Lateral gene transfer lies at the heart of some pressing practical problems, among them is the rapid emergence of multiply-resistant pathogens under the selection pressure of widespread (and sometimes inappropriate) use of antibiotics. Naturally spreading promiscuous plasmids of overlapping host range, combined with DNA transposition are arguably the major contributors to that process. The spread of pathogenicity islands is a similar example. More fundamentally, the nature and extent of LGT shakes the entire evolutionary concept of common descent and, along with it, the usefulness of its iconographic representation, the tree of life. Rather, the bacterial and the archaeal domains of life together form a densely woven genetic network along which smaller or larger packages of genes can move with a high enough degree of independence to leave frequent and conspicuous traces of this process in genome structures. Different genes may have come together in a common genome as the result of profoundly different ancestry trajectories. This mosaic nature of the genome potentially frustrates any attempt of deriving stringent phylogenetic relationships at the organismal level from DNA sequence data. As a substitute of low resolution, one may operate with the consensus tree topology one may find for the large majority of genes present in the genomes under consideration.

**Bibliography**
Cross-references
Metagenomics
Microbial Communities, Structure, and Function
Symbiosis

LEPTOTHRIX

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Definition
Leptothrix describes a genus of bacteria that have long been recognized as important in the biological oxidation of iron and manganese (van Veen et al., 1978). It is one of several genera that may be referred to as the “iron bacteria.” At present, there are five recognized species: L. ochracea, L. discophora, L. cholodnii, L. lopholea, and L. mobilis (Garrity et al., 2004). The genus is in the class Betaproteobacteria. The members of this genus are recognized for the production of an extracellular tubular sheath. The cells typically grow as chains of cells within the sheath, which they excrete as they grow. It is common for the sheaths to be coated in Mn oxides or Fe oxyhydroxides. L. ochracea is perhaps the most commonly recognized Leptothrix spp. due to copious production of very straight, iron encrusted sheaths. This species has never been cultivated in the laboratory and its exact relationship to other members of the genus is not known. Circumstantial evidence suggests that it is capable of lithotrophic growth using Fe(II) as sole energy source; however, this has not been proven. The evidence stems from its requirement for Fe-rich waters to grow. Typically, these are wetlands, springs, water wells, and like environments where anoxic groundwater enriched in Fe(II) comes into contact with air. Another interesting feature of L. ochracea, and also indicative of its use of iron as an energy source, is that only a small percentage (estimated at about 10%) of the sheaths contain cells during active growth (Figure 1). An interesting facet of these metal encrusted sheaths is that they can be preserved in the fossil record (Hofmann and Farmer, 2000). The other species of Leptothrix have been cultured in the laboratory and identified using classical microbiological techniques. All of these latter species are heterotrophic organisms utilizing organic carbon sources for growth. To date there is no compelling evidence that they are either lithoautotrophic or mixotrophic. They are typically found in wetlands and other aqueous habitats. Proteins with enzymatic activity for Mn-oxidation have been found in L. discophora and L. cholodnii (Adams and Ghiorse, 1987; Emerson and Ghiorse, 1992). Both species also accumulate Fe-oxyhydroxides on their sheaths, and a gene has been identified in L. discophora that is associated with iron oxidation (Corstjens et al., 1992). These Mn- or Fe-oxidizing proteins appear to be secreted from the cell into the surrounding sheath, the site of metal oxidation. The sheath of L. cholodnii is a complex fibrillar polymer consisting primarily of polysaccharides with a high concentration of the amino acid cysteine (Emerson and Ghiorse, 1993a). The fibrils are held together by extensive disulfide bonding, making it a very resilient structure (Emerson and Ghiorse, 1993b). A novel cysteine glycoconjugate is the primary building block of the sheath (Makita et al., 2006).

Bibliography
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Cross-references
Fe(II)-Oxidizing Prokaryotes
Manganese (Sedimentary Carbonates and Sulfides)
Magnetotactic bacteria (MTB) are a diverse group of Gram-negative, motile prokaryotes that align and migrate along the geomagnetic field. This magnetotactic behavior is based on intracellular organelles called magnetosomes, which are inorganic nanocrystals of either magnetite (Fe₃O₄) or greigite (Fe₃S₄), surrounded by a lipid bilayer membrane. The magnetosomes are typically arranged in one or more chains, conveying a magnetic dipole moment to the cell (Figure 1). Magnetotaxis is thought to be beneficial for the cell for finding its optimum position in vertical concentration gradients in aquatic habitats.

History
Bacteria that responded to magnetic fields were discovered independently by Bellini (1963) and Blakemore (1975), who coined the term magnetotaxis and noted the presence of crystals in the cells. Intracellular magnetite was identified and the physics of magnetotaxis was described by Frankel et al. (1979). Iron sulfide-producing MTB were found in 1990 (Mann et al., 1990; Farina et al., 1990). The ecology of MTB and the structures and morphologies of the crystalline components of magnetosomes have been widely studied since the discovery of MTB. In the past decade, significant progress has been made in elucidating the molecular, biochemical, and genetic bases of magnetosome formation (Schüler, 2007).

Ecophysiology
MTB are ubiquitous in both marine and freshwater aquatic habitats, where suboxic or anoxic conditions prevail, and dissolved iron is available. Because of their fastidious nature, only a few strains are available in pure culture. With one exception, all strains in pure culture have a respiratory form of metabolism. All known MTB are microaerophiles (they require a certain amount of oxygen for growth, but exhibit a negative tactic and/or growth response to atmospheric concentrations of oxygen) or anaerobes or facultatively anaerobic microaerophiles (Bazylinski and Frankel, 2004). The physiology of MTB appears to dictate their ecology: since MTB derive energy from the proximity of oxidants and reductants, magnetite producing organisms occur in largest numbers (up to 10⁶ cells/ml) in layers at or near the oxic–anoxic interfaces (OAI) of chemically stratified habitats. Iron sulfide-producing bacteria live below the OAI under anaerobic conditions (Simmons et al., 2004). Whereas magnetite-producing bacteria occur virtually in all types of water bodies, sulfide-producing organisms have been found in marine habitats only. Considering the large amount of iron concentrated in magnetosomes (10⁻¹⁵ to 10⁻¹³ g Fe per cell) and the estimated population density of MTB at and below the OAI, MTB are likely to contribute significantly to the flux of reduced Fe to sediments in chemically stratified marine basins.

Magneto-aerotaxis
Magnetotaxis means active motion guided by the magnetic field. MTB passively orient along the geomagnetic field and actively swim by means of flagella. A key element of magnetotaxis is that the cell should possess a large enough magnetic dipole moment to overcome the disorientating effects of thermal motions and be aligned parallel to the magnetic field. In the geomagnetic field, the magnetic energy of magnetotactic cells is typically ten times larger than thermal energy, which means that the cell can migrate along the field at 90% of its forward speed (Frankel et al., 1979).
Polar and axial magnetotaxis are distinguished (Frankel et al., 1997). Polar magnetotaxis is displayed by MTB that swim persistently toward one of the poles of a bar magnet under oxic conditions. Those that swim toward the "south" pole of a magnet have North-seeking polarity, since they would swim northward in the geomagnetic field. MTB from the northern hemisphere are predominantly North-seekers, whereas those from the southern hemisphere are predominantly South-seekers (Bazylinski and Frankel, 2004).

The observation of polar magnetotaxis led to the idea that MTB are guided by the geomagnetic field to less oxygenated regions. Since the geomagnetic field is inclined downward from the horizontal in the northern hemisphere and upward in the southern hemisphere under oxic conditions the cells with polar magnetotaxis swim downward in both hemispheres (Bazylinski and Frankel, 2004). However, if the cell is in a reducing environment, it reverses the direction of flagellar rotation and migrates upward. Since the motion of the cell is determined by both the magnetic field and the oxygen tension, the term "magneto-aerotaxis" was introduced (Frankel et al., 1997).

Axial magnetotaxis means that the axis of motion is determined by the magnetic field but its direction is not. As the cell swims, it samples the oxygen concentration and compares it with that in the recent past. Cells moving toward the optimal habitat have a decreased probability of reversing the sense of flagellar rotation, whereas those that are moving away from the optimal oxygen concentration have an increased probability of reversing the sense of flagellar rotation and thus their swimming direction (Frankel et al., 1997). The magneto-aerotic response provides an efficient mechanism for MTB to find the OAI in their chemically stratified habitats.

**Mineralogy and magnetism**

Magnetotaxis relies on intracellular grains of iron oxide or sulfide minerals. Magnetite (Fe₃O₄) is the only iron oxide mineral that has been described to date from magnetotactic cells. The sulfide-producing cells use greigite (Fe₃S₄) for navigation, but they also may contain nonmagnetic mackinawite (FeS) that rapidly converts to greigite through a solid-state phase transition (Pósfai et al., 2007).

Magnetite produced by MTB is chemically pure Fe₃O₄, in contrast to grains from rocks that usually contain other transition metals besides iron. Structurally, the nanocrystals are either perfect or contain twin boundaries. The morphologies of magnetite magnetosomes are typically highly regulated, strain-specific, and in many cases, unusual for a mineral with cubic symmetry (Pósfai et al., 2007). Whereas magnetotactic spirilla contain cubicohedral crystals, vibrio and cocci typically produce magnetite with prismatic habits (Figure 2a). Large, rod-shaped cells tend to contain arrowhead- or bullet-shaped magnetite crystals (Bazylinski and Frankel, 2004).

The sizes and shapes of magnetosomes are critical for magnetotaxis. Isolated, isometric magnetite crystals with diameters smaller than ~30 nm are superparamagnetic, i.e., they do not have a permanent magnetic moment. Crystals with diameters from ~30 to ~120 nm are magnetic single domains at room temperature, whereas grains larger than ~120 nm contain two or more magnetic domains, reducing the net magnetic moment of the crystal. Most magnetosomes are in the single-domain range, allowing the assembly of a highly efficient internal compass that consists of a chain (or chains) of single-domain magnets. Interactions between neighboring particles in chain configurations ensure that even those magnetite particles that are small enough to be superparamagnetic or large enough to contain two or more magnetic domains if they were isolated are also constrained to be single domains (Dunin-Borkowski et al., 2001). Since elongated particles are arranged with their axis of elongation (which typically coincides with their crystallographic easy magnetization axis) parallel to the chain of magnetosomes, shape anisotropy constrains the magnetic flux lines to be parallel to the axis of motility. Thus, in most magnetite-producing cells the effects of particle size, shape and magnetocrystalline anisotropy, and magnetostatic interactions among particles in chains combine to produce the possible largest magnetic moment (Figure 2b) (Pósfai et al., 2007).

The sizes, shapes, and orientations of iron sulfide magnetosomes are less distinct than those of magnetite magnetosomes. Greigite magnetosomes typically contain planar defects, as a result of an incomplete solid-phase transformation from mackinawite to greigite. The shapes and orientations of the crystals are random,
resulting in poorer confinement and greater variability in the directions of the magnetic induction lines in the cells than in magnetite-bearing bacteria. Remarkably, despite the different crystal structures, orientations, and arrangements of ferrimagnetic crystals, the magnetic moments of different cells are fairly constant (Pósfai et al., 2007), thereby satisfying the requirement for magnetotaxis.

Cell biology and genomic analyses
Magnetic enrichments have shown great morphological and ultrastructural diversity of MTB from a wide variety of aquatic environments. Magnetite-producing morphotypes include spirilla, cocci, vibrios, and rods of various sizes, moving at speeds ranging from 40 to 1,000 \( \text{m/s} \). A unique, greigite-producing magnetotactic organism, the “multicellular magnetotactic prokaryote” (MMP) consists of 15–45 cells organized around an internal acellular compartment (Keim et al., 2004). The aggregated cells of the MMP move together as one unit, with the coordinated motion of flagella on the outside of the cells.

The highly controlled, strain-specific properties of magnetite magnetosomes and their chains indicate that biomineralization is under genetic control, executed by specific proteins in the magnetosome membrane. Many details of magnetosome formation have been elucidated by studies of the biochemistry of the magnetosome membrane and genomic analyses of MTB. Workable genetic systems have been developed for two Magnetospirillum strains: \textit{M. magnetotacticum} AMB-1 and \textit{M. gryphiswaldense} MSR-1 (Jogler and Schüler, 2007). By genetic manipulation, including the generation of mutants and the expression of magnetobacterial genes in other hosts, the major steps of magnetite biomineralization have been identified in these systems. Magnetite nucleation is preceded by the formation of vesicles, which are invaginations of the cytoplasmic membrane (Komeili et al., 2006). Dissolved ferrous or ferric iron is transported into the cell by an unknown uptake system. Magnetosome membrane proteins (MamB/MamM) are involved in the transport of ferrous iron from the cytoplasm into the magnetosome vesicle, and then magnetite formation is activated by another protein (MamA). Within the vesicle, iron is oxidized and magnetite nucleation is triggered by the Mms6 protein. The least known aspect of the biomineralization process is the control over the distinct crystal morphology. The vesicles containing fully developed magnetite crystals are anchored to a filamentous cytoskeletal structure by the MamJ protein, resulting in an ordered magnetosome chain (Scheffel et al., 2006). Genomic analyses revealed that the genes that encode the magnetosome proteins are clustered in a 130 kb region, representing a “magnetosome island.” This genomic island appears to be conserved among different \textit{Magnetospirillum} strains and, to a lesser degree, in a magnetotactic coccus (Jogler and Schüler, 2007), raising the question of whether the “magnetosome island” is a universal feature of MTB.

Diversity
The term “magnetotactic bacteria” does not refer to a taxonomical unit. All known MTB belong to various groups within the domain Bacteria. Most magnetotactic microorganisms that are available in pure culture are affiliated with the \textit{Alphaproteobacteria}, including \textit{M. magnetotacticum} and \textit{M. gryphiswaldense}, a marine vibrio (MV-1), and a coccus (MC-1). In addition to the cultivated strains, 16S rRNA analysis of magnetic enrichments from many freshwater and marine samples revealed that a variety of magnetite-producing morphotypes are likely affiliated with the \textit{Alphaproteobacteria} (Amann et al., 2007). Two magnetite-producing candidate species, “\textit{Magnetobacterium bavaricum}” and “\textit{Magnetotacticum bremense},” are affiliated with the \textit{Nitrospira} phylum.

Sulfide-producing MTB could not have been cultured to date, but a multicellular organism was found to be closely related to the dissimilatory sulfate-reducing...
bacteria within the Deltaproteobacteria. Another sulfide-producing MTB was assigned to Gammaproteobacteria. These findings may suggest that magnetotaxis based on iron oxide and sulfide nanoparticles evolved independently; however, a magnetite-producing, sulfate-reducing organism (Desulfovibrio magneticus strain RS-1) is also affiliated with the Deltaproteobacteria (Amann et al., 2007).

The diversity of MTB indicates that magnetotaxis evolved independently in several phylogenetic groups. On the other hand, the discovery of a potentially mobil “magnetosome island” in the genome of magnetite-producing MTB could suggest that the trait of magnetotaxis was spread by lateral gene transfer among the various groups of MTB.

Magnetofossils, paleomagnetism
After MTB die, their intracellular magnetite or greigite particles are deposited in the sediment. If diagenetic conditions are favorable, the magnetosome crystals can be preserved as magnetofossils. Magnetofossils have been identified on the basis of the special properties of magnetosome crystals, including a narrow crystal size distribution, characteristic morphologies, chemical purity and, in some cases, chain configurations. Ferrimagnetic nanocrystals presumably originating from MTB have been described from a variety of sedimentary rock types, including clays, carbonates, marls, and evaporites. The magnetofossil record extends from present-day marine and lake sediments to the Cretaceous and with lesser certainty to the late Archean (Kopp and Kirschvink, 2008).

MTB carry a primary remanent magnetization, since they are aligned with the geomagnetic field, presumably even after cell death and burial in the sediment. Because magnetofossils are single magnetic domains, they have an optimized magnetic stability and can be important carriers of paleomagnetic information. Magnetofossils can serve as environmental proxies because MTB live under specific redox conditions. They can also be markers of ancient life, albeit dubious ones, as illustrated by a lengthy debate over whether nanocrystalline magnetite particles in the Martian meteorite ALH84001 represent magnetofossils or had formed inorganically (Winklhofer and Petersen, 2007).

Nanotechnological applications
Magnetic nanoparticles are widely used in technological applications, such as in spintronics, in magnetic inks, in high-density magnetic memory devices, and in medical applications (as contrast agents in magnetic resonance imaging and as magnetic carriers for drug targeting and delivery). Magnetite crystals produced by MTB have uniform sizes and shapes and can be separated from disrupted cells by magnetic methods with their magnetosome membrane intact. The membrane-bound bacterial nanoparticles have a large specific surface area and are well dispersed in aqueous solutions. Antibodies or proteins can be immobilized on the magnetosome membrane, producing functionalized magnetic nanoparticles (Matsunaga and Arakaki, 2007). Since bound and free analytes can be separated in a magnetic field, bacterial nanoparticles have been used for the rapid and sensitive detection of small molecules, including environmental pollutants, hormones, and toxic detergents. Immunomagnetic particles are also useful for sorting target cells from cell suspensions using a permanent magnet, and for using them in high-performance DNA/mRNA recovery and DNA discrimination analysis (Matsunaga and Arakaki, 2007).

Summary
MTB provide a fascinating example of microbial biomineralization and motivate interdisciplinary research at the junctions of biology, mineralogy, geology, geochemistry, and physics. The molecular and genetic bases of magnetic nanocrystal formation by MTB are now beginning to be understood. The results of research on MTB will be useful for understanding biomineralization processes and magnetic sensing mechanisms in more complex organisms, and for designing nanotechnological applications of bacterial nanocrystals.

Bibliography


Cross-references
Bacteria
Biosignatures in Rocks
Iron Sulfide Formation
Microbial Biomineralization
Nanocrystals, Microbially Induced

**MANGANESE (SEDIMENTARY CARBONATES AND SULFIDES)**

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**Synonyms**
Alabandite; Authigenic manganese minerals; Inca rose; Rambergite; Rhodochrosite

**Definition**
*Rhodochrosite*. Manganese(II) carbonate (MnCO₃; trigonal, calcite structure), named after the greek word for “rose-colored.” It was named as the state mineral of Colorado (USA) in 2002. Complete solid solution formation with calcite occurs at low temperatures (Böttcher, 1998), the “Ca-rhodochrosites” or “pseudo-kutnahorites” (Mucci, 2004).

*Kutnahorite*. Manganese(II)-calcium carbonate (CaMn (CO₃)₂; trigonal; ordered dolomite structure), named after the location KutnoHora (Peacor et al., 1987). Although physicochemically stable at low temperatures with respect to equally composed solid-solutions (Mucci, 2004), it is not formed in sediments, and only known from high-temperature hydrothermal and metamorphic environments.

*Rambergite*. Manganese(II) sulfide (μ-MnS; hexagonal), named after the mineralogist Hans Ramberg, is found as authigenic product in anoxic sediments of the Baltic Sea (Böttcher and Huckriede, 1997; Burke and Kemp, 2002).

*Alabandite*. Manganese(II) sulfide (α-MnS, cubic), named after the location Alabanda, Turkey, is found in sediments as minor intergrowth with rambergite (Lepland and Stevens, 1998).

**Manganese carbonate formation in suboxic/anoxic sediments**
Mixed manganese–calcium carbonates, with minor incorporation of magnesium and divalent iron, are formed in (brackish-marine) sediments as authigenic products of suboxic and anoxic early diagenesis, in the Baltic Sea deeps and the Panama Basin. Significant sedimentary Ca-rhodochrosite accumulation requires manganese(IV) dioxide pre-enrichment at the sediment–water interface by episodic water column oxidation, as shown for the temporarily euxinic deeps of the Baltic Sea (Huckriede and Meischner, 1996). The required dissolved carbonate species are formed in the pore water upon mineralization of organic matter, and aqueous Mn²⁺ is derived from microbial or chemical reduction of manganese(III, IV) oxides as electron acceptors (e.g., Banfield and Nealson, 1997). The carbonates occur as kidney-shaped globules, as overgrowth on foraminifers, detrital carbonates, and microbial cells or as idiomorphic crystals, depending on the microenvironment. Experimental studies reveal that the composition of manganese-rich carbonates is controlled by the pore water composition, the abundance of carbonate surfaces, and the presence of inhibitors (Böttcher, 1998; Mucci, 2004). Under equilibrium conditions, natural anoxic pore waters are in the stability field of coexisting rhodochrosite and iron sulfides (Figure 1), and no manganese sulfide should be formed during typically zoned early diagenetic processes.

**Manganese sulfide formation in anoxic sediments**
The only places worldwide where sedimentary manganese sulfides have been found so far are the Baltic Sea anoxic deeps (e.g., Baron and Debyser, 1957). Idiomorphic MnS (Figure 2) is associated with organic matter, Ca-rhodochrosite, and iron sulfides (Böttcher and Huckriede, 1997; Lepland and Stevens, 1998). Although MnS has been obviously formed under slow diffusion-controlled conditions where rhodochrosite formation was chemically inhibited, the exact process is still under debate. Occurrence of Ca-rhodochrosite replacing hexagonal MnS (Burke and Kemp, 2002) indicates possible re-adaptation of the metastable sedimentary system toward equilibrium conditions.
Conclusion

Sedimentary authigenic manganese(II) carbonate minerals, especially investigated in the nowadays brackish-marine Baltic Sea, are indicative of biogeochemical early-diagenetic processes in manganese-rich environments. They have gained a lot of attention in the past 2 decades because of their proxy potential for specific hydrological conditions leading to euxinic water column turnover or glacial-interglacial cycles that are controlled by climate change (e.g., Lepland and Stevens, 1998; Gingele and Kasten, 1994). One of the many open questions within the biogeochemistry and geomicrobiology of manganese in sediments is about the conditions leading to the metastable formation and preservation of manganese(II) sulfides. An interpretation of water profiles in sediments with high manganese load point toward complex interactions between chemical inhibitors, microbial processes, and rates of (temporary) mineral formation processes.

Bibliography


Cross-references

Carbonates
Divalent Earth Alkaline Cations in Seawater
Iron Sulfide Formation
Microbial Degradation
Sulfur Cycle
**MASS EXTINCTIONS, PHANEROZOIC**

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**Synonyms**  
Biotic crisis; Extinction event; Extinction-level event

**Definition**  
A *mass extinction* is a sharp decrease in biodiversity, speciation, and diversification rate among micro- and macro-organisms in the fossil record, often also related with a decrease of biomass. The microbial biodiversity, which probably dominates the entire biodiversity, however, is not to be considered under this aspect, because we have no idea about the real microbial biodiversity through time.

**Mass extinctions**  
Mass extinctions are generally important events, which have a large impact on evolutionary processes throughout earth’s history. Mass extinctions might have also occurred in the Precambrian, however, these are much more difficult to recognize and interpret, because most of them affected microbial communities. Profound extinction events in the Precambrian are related with the Great Oxidation Event 2.5 Gy ago, during which an unknown number of anaerobic microbial communities disappeared from oxygenated shelf areas and were restricted to the still anaerobic deeper parts of the oceans (Canfield Ocean, see Wiese and Reitner, Chapter *Critical Intervals in Earth History*, this volume). It can be suspected that the Neoproterozoic Cryogenian Snowball Earth situations (730–630 My) damaged early earth’s ecosystems as decreasing δ13C values prior to the glaciations suggest a decrease in bioproductivity (e.g., Moczydłowska, 2008; Le Guerroué, et al., 2006a, b; Hoffman, this volume). A first macrofossil extinction of eukaryotic multicellular organisms occurred in the Lower Cambrian, when the Ediacara organisms or Vendobionta, respectively (Raup and Sepkoski, 1982; Brasier et al., 1989; Jensen et al., 1998), became extinct.

Within the Phanerozoic, five major mass extinctions are known (Sepkoski, 1984, 1994; Alroy, 2004; Kiessling and Simpson, 2010), in the literature referred to as “the Big Five” (Figure 1). All of them have potentially different origins (e.g., impact, volcanisms, glaciations a.o.; see below) or are even multicausal. However, in most cases, the triggering mechanisms and feedback loops are often difficult to understand. Apart from cosmic impacts, mass extinctions are most likely no sudden events but have precursory ecosystem perturbations that prelude the phase of the biotic crisis (further reading see Wiese and Reitner, this volume).

**End-Ordovician mass extinction (ca. 443 My)**  
The end-Ordovician mass extinction event is the first major extinction event in the Phanerozoic. Brachiopods, bryozoans, trilobites, graptolites, corals, mollusks, conodonts, etc. were strongly decimated. It is estimated that roughly 50% of known taxa died out (further reading Krug and Patzkowsky, 2004; Sheehan, 2001; Sole and Newman, 2002; Webby and Droser, 2004).

**Possible origins**  
a. One reason might be the paleogeographic constellation of the continents at the Ordovician–Silurian boundary, when Gondwana was positioned at the South Pole. The position of large continental areas at the South Pole triggered a severe cooling, which terminated in the Sahara glaciation in the uppermost Ordovician (Hirnantian). Possibly, it was the heaviest glaciation during the entire Phanerozoic. The associated strong regression and a breakdown of oceanic circulation patterns brought up nutrients from the abyssal waters and affected the diversity of organisms negatively (Sheehan, 2001). A further idea is that the glaciation forced an equatorward migration of warmer water organisms, competition in remaining habitats and extinction of a large number of taxa. In a second step, a severe warming, succeeding the Sahara glaciation, forced a poleward migration of the few remaining cold water assemblages, which then also suffered heavy losses (see also Webby and Droser, 2004).

b. Melott et al. (2004) argued that the end-Ordovician mass extinction could have been caused by a gamma ray burst from a supernova 6,000 light years distant from the Earth. This burst should have destroyed half of the ozone layer, leading to an immediate kill by the heavy UV radiation. However, any hard data that confirm this model do not exist. As the ozone layer buffers the mutagen UV radiation, an increase of UV radiation should in some kind be visible in the fossil record by an increase of pathologically developed invertebrate macrofossils.

c. Young et al. (2009) speculated that CO2 might play a major role in the end-Ordovician mass extinction. Their idea is that through the late Ordovician massive volcanic CO2 outgassing was balanced by strong weathering of the uplifting Appalachian Mountains, which sequestered CO2. Finally, in the end of the Ordovician, the volcanic activity finished and the continued weathering caused a significant decrease of CO2. As a result, the Hirnantian ice age started.

**Late Devonian extinction events (ca. 370 My)**  
Within the late Devonian, several extinction events severely affected biodiversity. The most important ones were the Upper Devonian Kellwasser events in the Frasnian–Famennian boundary interval and the Hangenberg event at the Devonian–Carboniferous boundary (McGhee, 1996; Walliser, 1995; Sandberg et al., 2002). The
extinction seems to have only affected marine life. Reef-building organisms were almost completely wiped out, except some microbial reefs with occasionally few sponges. Roughly 60% of the known genera became extinct. The diversity of brachiopods, cephalopods, trilobites, benthic foraminifera, conodonts, rugose and tabulate corals, jawless fish, stromatoporoid sponges, etc. was strongly influenced by these events. However, the decline of biodiversity is probably a reduction in the formation of new species rather than an extinction of already existing taxa.

Possible origins
Different possibilities have been discussed for the late Devonian mass extinctions such as asteroid impacts, global anoxia, sea-level changes, climatic changes, etc. (Buggisch, 1991; Joachimski, 1997; Joachimski and Buggisch, 1993; Joachimski, 2001). The most intriguing hypothesis is the “Devonian Plant Hypothesis” (Algeo and Scheckler, 1998; Algeo et al., 1995, 2001). Due to the rapid and accelerating evolution of terrestrial plants from the Lower Devonian on, intensified chemical and physical weathering by roots and the increased continental bioturbation resulted in an intensification of pedogenesis. As a result, several feedback loops established. On the one hand, the increased runoff of nutrients caused eutrophication of the Devonian seas, the genesis of anoxia and black shales, and the extinction of deep water and shelf biota. On the other hand, due to silicate weathering and CO₂ withdrawal from the atmosphere, the alkalinity of the sea increased, enhancing precipitation of carbonates and further CO₂ withdrawal. As a result, a global cooling occurred, and the associated sea-level fall diminished shelf habitat significantly during the Kellwasser and Hangenberg events (e.g., Sandberg et al., 2002).

End-Permian mass extinction (ca. 251.4 My)
The Permian–Triassic extinction was the most severe extinction event within the Phanerozoic. Up to 84% of the known Permian taxa (genera) became extinct. Not only marine organisms were affected, but also terrestrial ecosystems. Many of the characteristic Paleozoic organisms like trilobites, representatives of the cephalopoda and corals, marine and terrestrial vertebrates, and also plants disappeared and died out. However, some of the Permian-style organisms like coralline sponges occurred again in the early–middle Triassic and are interpreted as Lazarus-taxa, a widely, only poorly understood phenomenon. The end-Permian extinction cannot be explained by one event only. The combination of various events is
possibly responsible for this tremendous mass extinction (Benton, 2005; Bowring et al., 1998).

Possible origins
a. Volcanic events are widely discussed to be the major controlling factor of the Permian–Triassic mass extinction. The most important volcanic events were flood basalt eruptions, which produced the Siberian Traps, some of the largest known volcanic provinces in earth’s history. These eruptions have an age of 251.2 ± 0.3 My, and they fit stratigraphically very well with the estimated age of the mass extinction. The Siberian Traps were estimated to exhibit 20% of pyroclastic material, which heavily reduced insolation by backscatter of sunlight. Furthermore, eruptions caused dust clouds, acidic aerosols, and acidic rain, which – in conjunction with the first – disturbed and disrupted photosynthesis on land and in the sea. The Siberian Trap lava intruded into calcareous sediments and large coal beds, and emitted large amounts of CO₂, causing a slight global warming (White, 2002; Saunders and Reichow, 2009; Reichow et al., 2009).

b. There is a worldwide negative excursion of δ¹³C_carb to values of ca. −10‰. There are various possibilities to explain this excursion. However, the release of huge amounts of methane possibly from collapsing marine and permafrost methane hydrates is the most probable reason (Palfy et al., 2001; Twitchett et al., 2001; Payne et al., 2004).

c. Several pieces of evidence can be brought forward for an impact event in the end of the Permian. There are possible impact craters, which have been proposed as possible causes of the extinction: the Australian Bedout crater and the Wilkes Land crater of East Antarctica. From these sites, shocked quartz and fullerenes, trapping extraterrestrial noble gases, are known. However, this evidence does not really prove the impact event, and the Wilkes Land crater is possibly younger than the Permian–Triassic boundary (Retallack et al., 1998; Becker et al., 2001, 2004). In this respect, the paper by Stanton (2002) is very intriguing, in which he suggests that the Gulf of Mexico is a very huge meteorite crater of end-Permian age – big enough to explain this fatal extinction event.

d. There is evidence of anoxic seawater conditions around the time of end-Permian extinctions. Major players in anoxic seawater are sulfate-reducing bacteria, which could produce high amounts of H₂S, which is, however, partly re-mineralized in iron sulfides (e.g., pyrite). Dissolved H₂S is a poison and could be responsible for aquatic mass killing. Perhaps, the release of H₂S in to the atmosphere weakened the ozone layer and increased the UV radiation with fatal effects for plants and land animals. There are indications that anaerobic photosynthesis via green sulfur bacteria occurred in the end of the Permian till the early Triassic (Kump et al., 2005; Grice et al., 2005). The triggering mechanism is believed to be the increase of volcanogenic CO₂, which lowered the pH value and increased chemical weathering. As a result, the continental runoff of nutrients increased dramatically, causing eutrophication and finally anoxia with the consequences listed above.

e. Last but not least, sea-level changes are also discussed to explain the mass extinction. Due to the Pangea situation, a very big marine regression is noticed. This reduced the shelfal areas, biodiversity, and the stability of food chains (e.g., Hallam and Wignall, 1999).

End-Triassic mass extinction (ca. 201.4 My)
The end-Triassic extinction defines the boundary between the Triassic and Jurassic, and it is one of the most important Phanerozoic extinction events. Ca. 47% of the known genera got extinct, such as the conodonts, nearly almost all Triassic taxa of ammonites except the stem groups of the Phylloceratina and the Lytoceratina. Many vertebrates such as a high number of large amphibians, Triassic archosaurs, many therapsids, etc. disappeared. The end-Triassic extinction event also destroyed the widespread shallow water coral reef environments, including many types of corals and especially coralline sponges. The recovery of this coral reef environment took a long time and was more or less completed in the Upper Jurassic (Hallam, 1987, 1997; Palfy et al., 2001; Ward et al., 2001).

Possible origins
There is only one seriously discussed reason for the end-Triassic extinction. During this time, a tremendous volcanic activity occurred, the Central Atlantic Magmatic Province (CAMP), which produced huge amounts of flood basalts, linked with a high amount of outgassing CO₂, sulfur gases, and volcanic ash (Hesselbo et al., 2002). This event heavily influenced the shallow water and terrestrial bioproduction and, probably due to acid rains, also biomineralization. The δ¹³C isotopic composition of organic matter from sediments from this time exhibits an unusual negative excursion (Deenen et al., 2010; Ruhl et al., 2009; Whiteside et al., 2010). Most probable trigger for the extinction is the CAMP-induced climatic change, acidification of the ocean surface waters, and a strong sea-level fall.

End-Cretaceous mass extinction (ca. 65.5 My)
The end-Cretaceous extinction event, also known as the Kreide–Tertiär (K-T) extinction event, was a very short-termed mass extinction event. However, the diversity of some groups of animals like ammonites, belemnites, various Cretaceous bivalves (rudists, inoceramids), dinosaurs, etc. decreased before the K-T event. The most important impact on biodiversity was the extinction of non-feathered dinosaurs, marine large reptiles (mosasaurs, plesiosaurs), pterosaurs, and the decrease of phyto- and zooplankton diversity and biomass in the ocean surface waters. Crocodilian archosaurs, turtles, birds, and small nocturnal mammalian survived the mass extinction event.
Extinction of most taxa of, for example, coccolithophorids and Cretaceous planktonic foraminifera led to a breakdown of primary productivity and calcareous sedimentation rates in the oceans. The K-T boundary layer is worldwide often documented as a black band, which exhibits geochemical anomalies, for example, an enrichment of iridium and in the Pt-group elements in general. Probably, ca. 47% of the known genera became extinct.

Possible origins
a. The most discussed reason is that huge impacts of cometary parts or asteroids caused the extinction. This assumption is evidenced by the geochemical anomalies (Ir, Pt-group) and the discovery of a very large impact structure in northern Mexico (Yucatan), the Chicxulub crater, which has an age corresponding to that of the K-T boundary. Some scientists assume that more impacts happened; however, no further impact structures are known. The ecological disaster shortly after the huge meteorite impact (estimated size of impactor more than 10 km) caused the extinction of the above-listed organisms.

b. Beside the bolide impact hypothesis, an increased volcanic activity is also discussed to be responsible for the big extinction event. The Deccan trap basaltts have the age of the K-T boundary, and this vast volcanic event might also have influenced the biodiversity and biomass production at that time, which then reflects a comparable scenario like for the Permian–Triassic boundary. It is easily possible that the conjunction of two events — the bolide impact and the Deccan trap volcanism — are responsible for this last large Phanerozoic mega extinction.

Conclusion
All five major extinction events within Phanerozoic are milestones in the evolution of organisms, community structures, and ecosystems. All of them are critical intervals (see contribution Wieser and Reitner this volume) and of fundamental paleobiological importance. All of the major extinction events are multicausal events and cannot be restricted to only one factor. Most of them are also not short-term events, except the K-T boundary event which is proved to be a large meteorite impact. However, also in this case, the extinction of some important taxa has a prelude in the form of successive decrease in diversity, and the K-T event finally wiped them out.

These catastrophic events are probably very important for the entire biotic evolution on earth and obviously one of major evolutionary driving force.

Bibliography


Cross-references
Asteroid and Comet Impacts
Biological Volcanic Rock Weathering
Comets
Critical Intervals in Earth History
Ediacaran Biota
Snowball Earth

MAT-RELATED SEDIMENTARY STRUCTURES

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Synonyms
Microbially induced sedimentary structures (MISS) (The acronym MISS was introduced by Noffke et al. (2001) for “microbially induced sedimentary structures.” Many of the structures addressed, however, are not truly “induced” by microbes, but rather by physical forces
acting on a biostabilized sediment surface or a microbial mat. Since, in this scenario, the biological component significantly influences the shape of evolving structures, it is suggested to write out the acronym MISS as “microbially influenced sedimentary structures.”

Definition
Mat-related sedimentary structures are small- to medium-scale sedimentary structures resulting from growth and extension of microbial populations and communities on a sediment surface, stabilization of surface sediments, trapping and binding of sediment particles, and from the impact of environmental factors, such as inundation, sedimentary deposition, subaerial desiccation, wind- or current-induced traction, on epibenthic microbial mats.

Introduction
Originally described from and applied to modern microbial mats (Gerdes et al., 2000; Noffke et al., 2001), mat-related sedimentary structures preferentially serve as proxies of the former existence of microbial mats in ancient siliciclastic sediments. Mat-related structures are in a way analogous to trace fossils (Schieber, 2004), whereby the former presence of mats can be inferred, e.g., from observations suggesting “unusual” sediment properties like original cohesiveness, tensile strength, and erosion-resistance of sand (Eriksson et al., 2007); uncharacteristic grain fabrics; and steep geochemical gradients on a mm-scale. Genetic explanation of the structures relies on an actualistic approach; preservation depends on intrinsic biological, and favorable environmental conditions.

In this chapter, the term “mat-related sedimentary structures” is applied to structures that form on, in, or below microbial mats with distinct properties, e.g., coherence, cohesiveness, minor thickness, and adaptation to periodic subaerial exposure. Further conditions include a fine-grained siliciclastic substrate (sand to silt) and lacking dominance of carbonate precipitation. The term also encompasses primary physical structures that have subsequently been microbially influenced.

Today, well-developed (cyanobacterial) microbial mats that meet the above conditions, are observed mainly in the intertidal to lower supratidal zones of low-gradient tidal flats where they grow in a wide range of climate zones from temperate humid to subtropical/tropical semi-arid and arid, at salinities ranging from brackish to hypersaline. Similar mats may also form in other environments, e.g., lacustrine, fluvial, where similar mat-related structures may partly form. Due to lacking vertical bioturbation in the Precambrian, the distribution of microbial mats may previously have been much wider, extending far into the subtidal zone.

Microbial mats are epibenthic, multilayered microbial communities including different functional groups of microorganisms (Stal, 2000) which act together in a kind of “joint venture” widely balancing organic matter production and destruction (van Gemerden, 1993). Thus, microbial mats in coastal environments normally do not exceed a thickness of a few centimeters. The “biological stratification” of microorganisms is superimposed by a biosedimentary laminating resulting from the interplay of microbial growth and seasonal, periodic, or episodic depositional events related, e.g., to tides or storms.

The top layer of the mat system, which periodically is exposed to the atmosphere, usually consists of oxygenic, filamentous cyanobacteria, frequently Microcoleus sp., which form a “felty” fabric of interwoven filaments (Gerdes et al., 2000), including also sediment grains incorporated by “trapping and binding” processes (Noffke et al., 2003a, with references). The felty layer may be overlain, or in some cases replaced, by a resistant (“leathery”) layer of colloid extracellular polymeric substances (EPS) produced mainly by cocccoid cyanobacteria. This top portion of the mat combines high mechanical resistance with low permeability and is the locus at which most of the mat-related sedimentary structures form. Deeper levels of the mat system are much less cohesive. They contain anaerobic bacteria among which sulfate-reducing bacteria produce sulfide leading to precipitation of iron sulfides (FeS) which gradually transform into pyrite. In the ancient record, the stratiform occurrence of pyrite, in some cases siderite or ferroan carbonate, within otherwise well oxidized sediments may serve as a proxy of the former existence of microbial mats.

Formation and types of structures
Mat-related sedimentary structures may form during all the stages from first microbial colonization of a sediment surface, through establishment and sustainment of a fully developed mat, to its destruction and final erosion.

Structures related to early microbial colonization
Microbial colonization leads to “biostabilization” (Krumbein et al., 1994) of a sediment surface which then can resist erosion to some degree. This property may lead to “palimpsest ripples” when new sediment is deposited on top a biostabilized rippled surface (Pflüger, 1999); to surfaces with “multi-directional ripple marks” (Noffke, 1998); and to “ripple patches” (Figure 1a) or “erosional pockets” when a biostabilized flat surface or mat is locally eroded (for ancient example, Noffke, 1999; McKenzie, 1972).

Structures related to microbial growth and mat growth
Depending on availability of water for some time, specific filamentous cyanobacteria (e.g., Lyngbya aestuarii) start to produce on the mat surface, a characteristic “reticulate growth pattern” of small, sharp-crested ridges stabilized by EPS (Figure 1b). Ancient structures of this type are named “elephant skin” texture (Gehling, 1999) (Figure 1c). There is considerable variability in respect of polygon size (few mm to few cm) and shape varying from nearly circular to narrow rectangular with almost linear crest orientation. A unifying feature, however, are the
narrow, sharp-crested ridges preserved on upper bedding surfaces.

A further type of structures related to mat growth are “petees” (Gavish et al., 1985; Bouougri et al., 2007), which deform the upper, cohesive part of the mat into bulges and domes, thus enlarging the mat surface (Figure 1d). Occasionally, the bulges may be arranged in a polygonal pattern. Provided, petees are filled by sediment rising up from below, the structures may be preserved. Ancient examples have been named “petee ridges” (Schieber, 2004).

Structures related to mat desiccation and shrinkage

Subaerial exposure causes dehydration and shrinkage of the EPS and eventually cracking of the mat. Incipient, isolated “shrinkage cracks” are sigmoidal and spindle-shaped, or tri-radiate starting from triple junctions, or occasionally circular in shape (Figure 1e). Crack propagation at progressive shrinkage leads to more or less complete networks of cracks. In general, shrinkage cracks in microbial mats develop a higher degree of curving unlike normal mud cracks. Ancient shrinkage cracks are also termed “sand cracks” referring to their occurrence in sandy sediment without shrinkable mud present. A specific type of shrinkage cracks, characterized by sinuous or sub-circular trends and developed mainly in ripple troughs, is referred to as “Manchuriophycus”-type in the ancient record (Porada and Löffler, 2000) (Figure 1f). A characteristic property of many ancient shrinkage cracks is their easy separability from the layers above and below, which is ascribed to soon microbial
overgrowth of the crack margins and of the sediment trapped in the crack.

Thick mats, frequently dominated by *Microcoleus chthonoplastes*, tend to form polygonal networks of wide cracks with “upturned” or involute, “curled margins” (Figure 2a); individual polygons may range in size from 10 to 60 cm across, depending likely on the thickness of the mat. Ancient examples exhibit a “chaotic” upper surface with irregularly oriented bedding (Figure 2b) and resemble sedimentary structures ascribed to seismic events (Donaldson and Chiarenzelli, 2007).

Thin mats, frequently dominated by coccoid cyanobacteria and vested with a strong EPS top layer, tend to form circular openings with curled margins around (Figure 2c). Ancient examples have been described from thin siltstone layers within heterolithic deposits (Bouougri and Porada, 2007) (Figure 2d).

**Structures related to mat deformation, destruction, and erosion**

Cohesive microbial mats may become torn, detached from the substratum and tightly folded, and eventually eroded by strong currents, e.g., during storm and flooding events. Mats are also destructed by complete desiccation after cessation of groundwater supply, and dried mat fragments

*Mat-Related Sedimentary Structures, Figure 2* Structures related to mat desiccation and deformation. (a) Polygonal pattern of shrinkage cracks with upturned margins in thick microbial mat. Scale (knife) is 8 cm. Locality: Trucial Coast, west of Abu Dhabi, U.A.E. (b) Surface outcrop of Holocene microbial mat exhibiting relics of polygons with upturned margins. Scale (knife) is 8 cm. Locality: Trucial Coast, west of Abu Dhabi, U.A.E. (c) Irregular to subcircular openings with curled margins, formed in a thin microbial mat due to desiccation and shrinkage. Note newly overgrown margin in lower right of photograph. Locality: Sabkha El Gourine, Mediterranean coast of southern Tunisia. (d) Upper surface of siltstone layer exhibiting irregular to circular cracks. The structures are interpreted as openings in a previous thin mat that underwent desiccation. Locality: Terminal Proterozoic Vingerbreek Member, Nama Group; Farm Haruchas, Namibia. (e) Microbial mat, torn and partly removed by current or wind action. Flip-overs of mat margins are indicated by “F.” Note small-scale desiccation cracks in exposed mat substratum. Locality: Sabkhet Mjasser, Mediterranean coast of southern Tunisia. (f) “Roll-up” structure (“jelly-roll”) consisting of rolled-up microbial mat and adhering sediment. Scale (coin) is 23 mm. Locality: Bhar Alouane, Mediterranean coast of southern Tunisia.
may be transported by wind over wide distances and be deposited in environments where mats usually do not grow.

Mat destruction by currents leads to typical structures repeatedly observed in modern environments and in the ancient record: (1) “flip-over” structures (Figure 2e) result when a mat’s edge is flipped over; (2) cigar-shaped “roll-up” structures, also named roll-ups or “jelly-rolls” (Figure 2f), may develop when curled margins or flip-overs undergo additional rolling due to current action; and (3) irregular or arcuate belts of “mat deformation folds” form when a torn and detached mat is crumpled by tractional forces (Figure 3a); similar folds may also result from mat slumping on steep slopes, e.g., along tidal channels. Roll-up structures can withstand transport over some distance and may gather along trash lines, whereas the other structures largely remain in situ. For ancient examples see Schieber et al. (2007).

“Mat chips” (Gerdes et al., 2000) are small fragments of eroded mats or biostabilized sediment. They primarily are irregular in shape with frayed edges, but may become pebble-shaped with transport (Figure 3b). Ancient examples have been named “microbial sand chips” (Pflüger and Gresse, 1996) or “sand clasts” in contrast to intraformational mud clasts (Figure 3c).

Structures formed beneath microbial mats
From the hydraulic perspective, the sediment beneath cohesive and coherent microbial mats may be addressed and treated as a confined aquifer in which, at sufficient hydraulic head, a potential for liquefaction may develop (Porada et al., 2007). In such case, hydraulic upward

Mat-Related Sedimentary Structures, Figure 3 Structures related to mat deformation and erosion, and subsurface structures. Scale (knife) in a, b, e, and f is 8 cm. (a) Detached, thin microbial mat, torn and strongly deformed by current action. Locality: Coastal sabkha between Gabes and Skhirat, Mediterranean coast of southern Tunisia. (b) Modern sandy-pebbly sediment surface with subrounded to rounded “mat chips”. Locality: Salins du Midi, Réserve Nationale Camargue, southern France. (c) Upper surface of sandstone bed carrying subrounded to rounded “sand clasts,” interpreted as previous “mat chips.” Locality: Terminal Proterozoic Vingerbreek Member, Nama Group; Farm Haruchas, Namibia. (d) Morphological details of a mat subsurface, exposed after removal of the mat. Scale (coin) is 24 mm. Locality: Sabkhet Mjasser, Mediterranean coast of southern Tunisia. (e) Upper surface of sandstone layer exhibiting irregular bulges and domes. The structure is considered to represent morphological features of a previous mat subsurface. Note tri-radiate crack in well-developed dome just below scale. Locality: Neoproterozoic Tizin-Taghatine Group; Taghdout area, Anti-Atlas, Morocco. (f) Upper surface of sandstone layer with “Kinneyia” structure. Locality: Terminal Proterozoic Vingerbreek Member, Nama Group; Farm Haruchas, Namibia.
pressure will lead to slow, upward movement of sediment grains. As a result, bulges and domes developed in the mat will gradually be filled from below; “petee ridges” have been quoted as an example above.

Thin microbial mats, particularly those dominated by coccoid cyanobacteria, may develop very irregular surfaces with numerous small domes and buckles which also appear as positive features on the subsurface (Figure 3d). Ancient examples of such “subsurface structures” strongly resemble load structures but are clearly distinguished from these by their occurrence as positive features on upper bedding surfaces (Figure 3e).

“Kinneyia” (Figure 3f) is a further structure developed in the sediment beneath a microbial mat. The structure is characterized by millimeter-scale, flat-topped, steeply sided, winding ridges separated by equally sized round-bottomed troughs and pits. It resembles small-scale interference ripples including crest-dominated linear and pit-dominated honeycomb-like patterns. The structure has been described from the Archean (Noffke et al., 2003b) to the Jurassic (Bloos, 1976), but not yet from the Recent. Genetic interpretation is thus difficult. Recent models suggest formation by trapping of gas underneath a sealing mat (Pflüger, 1999) or by reversals of groundwater flow in the liquefied mat substratum (Porada et al., 2007).

Wrinkle structures
The term “wrinkle structure” (Hagadorn and Bottjer, 1997, 1999) is currently used as a collective term for various small-scale irregularities developed on ancient, siliciclastic sediment surfaces. Application of the term implies that a microbial participation in the formation of the structure is suspected, at the least. Within this broad definition, wrinkle structures may originate from very different processes including microbial growth, mat deformation and subsurface processes (Porada and Bouougri, 2007). Included in the term are also structures like elephant skin and Kinneyia which are well defined and for which, if clearly identified, usage of the proper name is recommended.

Preservation of mat-related structures
As most of the organic matter produced by the cyanobacteria is soon decomposed and mineralized so that organic components or even carbon are rarely preserved in the ancient siliciclastic record, preservation of mat-related structures is largely dependent on the volume of inorganic material bound or produced by the mat or trapped by the structure. Microbially supported processes introducing inorganic material into the mat include “trapping and binding” of silt- to sand-sized grains and possibly clay minerals, formation of authigenic clay minerals during bacterial lysis, formation of iron sulfides and pyrite, and precipitation of carbonates. The mat-related structures themselves, being either positive or negative features on the mat surface, may act as sediments traps. Thus, shrinkage cracks may become filled with sediment by currents or eolian action, and involute (curled) margins may trap grains in their windings.

Another mechanism by which some mat-related structures, e.g., domes and bulges developed in the mat, may become preserved is “filling from below.” This process requires comparatively high hydraulic upward pressure enabling liquefaction of the sediment below the sealing mat so that grains are lifted and moved upwards. As a result, a previously flat surface of a sedimentary layer may be transformed into an irregular surface mimicking morphological features of the mat.

Conclusions
Simplistically subsumed under the main groups of “wrinkles,” “unusual cracks,” and mat chips in the fossil record, mat-related sedimentary structures exhibit a wide range of shapes and patterns resulting from various processes and interactions between conflicting biological and physical factors. Due to local microtopographic features and the intrinsic inhomogeneity of microbial mats on a small scale, even well-defined structures, like elephant skin or Kinneyia, may show a high degree of variability. Thus, one structure almost never is like the other and the paradigm of reproducibility is not strictly applicable to them. Nevertheless, the structures are distinctive as exemplified on Figures 1–3.

Mat-related sedimentary structures may serve as tools for detailed facies analysis in siliciclastic peritidal settings. Based on modern analogs, most of the structures form in the intertidal to supratidal zones, but mat extent may have been wider in the Precambrian. Structures requiring (at least temporarily) subaerial exposure for their formation, e.g., types of shrinkage cracks with or without upturned or curled margins, clearly indicate intertidal to supratidal positions, whereas others remain less unequivocal. Subsurface structures including Kinneyia may form down to the shallow subtidal zone, as long as coherent, cohesive mats are present.

In the fossil record, the significance of mat-related sedimentary structures increases greatly if several types are observed in association. Some mat-related structures, e.g., reticulate growth patterns, preferentially occur in heterolithic successions of thinly laminated siltstone and mudstone which, in some cases, may be addressed as “siliclastic biolaminites” resulting from the interaction of microbial mat growth and sediment supply (Bouougri and Porada, 2007), whereas sand cracks are usually developed on sandstone layers intercalated in the succession. In contrast, Kinneyia structures exclusively occur on upper surfaces of sandstone beds which frequently represent event deposits after storms or floods.

Bibliography


**Cross-references**

Biofilms

Extracellular Polymeric Substances (EPS)

Iron Sulfide Formation

Microbial Communities, Structure, and Function

Microbial Mats

Sulfate-Reducing Bacteria

Tidal Flats

**METAGENOMICS**

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**Definition**

Metagenomics is a research discipline that uses molecular biology methods to acquire, analyze, and interpret sequence data and/or functional data from the metagenomes (the collectivity of genomes) present in the environmental samples, without the need to cultivate the organisms in the samples. Related methodologies are so-called metatranscriptomics and metaproteomics that focus on the collective transcriptomes and proteomes, respectively, rather than on the genomes.

**Introduction**

Microorganisms constitute major life forms on earth with respect to cell numbers and total biomass, and fulfill essential functions in global element cycles. It has been estimated that the earth’s biosphere harbors at least two to three orders of magnitude more prokaryotic cells (4–6 × 10^30) than the number of plant and animal cells combined. The total carbon fixed in prokaryotic cells amounts to 3.5–5.5 × 10^17 g of C which is 60–100% of the estimated total carbon in plants (Whitman et al., 1998).

For many decades since the beginnings of microbiology, research in this field has depended on the isolation and cultivation of individual microorganisms (best illustrated for the area of medical microbiology with the work of Robert Koch on the isolation and characterization of pathogenic bacteria in monocolours, leading to the Koch’s postulates) or well-defined syntrophic consortia, and the subsequent in-depth analysis of pure cultures. Until today, microbiological research with traditional cultivation and
subsequent chemical, genetic, and physiological characterization has led to the description of roughly $10^4$ bacterial and archaeal species deposited as pure cultures in microbial culture collections. However, in the course of the past 2 decades, it has become more and more evident that the microbial diversity in most aquatic habitats, soil, as well as oceanic and terrestrial subsurfaces is enormous (Amann et al., 1995; Hugenholtz et al., 1998; Quince et al., 2008; Sleator et al., 2008) and that at least at present the majority (>99%) of microorganisms in many complex habitats are not cultivated by using standard techniques (Amann et al., 1995). Thus, it must be acknowledged that the number of described species contrasts by orders of magnitude with estimates about the number of prokaryotic taxa on earth which may amount to $10^6$–$10^8$ distinct genospecies (Simon and Daniel, 2010). Metagenomics, a relatively young research discipline, can aid in the detection and acquisition of data about this vast majority of uncultured microorganisms. For this methodology, which importantly is independent of cultivation, the collectivity of genomes (the metagenome) present in a given environmental sample is extracted and subjected to completely random or gene-targeted sequence analysis (Figure 1).

Although bacteria and archaea normally are unicellular, they usually occur as highly diverse but often poorly characterized consortia in their natural habitats. Individual environmental samples can contain a breathtaking prokaryotic diversity. For example, the numbers of different molecular species or operational taxonomic units (OTUs) in soil samples could be over $10^6$ (Curtis et al., 2002). Statistics-based predictions for a specific Canadian soil sample range from $2 \times 10^4$ to $1.4 \times 10^5$ taxa (Quince et al., 2008). Highly interesting, based on taxonomy assessment results from deep-sea hydrothermal vent samples and statistical follow-up considerations, even samples from extreme environments—which often are believed to contain only limited biodiversity due to the extreme conditions—have revealed enormous microbial biodiversity, for example, $3 \times 10^5$ bacterial taxa at one specific sample site (Quince et al., 2008). These numbers illustrate the problems one faces to reach near-complete metagenomic datasets for complex natural habitats with today’s analysis techniques. Thus, even with high-throughput methods, it is difficult if not impossible to generate a complete microbial inventory of a habitat including also most of the rare taxa. However, the methods available do allow the reliable

![Flowchart summarizing steps of environmental meta-analysis](image-url)
identification of the abundant taxa that presumably account for the major biogeochemical conversions.

**Uses and applications of metagenome analysis**

One major advantage of metagenomic analysis is that one can obtain a wealth of genetic information about organisms that live together in more- or less-complex communities, most of which cannot be cultivated with today’s standard techniques. It can be used for extremely diverse purposes, such as the analysis of fundamental aspects of microbial community structure, function, and adaptation in environmental microbiology, but also to explore and exploit the microbial genetic, metabolic, and enzymatic diversity space for the development or improvement of biotechnological processes. Examples for the application of metagenomic techniques in geobiology are the analysis of microbial communities in rock material, sediments, aquifers, as well as in biofilms, on rock surfaces or in subsurface caves and mines, etc. It must be stressed that it is of utmost importance for most metagenomic studies to comprehensively record the sample-associated metadata (the data about the data), like date and position of sampling, physical and chemical characteristics, etc., as has been pointed out by Wooley et al. (2010).

Principally, metagenome analysis can be applied to any kind of habitat, irrespective of the complexity of the microbial communities present. For low-diversity habitats, high-throughput metagenome sequencing can yield a comprehensive overview of genetic information present and can even allow the near-complete or complete assembly of whole prokaryotic genomes, which was nicely shown for the organisms present in acid mine drain fluids (Tyson et al., 2004) or community genome sequencing of an anammox bacterium (Strous et al., 2006). However, the more diverse the community, the higher the sequencing and/or screening effort has to be to arrive at results that cover as comprehensively as possible the target genes of interest present in the sample. In some cases, it is possible to reduce the amount of work and costs by choosing a more specific than a completely random metagenome analysis. For example, if the main interest of a study lies in the analysis of genes involved in a certain metabolic trait (like breakdown and utilization of a specific substrate), one can try to enrich for the corresponding genes by carrying out enrichment cultures wherein the growth of organisms which display this metabolic trait is favored (e.g., by supplying the said specific substrate as the sole carbon source). Enrichment cultivation also has the advantages that (i) more cell material becomes available and that (ii) less organic and inorganic contaminating compounds from the original habitat sample that may interfere with DNA isolation yield and quality are present. An alternative is to enrich for genomic DNA or RNA from microorganisms with a certain substrate utilization capability by feeding a natural microbial community with \(^{13}\)C- or \(^{15}\)N-labeled substrate and then to isolate and process only the heavy nucleic acid that stems from organisms that utilized the added \(^{13}\)C- or \(^{15}\)N-substrate and incorporated the label into their nucleic acids (stable isotope probing) (Friedrich, 2006; Neufeld et al., 2007). Using enrichment cultures to preselect for microorganisms with certain physiological traits has been successfully done in several cases (e.g., Entcheva et al., 2001) and may be of advantage especially for very complex microbial communities, but it has to be kept in mind that any kind of cultivation step before DNA isolation can result in dramatic changes in community composition and diversity.

**Assessment of phylogenetic and functional diversity**

Sequence analysis of conserved marker genes, usually 16S rRNA genes, is widely used for community diversity assessment. To this end, 16S rRNA gene sequences can be amplified with PCR directly from metagenomic DNA samples, followed by cloning into appropriate vectors and sequence analysis. Also, the sequences of conserved marker genes (rRNA genes, but also protein-encoding marker genes) can be retrieved from large sequence datasets compiled by large-scale random sequencing of metagenome DNA and then used for phylogenetic community composition assessment (von Mering et al., 2007).

Recently, massively parallel sequencing specifically of rRNA gene fragments amplified from metagenomic DNA has become possible by the use of the 454 Life Sciences (Roche) high-throughput tag pyrosequencing technology (Huse et al., 2008), but due to the fact that read-length limitations presently only allow the sequencing of incomplete rRNA gene fragments, this technology is suited for the trustworthy phylogenetic assignment at the phylum level but is not as reliable at the genus or species level. Nonetheless, this method allows interesting insights into the affiliation of microorganisms from complex communities to the large phylogenetic lineages, and technological improvements pertaining to the read lengths will surely allow more precise analyses at the genus or species level in the near future.

Bioinformatic tools are available for the comprehensive phylogenetic tree calculation from alignments of conserved marker sequences, for example, the ARB software package (Ludwig et al., 2004). Also, other sequence information than merely phylogenetically conserved genes available from large metagenomic random DNA sequence datasets can be used for diversity assessment. In the past years, various software tools have been developed that are useful for the so-called taxonomic binning of such sequence information (see Simon and Daniel, 2010). While some algorithms work on the basis of oligonucleotide sequence frequencies (composition-based binning, e.g., PhyloPhytia, TETRA; Teeling et al., 2004; McHardy et al., 2007), which are known to vary between different genomes, others use comparative orthologous ORF analysis for this purpose (CARMA, MEGAN, Sort-ITEMS; Huson et al., 2007; Krause et al., 2008; Monzoorul et al., 2009). Importantly, however, since most of these
bioinformatic tools depend on genome sequences and annotations from the available reference genome databases, which presently cover only a minority of the (readily) culturable species (not to speak of the enormous uncultured species diversity present in nature [see above]), our present possibilities for the assignment of short metagenomic DNA sequences to microbial taxa are quite limited.

Current large-scale efforts to sequence the genomes of the prokaryotic species deposited in major microbiological culture collections (GEBA, a Genomic Encyclopedia of Bacteria and Archaea; http://www.jgi.doe.gov/programs/GEBA/; Wu et al., 2009) will surely dramatically improve the reference genome sequence database necessary for a more accurate phylogenetic assignment of metagenomic sequence fragments. Nevertheless, considering that there are still in some cases not a single, in other cases only very few cultured representatives for some deep-rooting phyla, severe limitations in our ability to reliably assign short metagenomic DNA sequences to phylogenetic taxa will remain at least in the near future.

Screening for genes in metagenomes

Metagenomic studies often include the screening of metagenomic libraries or metagenomic sequence datasets for genes of interest, for example, for genes encoding key enzymes characteristic for habitat-specific physiological traits, or for genes for biotechnologically relevant enzymes. Two fundamentally different approaches are used for this: the sequence-based approach and the function-based approach. Both methods have individual advantages and disadvantages and are explained briefly in the following.

The sequence-based approach uses in silico sequence mining, PCR amplification or hybridization methods to detect and isolate genes of interest. Metagenomic sequence datasets can be scored for fragments encoding orthologs of known genes deposited in the available reference databases. Various bioinformatic tools such as BLAST, Pfam, SEED, TIGRFAM, MEX, RAMMACAP, or MG-RAST are available which are helpful for the functional annotation of metagenome sequence data (see Simon and Daniel, 2010; Wooley et al., 2010). Another sequence-driven approach is to design degenerate PCR primers directed against conserved sequence motifs in a gene family of interest and to amplify partial ORFs from metagenomic DNA. The fragments can then be used to isolate the complete ORFs, for example, by using them as hybridization probes to identify clones or metagenomic DNA fragments containing the adjacent, missing ORF sequences, or by metagenome walking (see Simon and Daniel, 2010). Alternatively, DNA microarrays carrying oligonucleotides derived from consensus sequences of known genes have been used for the sequence-directed screening of a metagenomic library for a light, oxygen and voltage (LOV) domain blue light photoreceptor gene (Pathak et al., 2009). The major disadvantage of sequence-based gene detection is that this method depends on prior sequence information from known gene families. Therefore, truly novel genes unrelated to the already known genes encoding for a given function cannot be identified with this approach.

In the function-based screening approach, clones from metagenomic libraries are subjected to activity-based assays that indicate the function of interest. The major advantage of this approach is that it allows the recovery of complete genes or gene clusters and of novel types of enzymes without any prior knowledge of the sequence. However, in order to work efficiently, the genes of interest, which often originated from uncultured microorganisms with unknown genetics and physiology, must be compatible with the expression machinery provided by the heterologous screening host with respect to transcription, translation, protein folding, and eventually secretion (Gabor et al., 2004). Therefore, the fraction of the existing enzymatic diversity that can be accessed via functional screening approaches is strongly influenced by the choice of the host organism used. The phylogenetic distance between the gene donor and the cloning/expression host may be crucial for the success of gene expression. Mostly, Escherichia coli is used to construct metagenomic libraries for functional screening, but it is clear that the expression of metagenomic genes can be severely biased in this host. Therefore, it is important to establish and improve an alternative host/vector systems in order to enhance the probability of detecting genes from metagenomic samples (Angelov et al., 2009; Steele et al., 2009; Uchiyama and Miyazaki, 2009).

Some restrictions and problems

Despite the power of metagenomic studies to unravel genetic information from microbes in communities that are not amenable to cultivation and in-depth characterization, a number of restrictions and problems remain, only some of which are listed hereafter.

Huge amounts of data are generated, especially in metagenome random sequencing projects. These data need to be generated, processed, and stored in a complex succession of steps. Special care must be taken with respect to library construction and sequencing quality. Exemplarily, the procedure of a metagenomic sequencing project is described by Kunin et al. (2008). A useful compilation of software tools for composition-based and similarity-based taxonomic binning, functional annotation of metagenomic data, and comparative metagenomics can be found in Wooley et al. (2010).

Interpretation of the sequence data and their connection with biochemical functions of biocatalysts remains a challenging task. The tools for ORF extraction from metagenomic sequence data and bioinformatics-driven function prediction are being improved constantly, but it is of course not feasible to check all gene function predictions experimentally, for example, by heterologous expression and biochemical analysis. Also, if only metagenomic DNA sequence data are available, it is unclear if the genes found are expressed at all. Thus, it has to be kept in mind that in many cases sequence data
obtained from metagenomes only indirectly point to (predicted) enzymes/pathways of the organisms in the sample. In future, performing metatranscriptomics and/or metaproteomics experiments in parallel with metagenomics can help to overcome this problem.

Also, linking phylogenetic and functional metagenomic data with the physiology of living microorganisms in the environmental sample is a crucial but not a trivial step for many studies attempting to correlate environmental data with a microbial community. Due to the species complexity in most microbial communities, it is only rarely possible to obtain assembled genomes. Even if conserved marker sequences point to close relatives of certain well-known microorganisms this does not necessarily prove that the organisms from which the metagenomic sequence information was derived have identical physiologies as the reference organisms. Also, some traits believed to be characteristic for certain phylogenetic groups of microorganisms can get lost by simple mutations. Still, it is possible to extract environment-specific gene fingerprints from unassembled random environmental DNA sequencing data that reflect known characteristics of the sampled environments, thus linking metagenomic sequence information with metabolic functions of the organisms present (Tringe et al., 2005).

Finally, since most conclusions reached from metagenome data analysis rely on comparative sequence analysis and thus are indirect, it will be important to develop and improve new microbiological cultivation and characterization techniques that also allow the cultivation and in vivo analysis of physiologically and quantitatively dominant microbial representatives within the studied communities.

Summary

Considering the enormous microbial diversity in most environmental samples on one hand and the current shortcoming of the microbiologists’ ability to cultivate the vast majority of these organisms on the other hand, it makes sense that metagenomics has become one of the key methods to study the composition and functions of complex microbial communities. The rapid advance of massively parallel sequencing and high-throughput screening technologies will enable even faster and cheaper access to environmental sequence datasets in the future, but connecting the metagenomic data with the physiology of the microorganisms and the environmental data remains challenging, and it will be crucial that the development of experimental setups and bioinformatic tools keep pace in order to be able to derive meaningful conclusions from metagenomic sequence data.

Bibliography


Metalloenzymes in early evolution

In early evolution, precursors of metal enzymes may have played a key role in catalytic reactions relevant for the synthesis of prebiotic macromolecules. According to the hypotheses originally postulated by Wächtershäuser, surface-bound iron, cobalt, nickel, and other transition metal centers with sulphido, carbonyl, and other ligands were catalysts involved in the synthesis of macromolecular compounds by carbon fixation, thus promoting growth of organic “superstructures” (Wächtershäuser, 1990). The metabolic type of this prebiotic scenario was chemolithoautotrophic, driven by the reducing potential of the outflow from deep-sea hydrothermal vents during
the Hadean period. Namely, the acetyl-CoA biosynthetic pathway may be seen as a model for prebiotic energy metabolism, since in this pathway enzymes and other proteins with FeS and (Fe,Ni)S centers are involved (acetyl CoA-synthethase/carbon monoxide dehydrogenase, hydrogenases, ferredoxin); these metal centers are in principle the cubic Fe₄S₄ as well as (SNiS)(Fe₄S₄)(SFeS) units of greigite (Fe(II)Fe(III)₂S₄). Later on in evolution, the prebiotic metal centers became parts of peptides and, finally, defined proteins (Wächtershäuser, 1990; Russell and Martin, 2004). Among all metalloproteins, iron metalloproteins and, within this class, iron–sulfur proteins are supposed to be the most important group and ubiquitous in organisms.

Besides FeS-centers, tetrapyrroles as metal-chelating prosthetic groups are interesting from the evolutionary viewpoint. Tetrapyrroles are as cyclic molecules (corrins and porphyrins; e.g., Figure 1e) ideal chelators of cobalt, copper, iron, manganese, and nickel. At the same time, the highly conjugated porphyrin molecules increase the catalytic activities of transition metal ions in electron transfer reactions by several orders of magnitude. Since porphyrins are present in all kingdoms of life, they must have evolved early in evolution, perhaps even in the

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**Metalloenzymes, Table 1** Abundance of some metals in bacterial and algal organisms in g/100 g (dry weight) and some important key functions in enzymes (Barton et al., 2007)

<table>
<thead>
<tr>
<th>Element</th>
<th>Algae</th>
<th>Bacteria</th>
<th>Some specific roles in enzymes</th>
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<tbody>
<tr>
<td>K</td>
<td>1.81</td>
<td>1.7</td>
<td>Protein stabilization</td>
</tr>
<tr>
<td>Ca</td>
<td>0.55</td>
<td>0.1</td>
<td>Protein stabilization</td>
</tr>
<tr>
<td>Mg</td>
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<td>0.25</td>
<td>Protein stabilization</td>
</tr>
<tr>
<td>Fe</td>
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<td>0.015</td>
<td>O₂ transport, storage and/or activation; electron transport; superoxide breakdown</td>
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<td>0.005</td>
<td>O₂ evolution; peroxide and superoxide breakdown</td>
</tr>
<tr>
<td>Zn</td>
<td>0.001</td>
<td>0.005</td>
<td>Protein stabilization; hydrolytic cleavage</td>
</tr>
<tr>
<td>Cu</td>
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<td>0.001</td>
<td>O₂ transport, storage and/or activation; electron transport; superoxide breakdown</td>
</tr>
<tr>
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<td>0.001</td>
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</tr>
<tr>
<td>Co</td>
<td>0.0003</td>
<td>0.001</td>
<td>Free radical reactions; nucleophil</td>
</tr>
</tbody>
</table>

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**Metalloenzymes, Figure 1** Various metal centers (from Degtyarenko, 2000) (a) mononuclear iron center (nitrile hydratase), (b) mononuclear magnesium center (Ni-Fe-hydrogenase from Desulfovibrio), (c) dinuclear copper center (oxyhemocyanin), (d) polynuclear iron–sulfur center, (e) heme iron coordination (cytochrome P450), and (f) molybdopterin (sulfite oxidase).
prebiotic state. As for FeS centers, the prosthetic groups are found in otherwise completely unrelated metalloproteins, such as methyl-coenzyme M reductase and chlorophylls (see below; Mauzerall, 1998).

Until the Paleozoic era, availability of bioessential transition metals changed by several orders of magnitude. Whereas Cu, Zn, Ni, and Mo concentrations increased in the ocean water, Co, Mn, and particularly iron decreased by several orders of magnitude during the Proterozoic eon (Anbar, 2008). These changes must have had an impact on the biogenic production of greenhouse gases such as nitrous oxide (Cu-dependant) or methane (Ni-dependant) and, hence, respective impact on global climate. The molybdenum cofactor (see below), in particular, plays a key role in the biological nitrogen cycle. It may even be speculated that molybdenum availability in oceans had a tremendous impact on early evolution. Under the reducing atmosphere, molybdenum was depleted in the proterozoic ocean, generating a “bottleneck” for the nitrogen cycle. The low availability of nitrogen must have had a retarding effect on total biomass production of all organisms (especially non-iron-fixing eukaryotes), until the oxidizing atmosphere also provided high molybdenum availability and stopped nitrogen limitation (Zerkle et al., 2006; Anbar, 2008). Limited molybdenum availability may also have been overcome in part by (less effective) molybdenum-independent nitrogenases. The evolutionary history of the enzymes is, however, difficult to reconstruct. There is no phylogenetic evidence that these “alternative” nitrogenases preceeded the molybdenum-dependant enzymes (Raymond et al., 2004).

In recent oceans, though, most metal ions relevant for enzyme metal centers are just present in trace amounts in seawater, and/or form hardly soluble salts. Thus, most organisms utilize specialized chelating compounds, uptake and transport systems for metal ion intake.

**Biomimetic metalloenzymes**

Metal centers from enzyme proteins may give essential data for the design of biomimetic enzymes in bioorganic synthesis. Biomimetic metal centers from enzymes such as cytochrome oxidases (with Fe-porphyrin), catechol oxidase (based on Mn(IV)-complexes with o-aminophenol ligands), or hydrogenase (based, e.g., on six-coordinate Ni-Ru-centers with hydrogen as one of the bridging ligands for NiFe hydrogenase) reveal the biotechnological potential of catalysts derived from biological metal enzymes (Zbaida and Kariv, 1989; Chaudhuri et al., 2005; Song et al., 2005; Ogo et al., 2007).

**Classification of metalloenzymes**

Since metalloenzymes are found in all enzymes families, simple classification of metal-containing subgroups based on sequence similarities cannot be applied. Reasonable classification schemes are based upon the bioinorganic motifs (Degtyarenko, 2000; Figure 1), i.e., the metal atom and its ligands (Degtyarenko and Contrino, 2004).

Metal centers may be classified by the number of bound metal atoms as mononuclear (for one metal atom, Figure 1a and b) or polynuclear (more than one metal atom, Figure 1c and d) with endogenous ligands (i.e., the polypeptides of the enzyme molecule form a mostly polydentate coordination shell) or exogenous ligands (non-protein component of a conjugated protein such as heme or molybdopterin; Figure 1e and f).

The description given below is, for the sake of simplicity, based on the central atoms. It has to be kept in mind that different metal atoms can be bound to identical or similar bioorganic motifs (e.g., cobalt, copper, iron, magnesium, and nickel by porphyrins or corrins), and different motifs coordinate the same type of metal atom (e.g., either molybdopterin or Fe₇MoS₉ homocitrate cofactor for molybdenum; see below).

**Metal centers in metalloenzymes and other metal proteins**

**Mononuclear iron proteins** are oxidoreductases (e.g., aromatic amino acid hydroxylases, aromatic ring cleavage dioxygenases, Fe-superoxide dismutase, lipoxigenases, nitrite hydratase, and Rieske oxygenases), or involved in electron transfer (desulfoferredoxins, rubredoxins, and photosynthetic reaction centers). The amino acid ligands are mostly histidine and/or cysteine such as in nitrite hydratase with three cysteine residues and two amide nitrogens (Figure 1a). **Diiron carboxylate proteins** bind a coupled binuclear iron center using, e.g., four carboxylate and two histidine residues. Oxidoreductases (alkene hydroxylase, methane monoxygenase, and phenol hydroxylase), iron storage (bacterioferritin, ferritin), and oxygen storage/transport proteins are members of this group (hemerythrin, myohemerythrin).

**Hemoproteins** are characterized by an iron porphyrin as prosthetic group (Figure 1e). While four of the iron ligands are occupied by the porphyrin ring, the other two are used for binding of the protein. Hemoproteins are oxidoreductases (catalases, peroxidases), electron transfer proteins (cytochromes), or oxygen transport and storage proteins (globins) (Nordlund and Eklund, 1995; Que and Ho, 1996).

**Iron–sulfur proteins** are characterized by the presence of iron–sulfur clusters containing sulfide-linked di-, tri-, and tetrairon centers in variable oxidation states (Figure 1d). Iron–sulfur clusters are found in ferredoxins, NADH hydrogenases, dehydrogenases, cytochrome c reductases, nitrogensases, and other proteins. Proteins with iron–sulfur clusters have key roles in nearly all respiratory chains, either in bacteria and archaea or in mitochondria of eukaryotic organisms. Other functions include catalysis of stereo-specific isomerization of citrate to isocitrate (aconitase) in the tricarboxylic acid cycle or the redox-dependent gene regulation. The simplest iron–sulfur cluster type consists of two iron ions bridged by two sulfide ions and is coordinated by four cysteiny1 ligands or by two cysteines and two histidines. The oxidized proteins
contain two Fe\(^{3+}\) ions, whereas the reduced proteins contain one Fe\(^{3+}\) and one Fe\(^{2+}\) ion. These species exist in two oxidation states, Fe(III), and Fe(III)Fe(II). Besides [2Fe-2S], [3Fe-4S], [4Fe-4S], or [8Fe-7S] clusters are known to contain two Fe\(^{3+}\) in galactose oxidase.

Also in copper-containing proteins, several metal center types occur. Similar to iron proteins, copper proteins are involved in oxygen transport or activation processes and electron transport. **Type I copper centers** have a single copper atom coordinated by two histidine residues and a cysteine residue in a trigonal planar structure and a variable axial amino acid ligand. Type I proteins are, e.g., ascorbate oxidase, ceruloplasmin, laccase, nitrite reductase, auracyanin, and azurin. **Type II copper centers** are square planar coordinated by N or N/O ligands (e.g., cytochrome c oxidase, auracyanin, and azurin). **Type III centers** occur in anaerobic bacteria and archaea (CO-dehydrogenases from aerobic bacteria are molybdopterin enzymes), whereby CO is bound by the nickel atom and hydroxyl oxygen (from water) is transferred to the bound carbon atom. After CO\(_2\) is released, nickel is reduced (Ni\(^{2+}\) → Ni\(^{+}\)) and transfers two electrons to ferredoxin.

In the methyl-coenzyme M-reductase reaction, methane and a heterodisulfide are generated from the substrate methyl coenzyme M that reacts with coenzyme B (7-thioheptanoylthreoninephosphate). In nickel-iron hydrogenases, nickel of the Ni-Fe center is coordinated by one apical and three equatorial S-cysteins. The Ni-Fe hydrogenases catalyze both \(\text{H}_2\) evolution and uptake in vitro, with cytochromes acting as either electron donors or acceptors. Acetyl-CoA synthase catalyzes the reversible formation of acetyl-CoA from carbon monoxide, a methylated corrinoid protein and coenzyme A, and is complexed with a nickel CO dehydrogenase (Ermel et al., 1998).

**Manganese in metalloenzymes** may also act as a simple Lewis acid catalyst. Manganese (normally Mn(II)) can also be oxidized to the (III)- or (IV)-state in biological systems, which allows redox-cycling reactions. **Manganese metal centers** may be mono-, bi-, tri-, or tetranuclear. Mn-superoxide dismutases (SODs) exhibit *mononuclear* centers and are antioxidant metalloenzymes catalyzing the redox disproportionation of the superoxide radical, \(\text{O}_2^-\). SOD decomposes the highly reactive superoxide anion by a two-step disproportionation reaction which generates oxygen hydrogen peroxide. The active site manganese is five-coordinate, with the metal ligands arranged in distorted trigonal bipyramidal geometry. Two known enzymes with *bimolecular* centers are arginase and manganese catalase. Arginase catalyzes the conversion of arginine to urea and ornithine. The manganese ions are five- and six-coordinate, respectively, with square-pyramidal and (distorted) octahedral coordination symmetries. The metal centers are interconnected by a solvent-derived hydroxide and carboxylate residues. This metal-bridging hydroxide acts as nucleophile that attacks the arginine guanidinium carbon. Other bimolecular manganese enzymes are aminopeptidase P from *Escherichia coli* and the dinitrogenreductase-activating glycohydrolyase from *Rhodospirillum rubrum*.

One of the most important biochemical catalytic reactions in our biosphere, the oxygen-generating photolysis of water, is brought about by the *tetranuclear manganese cluster* in oxygengenerating photosynthesis of cyanobacteria, eukaryotic algae, and higher plants. In oxygenic photosynthesis, water is oxidized by a cluster of Mn ions. Light is captured by antenna chlorophyll and funneled to the primary reaction center, which contains chlorophyll (P680). The oxidized P680 first oxidizes a redox-active tyrosine. Subsequent oxidation of the Mn cluster occurs via this
generated tyrosine radical. The enzyme can exist in five oxidation levels, corresponding to various oxidation states of the cluster (Wieghardt, 1994; Yocum and Pecoraro, 1999; Carrell et al., 2002).

Molybdenum and tungsten enzymes catalyze basic metabolic reactions in the nitrogen, sulfur, and carbon cycles. With the exception of the nitrogenase cofactor, molybdenum is associated with the heterocyclic pterin derivative (molybdopterin) that contains a mononucleotide center, coordinated to the thiols of the cofactor (Figure 1f). These molybdenum-cofactor–containing (MoCo) enzymes catalyze the transfer of an oxygen atom, derived from or incorporated into water, to or from a substrate in a two-electron redox reaction. Three classes of MoCo enzymes are now distinguished, represented by xanthine oxidase and sulfite oxidase families (with single pterin ligands and either ligand-Mo(VI)OS(OH) or ligand-Mo(VI)O2(S-cys) cores), and DMSO reductase family (with two pterin ligands). The nitrogenase molybdenum metal center is not related to the pterin derivatives. In molybdenum nitrogenases, each Mo forms a part of a polynuclear cluster containing 1 Mo, 7 Fe, and 9 S organized into an elongated structure. Within the protein the terminal Fe of the cluster is coordinated by a cysteine residue, while the Mo is coordinated by a histidine residue and a homocitrinate ligand.

Tungsten, which lies in the chromium group of the periodic system immediately below molybdenum exhibits similar chemical properties and is complexed in a similar way to the pterin cofactors as described for molybdenum. Due to the higher sensitivity of tungsten toward high redox potential, tungsten enzymes are found in (thermophilic) anaerobic bacterial and archaeal microorganisms (Hille, 2002; Williams and Fausto da Silva, 2002).

Cobalt is the central metal ion in the tertrapyrrolic corrin ring in the coenzyme B12. Similar to porphyrin rings, four coordination sites are provided by the corrin ring. The B12-bearing enzymes catalyzes either the transfer of methyl groups between two molecules or isomerization reactions, i.e., rearrangements of hydrogen atoms with concomitant exchange of a substituent (e.g., an hydroxyl group) between two adjacent carbon atoms in a molecule. In non-corrin cobalt enzymes, cobalt is complexed by aminoacids such as in nitrate hydratase (catalyzing the hydration of nitriles to amides; Kobayashi and Shimizu, 1999).

Vanadium occurs as V(III), V(IV), or V(V) in biological systems. The respective enzymes catalyze the two-electron oxidation of a halide by hydrogen peroxide. Besides the vanadium-containing enzymes, also heme-containing haloperoxidases and metal-free enzymes are known. In tunicates (ascidians) vanabin binds 20 V(IV) ions. However, the role of vanabins in vanadium accumulation by the ascidian has not yet been determined. In vanadium nitrogenases, vanadium, instead of molybdenum is present in a FeVCo-center (Michibata et al., 2003; Crans et al., 2004).

Though frequently not classified as metalloenzymes in the strict sense, numerous (enzyme) proteins bind Na⁺ and K⁺ ions. The structures of Na⁺ and K⁺-complexes with proteins have a variety of coordination schemes and functions. Usual coordination numbers are five and six. Though the ions are not directly involved in catalytic steps, they activate numerous enzymes. Prominent examples are Na⁺-activated β-galactosidase or the K⁺-dependent activity of pyruvate-kinase and glycerol dehydratases (Page and Di Cera, 2006).

Specific binding of calcium ions frequently leads to conformational changes and increased protein stability. One large family of calcium-binding proteins is denoted EF-hand proteins, comprising a calcium-binding structural domain (“helix-turn-helix” motif). In an EF-hand loop the calcium ion is coordinated in a pentagonal bipyramidal configuration, by atoms of the protein backbone and side chains especially of aspartate and glutamate. Examples for EF-hand proteins are calmodulin and parvalbumin. In non-EF-hand calcium-binding proteins, the binding motifs are not conserved, such as α-lactalbumin and thermolysin (Yang et al., 2002).

Also magnesium has a key function in activation and stabilization of enzymes. Isocitrate lyase and glutamine synthetase are two examples of Mg²⁺-activated metabolic enzymes. Enzymes catalyzing phosphorylation of proteins typically use magnesium chelates of ATP as cosubstrate. The bound Mg²⁺ serves to facilitate nucleophilic attack at the γ-phosphate of the ATP substrate. Phosphate and phosphoryl transfer reactions require Mg²⁺ as an essential cofactor. In chlorophyll molecules of photosynthetic reaction centers, magnesium ions are coordinated in a terrapyrrole ring system and to an axial N-histidin, O-aspartate, O-formyl-methionin, O-leucin, or water as ligands (Figure 1b; Cowan, 2002).

Conclusion

Since metal centers are present in all enzyme families, and enzymes with metal centers also catalyze a wide variety of different reactions, metal enzymes cannot be assigned to a special group of enzymes or proteins. Thus it is very likely that metal centers developed early in evolution and evolved in several different lineages. Since free transition metal ions are ideal catalysts for numerous reactions in organic chemistry (and biochemistry) at moderate temperatures, they appear to be the natural “precursors” of functional enzyme proteins. Peptides and proteins are, on the other hand, ideal chelators for metal ions. Thus, protein and metal partners may have easily come together frequently during evolution.

Bibliography


Cross-references

Anaerobic Oxidation of Methane with Sulfate
Methane, Origin
Methane Oxidation (Aerobic)
Nickel, Biology
Siderophores
Zinc

METALLOGENIUM

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Definition

Metallogenium s.str. is a star-shaped enigmatic bacterium oxidizing Mn and iron. It preferentially lives in limnic environments.

Metallogenium is a relatively large (2–10 µm) microorganism with bacterial affinities that concentrates manganese and iron oxides in significant amounts. The genus Metallogenium has not yet been assigned to any taxonomic group. The cells lack rigid walls and develop flexible, tapering threads from which new cells probably arise. The organism is therefore, star-shaped and is found in various facies, predominantly in limnic environments (Figures 1 and 2). The threads are often heavily encrusted with oxides of iron and/or manganese. The oldest
Metallogenium-like fabrics are known from the 1.88 Gy old Paleoproterozoic Gunflint Formation (Barghorn and Tyler, 1965).

The first detailed description of Metallogenium was carried out byPerfil’ev and Gabe (1961) who called the type species Metallogenium personatum. There is an enormous record of publications about this microorganism (Zavarin, 1964a, b; Dubinia, 1970; Schmidt and Overbeck, 1984), and many speculations have been made about its systematic position, for example, within the Mycoplasmatales (Zavarzin and Hirsch, 1974), Chlamydbacteria (Klaveness, 1977), and Hyphomicrobiales (Perfil’ev and Gabe, 1961). Clasen (1969), Schmidt and Overbeck (1984), and Zavarzin (1989), suggested a relationship with the iron bacterium Leptothrix echinata (Beger, 1935) which shows a considerable morphological similarity to Metallogenium. Unfortunately, valid molecular biological examinations presently do not exist to clarify the phylogenetic position of this organism, although it is common especially in many freshwater lakes (e.g., Klaveness, 1999; Schulte, 1994). Besides Metallogenium personatum, which is undoubtedly a prokaryotic organism, “Metallogenium symbioticum” was described (Zavarzin, 1964b), which is also an Mn-oxidizing prokaryote preferentially growing on fungal microorganisms (Schweisfurth, 1971).

Bibliography
METALS, ACQUISITION BY MARINE BACTERIA

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Definition
Oceanic bacteria produce siderophores to acquire iron. Given that the iron concentration in surface ocean water is very low, siderophores produced by marine bacteria are tailored to the physical and chemical constraints of the marine environment.

Introduction
Microbial iron uptake
Virtually, all bacteria require iron for growth. Bacteria growing under aerobic conditions utilize a variety of pathways to acquire iron, but under conditions of iron starvation, bacteria often produce siderophores (Crosa et al., 2004). Siderophores are low molecular weight, chelating compounds produced by bacteria to facilitate iron(III) uptake (Crosa et al., 2004; Kraemer et al., 2005; Butler, 2007). The Fe(III)-siderophore complex is recognized by a specific outer membrane protein receptor, which catalyzes the transport of the Fe(III)-siderophore complex across the outer membrane (Figure 1).

The study of marine microbial siderophores is relatively new (Butler and Martin, 2005), yet certain characteristic structural features have already begun to emerge: suites of amphiphilic siderophores and photoreactive Fe(III)-siderophores.

Marine Siderophores
Amphiphilic marine siderophores
One characteristic of marine siderophores is the predominance of suites of amphiphilic siderophores, comprised of a hydrophilic head group, which coordinates with Fe(III), and one of a series of hydrophobic fatty acid appendages, which confers amphiphilicity (Figure 2). Two types of suites of amphiphilic siderophores are produced by marine bacteria. The aquachelins, marinobactins, and amphibactins are amphiphilic peptidic siderophores produced by distinct genera of marine bacteria (Figure 2) (Martinez et al., 2000, 2003). The relative amphiphilicity of each of these siderophores is defined by both the number of amino acids in the peptide fraction, compared to the fatty acid chain length (e.g., C12–C18). The amphibactins with only four amino acids and relatively long C18 fatty acids are the most hydrophobic of the amphiphilic peptide siderophores. As a result, they remain cell associated and are extracted from the bacterial pellet during the isolation process (Martinez et al., 2003). In contrast, the marinobactins and aquachelins, with five and six amino acids in the head group and a predominance of the shorter fatty acids, are more hydrophilic and are thus isolated from the supernatant following centrifugation of the bacterial culture to pellet the cells (Martinez et al., 2000).

The synechobactins and ochrobactins (Figure 2) are different suites of amphiphilic siderophores, in which the head group is based on citric acid, as opposed to a peptide. The head group is thus smaller than in the peptidic amphiphilic siderophores. The fatty acids are also shorter than observed for the peptidic amphiphilic siderophores. Nevertheless, the ochrobactins are quite hydrophobic as a result of two fatty acid appendages; these siderophores remain associated with the bacterial pellet (Martin et al., 2006). The synechobactins, with
only one fatty acid appendage, partition between the culture supernatant and the bacterial pellet (Ito and Butler, 2005).

Photoreactive Fe(III)-siderophores

The other characteristic of marine siderophores is the predominance of \(\alpha\)-hydroxycarboxylic acid moieties in the form of \(\beta\)-hydroxyaspartic acid or citric acid (Figures 2 and 3).

The Fe(III)-complex of an \(\alpha\)-hydroxycarboxylic acid moiety is photoreactive in UV light, including natural sunlight, which is a condition experienced by the mixed layer of the upper 30–40 m of the ocean. Upon UV photolysis of the Fe(III)-siderophores containing citric acid, such as the ochrobactins (Martin et al., 2006), the synechobactins (Ito and Butler, 2005), the petrobactins (Bergeron et al., 2003; Hickford et al., 2004), and aerobactin (marine *Vibrio* sp. DS40M5) (Küpper et al., 2006; Haygood et al., 1993), citrate is oxidized to 3-ketoglutamate and Fe(III) is reduced to Fe(II). The resulting ligand photoprodut retains the ability to coordinate Fe(III) with remarkably high stability constant as a result of the 3-ketoglutate moiety and the remaining hydroxamic acid groups (Figure 4, for aerobactin (Küpper et al., 2006).

Other marine siderophores such as the aquachelins, the marinobactins, the alterobactins, and the pseudoalterobactins contain \(\beta\)-hydroxyaspartic acid as the \(\alpha\)-hydroxycarboxylic acid group. UV photolysis of the Fe(III)-aquachelins results in ligand oxidation, specifically loss of \(\beta\)-hydroxyaspartic acid, as well as reduction of Fe(III) to Fe(II) (Barbeau et al., 2001). This peptide photoprodut retains the two hydroxamate groups and thus the ability to coordinate with Fe(III), although the conditional stability constant is somewhat reduced (Barbeau et al., 2001). Only catalytic amount of Fe(III) is required to effect the complete oxidation of the aquachelins in aerobic conditions, because the Fe(II) that is produced is oxidized back to Fe(III) and thus can coordinate with another apo aquachelin. The ferric complexes of the alterobactins A and B and the marinobactins are also photoreactive (Barbeau et al., 2003), although the
Metals, Acquisition by Marine Bacteria, Figure 3 Marine siderophores with \(\alpha\)-hydroxycarboxylic acid groups. The aquachelins, marinobactins, ochrobactins, and synechobactins shown in Figure 2 also contain \(\alpha\)-hydroxycarboxylic acid moiety. Alterobactins (Reid et al., 1993); pseudoalterobactins (Kanoh et al., 2003); aerobactin (Haygood et al., 1993); petrobactin (Bergeron et al., 2003); petrobactin-SO\(_3\) (Hickford et al., 2004); vibrioferrin (Amin et al., 2007). The circled portion shows the \(\alpha\)-hydroxyacid in citric acid.

Metals, Acquisition by Marine Bacteria, Figure 4 Reaction products in the photolysis of Fe(III)-aerobactin. (Adapted from Küpper et al., 2006).

structures of the oxidized ligands have not been completed yet. The ferric complexes of pseudoalterobactins A and B, which are chemically related to the alterobactins (Kanoh et al., 2003), would also be expected to be photoreactive.

Summary

The preponderance of suites of amphiphilic siderophores and of \(\alpha\)-hydroxycarboxylic-acid-containing siderophores produced by marine bacteria suggests that these properties have evolved as common iron acquisition strategies for marine bacteria, although details of the biological advantages conferred by these molecular properties are yet to be elucidated.

Bibliography


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**Cross-references**

- Bacteria
- Cyanobacteria
- Fe(II)-Oxidizing Prokaryotes
- Metalloenzymes
- Siderophores

**METEORITICS**

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**Definition**

Meteorites are fragments of extraterrestrial material that fall on the Earth’s surface. Most meteorites are parts of asteroids propelled into Earth-crossing orbits by relatively recent collisions in the asteroid belt, initiated by the gravitational effects of Jupiter’s orbit. Relatively small numbers of meteorites originate from larger objects such as the Moon and Mars. The Earth acquires $10^2$ to $10^3$ t of such material each day, but only 1% or less arrives in pieces large enough for identification and recovery (Dodd, 1981). The surface of the meteorite usually melts and emits a glowing tail and a trail of smoke, but the interior of the meteorite is unaffected by passage through the Earth’s atmosphere and remains at the temperature of interplanetary space.

**Collection of meteorites**

“Falls” are meteorites that have been observed to fall and were subsequently collected; “finds” are meteorites that were not seen to fall. Before the 1970s, the total number of known meteorites amounted to less than 1,000 falls and 1,706 authenticated finds. Since then, it has been recognized that certain parts of the Earth’s surface preserve and concentrate meteorites. To date, tens of thousands of meteorites have been collected from the Antarctic ice. Similar numbers of meteorite fragments have also been collected from hot deserts. Most recently, telescope and camera network surveys have produced meteorites with photographed fireballs allowing the calculation of their orbits and assignment to a particular source within the solar system (Bland et al., 2009).

**Chondrites**

About 85% of the meteorites observed to fall on the Earth are chondrites, conglomerate rocks with an overall elemental composition similar to the Sun, if the loss of the most volatile elements is ignored. When their near solar makeup is considered alongside radiometric ages of $4.56 \times 10^9$ years, it becomes clear that chondrites are rocks formed during, or shortly after, the birth of the Sun.

**The record in chondrites**

Their status as some of the earliest formed solids in the solar system combined with a lack of subsequent processing ensures that chondrites represent a valuable record of extraterrestrial environments. Contributing regions include interstellar space, the solar nebula and the asteroidal meteorite parent bodies.
Carbonaceous chondrites
Of all the chondrites, the carbonaceous chondrites are considered the most primitive and these objects represent an enthralling window back in time providing views of the earliest solar system (Figure 1). The name “carbonaceous chondrite” is highly descriptive as these meteorites contain a carbon-rich matrix composed primarily of clay minerals, oxides, and organic matter, and most contain spherules of silicate glass termed chondrules.

Classification of carbonaceous chondrites
The carbonaceous chondrites are classified according to their primary and secondary characteristics (Figure 2). The primary classification (CI, CM, CR, CO, CV, CK) reflects the original properties of the meteorites such as bulk chemistry and mineralogy. The label is derived from the carbonaceous chondrite accepted as the type specimen, for example, CI = Ivuna. The secondary numerical classification reflects petrographic type, which indicates the extent and type of alteration that the meteorite has undergone on its parent body.

Carbon in meteorites
The carbon in carbonaceous chondrites is present in several forms and each has its specific source region(s) (Table 1; Sephton et al., 2003). Materials like silicon carbide and graphite condensed from the atmospheres of stars that existed long before our Sun. Nanometer-sized diamonds also originate as condensation products of stellar outflows as well as from catastrophic supernova explosions. Carbonate minerals were formed in the early solar system as aqueous alteration took place on asteroids. Yet all these components are quantitatively subordinate to the main carbonaceous material in carbonaceous chondrites – organic matter.

Types of organic matter in meteorites
Much of our current understanding of meteoritic organic matter has come from investigations of the Murchison carbonaceous chondrite, approximately 100 kg of which fell in Australia in 1969 (Figure 1). Not all of the organic molecules in meteorites are present in the same form. A minor portion of the organic matter in meteorites is present as small “free” molecules, including various hydrocarbons and the biologically useful amino acids and carboxylic acids (Figure 3), but the majority of organic matter in the carbon-rich meteorites is present as a highly cross-linked organic network or “macromolecular material”.

![Meteoritics, Figure 1: The Murchison carbonaceous chondrite.](image1)

![Meteoritics, Figure 2: The classification system for carbonaceous chondrites.](image2)
Free compounds

Amino acids

The amino acids found in meteorites have attracted a great deal of attention owing to their role as building blocks for proteins. The search for amino acids in meteorites accelerated in the 1960s (Degens and Bajor, 1962; Kaplan et al., 1963) following reports of microfossils in certain samples (Claus and Nagy, 1961). Amino acids were discovered, but these initially promising results were later found to be the result of terrestrial contamination. It was only when the organic-rich Murchison meteorite fell and was rapidly analyzed that both protein and, importantly, nonprotein amino acids were found in this fresh sample (Kvenvolden et al., 1970). Today it is known that the amino acids in Murchison are present at around 60 ppm. Compounds containing two through eight carbon atoms exist and they exhibit several features, which suggest that they are not terrestrial contaminants but are extraterrestrial and nonbiological or “abiotic” in origin. The meteoritic amino acids display broad structural diversity, a decline in abundance with increasing molecule size, and the presence of unusual and even unique molecular configurations.

A particular characteristic of amino acids is chirality or handedness. One chiral form rotates the polarized light to the left and is termed levorotatory or “L,” while the other form rotates the light to the right, and is called dextrorotatory or “D.” Chirality is important for theories that link extraterrestrial organic matter with the origin of life, because terrestrial biology preferentially generates L-amino acids whereas abiotic reactions generally have no preference and produce racemic mixtures with equal amounts of L and D forms.

The discussion of whether amino acids in meteorites were racemic (with equal amounts of L and D forms) or nonracemic (with a preference for one or the other) began in the 1950s and continued through the 1960s, (e.g., Mueller, 1953; Nagy et al., 1964) but early results implied that the dominance of L forms was caused by terrestrial contamination and analytical artefacts. Yet following the fall of Murchison in 1969, four protein and seven

Meteoritics, Table 1 The carbon-bearing components in carbonaceous chondrites

<table>
<thead>
<tr>
<th>Carbon-bearing component</th>
<th>Abundance (wt. %)</th>
<th>Environment of formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic matter</td>
<td>2.0</td>
<td>Interstellar space, solar nebula, asteroids</td>
</tr>
<tr>
<td>Carbonate</td>
<td>0.2</td>
<td>Asteroids</td>
</tr>
<tr>
<td>Diamond</td>
<td>0.04</td>
<td>Stars, supernovae</td>
</tr>
<tr>
<td>Graphite</td>
<td>0.005</td>
<td>Stars</td>
</tr>
<tr>
<td>Silicon carbide</td>
<td>0.009</td>
<td>Stars</td>
</tr>
</tbody>
</table>

Meteoritics, Figure 3 Some free compound classes and examples found in carbonaceous chondrites.
nonprotein amino acids were found to be racemic (Kvenvolden et al., 1970, 1971). More modern analyses have reinvigorated the debate over the racemic nature of meteorite amino acids and a slight L-excess in both protein and nonprotein amino acids has been reported (Engel and Nagy, 1982; Pizzarello and Cronin, 2000).

Proposed mechanisms for producing this L-excess in meteoritic amino acids rely on selective destruction of the right-handed form of the amino acids. Currently, popular hypotheses involve ultraviolet circularly polarized light from a neutron star or reflection nebulae (Bailey, 2001; Bonner and Rubenstein, 1987). It is a necessary consequence of this theory that at least some amino acids were already formed in interstellar space so that preferential destruction of D-amino acids could take place. The recent detection of the simplest amino acid, glycine, in interstellar spectra supports this assertion (Kuan et al., 2003).

Carboxylic acids
Carboxylic acids are another type of biologically useful molecule found in meteorites. In life, they are a convenient and compact store of energy and are common cell membrane molecules. Carboxylic acids in Murchison contain between two and five carbon atoms and display complete structural diversity, a general decrease in abundance with increasing carbon number, and an equal concentration of branched and straight-chain isomers (Lawless and Yuen, 1979; Yuen and Kvenvolden, 1973). The monocarboxylic acids are present in a much higher concentration (332 ppm) than the amino acids and are the most abundant solvent extractable compounds. A structurally diverse suite of dicarboxylic acids containing up to nine carbon atoms are also present in much smaller amounts (26 ppm) (Lawless et al., 1974).

Hydroxyacids
Hydroxyacids (or hydroxyacids) in carbonaceous chondrites contain up to nine carbon atoms and are structurally diverse and present in concentrations of 15 ppm (Peltzer and Bada, 1978). Intriguingly, the hydroxyacids correspond in structure to the more abundant amine acids suggesting that some hydroxy and amino acids may have been produced together on the parent asteroid. The relevant chemical reaction is known as the Strecker-cyanohydrin synthesis. Hydrogen cyanide and aldehydes combine in aqueous solution to produce hydroxyacids but if ammonia is also present then the products become amino acids.

Sugar-related compounds
Collectively, sugars and their related compounds are called polyhydroxylated compounds or “polyols.” They are compounds containing a number of hydroxyl (OH) groups attached to their carbon skeleton. Sugars are important biologically as a source of energy for organisms and, when combined to form larger molecules, they can act as food stores and give structural support. Experiments in the 1960s detected polyols in carbonaceous chondrites, but terrestrial contamination was implied (Degens and Bajor, 1962). More recent experiments on Murchison have discovered a number of sugar-related compounds (sugars, sugar alcohols, and sugar acids) in similar abundances to the amino acids (Cooper et al., 2001). The Murchison polyols display many of the features of extraterrestrial compounds. Progressively larger polyols become less abundant, display almost complete structural diversity, and contain some terrestrially rare compounds implying an abiotic source.

Aliphatic and aromatic hydrocarbons
Short aliphatic molecules in Murchison are present in amounts of 1.4 ppm and display many nonbiological features, which imply they are indigenous to the meteorite, such as structural diversity and a decrease in amount with increasing molecule size (Yuen et al., 1984). Aromatic hydrocarbons, in free form are present in carbonaceous chondrites at around 30 ppm (Perring and Ponnamperuma, 1971). Compound sizes range from one to seven aromatic rings with the smaller compounds generally being most abundant (Sephton et al., 1998).

Amines and amides
Amines are derivatives of ammonia (NH₃) where hydrogen atoms are replaced by other structures. In Murchison, amine concentration is 8 ppm, amounts of individual amines decrease with increasing carbon number and structural diversity is observed with almost all amine isomers with up to five carbon atoms present (Pizzarello et al., 1994).

Two possible sources of the amines in Murchison have been proposed: they may be directly inherited from the presolar molecular cloud, an assertion supported by the detection of methylamine in interstellar space, or as with the hydroxyl acids, the amines may share a common origin with the amino acids. By simply removing the carboxylic acid group of amino acids, 16 of the 20 amines can be made, a reaction that would take place if the molecules were heated on their parent asteroid.

Amides are structurally analogous to carboxylic acids and, in Murchison, the amide equivalents of monocarbonyl acids, dicarboxylic acids, and hydroxyacids are found in concentrations greater than 70 ppm (Cooper and Cronin, 1995). The amides display complete structural diversity up to and including molecules with eight carbon atoms, a decline in abundance with carbon number, and the presence of many compounds with no terrestrial source. Cyclic amides (lactams) are less abundant (2 ppm) but they are very interesting to
investigators of the origin of life. The chemical structures of cyclic amidases permit hydrogen-bonded pair formation and may have acted as parts of a primitive genetic coding apparatus, possible forerunners of the present day nucleic acids.

Nitrogen heterocycles
Another compound class essential for terrestrial life is that of the nitrogen heterocycles. Some of these compounds are building blocks for nucleic acids that store genetic information. The possible presence of nitrogen heterocycles in water extracts of carbonaceous meteorites was first reported in the early 1960s but the initial optimism was dispelled when they turned out to be laboratory contaminants (Oró, 1963). Later work did confirm the presence of nitrogen heterocycles in meteorites such as Murchison and several classes, including purines, pyrimidines, quinolines/isoquinolines, and pyridines were found. All of the purines and pyrimidines found are biologically common and together account for 1.3 ppm in Murchison (Stoks and Schwartz, 1979, 1981). The quinolines, isoquinolines, and pyridines are structurally diverse, contain a large number of isomeric alkyl derivatives, and are present at concentrations of 7 ppm (Pizzarello et al., 1982). Recent work has confirmed the presence and extraterrestrial origin of xanthine and uracil in the Murchison meteorite (Martins et al., 2008).

Sulfur heterocycles
Murchison contains small amounts of sulfur-containing compounds such as thiophenes, benzothiophenes, dibenzothiophenes, and benzonaphthothiophenes. The abundance pattern of thiophenes in Murchison is markedly different from those in terrestrial sediments, indicating an abiotic origin for these molecules (Shimoyama and Katsumata, 2001).

Phosphorus and sulfonic acids
Phosphorus compounds have many biological roles and play a part in cell membranes, energy transactions during metabolism, and the storage and transfer of genetic information. Sulfur compounds are also common in living systems and take part in key biochemical reactions. In Murchison, a series of alkyl phosphonic acids and alkyl sulfonic acids with up to four carbon atoms have been detected (Cooper et al., 1992). As observed for many of the other compound classes in Murchison, the sulfonic and phosphonic acids display an exponential decline in amount with increasing carbon number and exhibit complete structural diversity.

Macromolecular materials
The solvent soluble substances are only trace components, yet they are often the focus of studies on meteorite organic matter due to their analytical amenability. Essentially, the macromolecular materials can be considered as a cross-linked agglomeration of some of the free compounds but with an overwhelming dominance of aromatic hydrocarbons. The macromolecular materials are generally assumed to be completely indigenous to their meteorite host due to their high molecular weight and immobility. As the major organic component, the macromolecular material is key to theories of the origin of meteoritic organic matter as a whole. Hence it is interesting to consider that meteoritic organic matter is often compared with that observed in interstellar space. Some scientists believe that the molecular cloud that collapsed to form the solar system bequeathed a significant amount of interstellar organic matter, aliquots of which are preserved in primitive asteroids and the meteorites derived from them. For many years, only one- to four-ring polycyclic aromatic hydrocarbons (PAH) were commonly observed in Murchison macromolecular material breakdown products (Sephton et al., 1998), which contrasted sharply with the greater than 20-ring PAH proposed for the interstellar medium (Pendleton and Allamandola, 2002). Recently, using modern analytical techniques the meteoritic macromolecular material has yielded more information. Up to seven-ring PAH units have been liberated from the macromolecular material in Murchison and it appears that even larger entities are present in the experimental residue (Sephton et al., 2004). These discoveries partly reconcile the apparent disharmony between the meteoritic and interstellar organic inventories and point to a partly presolar origin for this abundant organic component.

Terrestrial contamination
Although some compound classes in meteorites may contain a record that extends back to interstellar environments before the solar system formed others can be much more recent in the form of terrestrial contamination. Unusual and subtle contamination sources exist and an example is provided by data from the Orgueil carbonaceous chondrite, which has a long curation history since its fall in 1864. Terpene-related compounds have been identified in this meteorite (Watson et al., 2003) and these compounds are structurally specific and are unlikely to be produced by abiotic reactions. Terpenes are common in essential plant oils, used extensively in cleaning products, and may have percolated their way into the porous Orgueil stones.

A more widespread type of terrestrial contaminant is provided by the long-chain aliphatic hydrocarbons (normal alkanes) in meteorites (Sephton et al., 2001). Unlike the short-chain aliphatic hydrocarbons, the normal alkanes are structurally specific with all carbon atoms arranged in a straight chain. Normal alkanes in meteorites were first detected in Orgueil and were attributed to extraterrestrial biology based on similar distributions.
for molecules in the meteorite and those found in butter and marine sediments (Nagy et al., 1961). Subsequent analyses on many different meteorites led to the discovery that these compounds were present in several tens of ppm, were concentrated near to the surfaces of samples, and that they were frequently associated with other, obviously terrestrial, compounds (Oró et al., 1966). A subsequent revival in the belief that these compounds were indigenous occurred when normal alkanes were interpreted as products of an abiotic catalytic reaction called the Fischer–Tropsch synthesis (Studier et al., 1968). Modern measurements of individual normal alkanes reveal terrestrial values that are distinct from the majority of meteoritic organic matter (Sephton et al., 2001) and imply that normal alkanes are simply contributions from our terrestrial environment once the meteorite has fallen on the Earth.

The location of organic matter in meteorites
In chondrites, organic matter is present within the fine-grained inorganic meteorite matrix, which contains evidence of alteration by liquid water on the parent asteroid. Hence, a general relationship between extraterrestrial organic matter and inorganic aqueous alteration products in carbonaceous chondrites has been recognized for some time. Recently, however, it has been discovered that the association is highly specific to a particular inorganic phase – clay minerals (Pearson et al., 2002). Clay minerals, therefore, may have trapped and concentrated organic matter in the early solar system and organic–inorganic interactions may have played a role in the assembly of increasingly complex organic entities 4.56 × 10⁹ years ago.

Relevance to the origin of life on Earth
With the recognition that meteorites contain many biologically useful molecules it is enlightening to consider that the amounts of organic matter extraterrestrial objects can deliver to the Earth’s surface are considerable. For example, calculations indicate that comet Halley contains organic matter equivalent to approximately 10% of the current biomass of the Earth (Greenberg, 1993). A connection between life and extraterrestrial material was first proposed in the early 1960s by the biochemist Jan Oró (Oró, 1961). It was suggested that extraterrestrial objects may have seeded the early Earth with an extensive list of ingredients for use in the recipe of life. Modern theories imply that the early Earth had a nonreducing atmosphere composed mainly of carbon dioxide and nitrogen in which the in situ generation of organic matter would be rather sluggish and inefficient. Even if only a small fraction of the molecules delivered by extraterrestrial infall proved biologically relevant, they would have had a significant impact on the prebiotic chemistry of the Earth.

Outlook
Until space missions begin to return large quantities of extraterrestrial material back to the Earth for analysis in laboratories, meteorites will remain the most accessible and most frequently studied objects to reveal the origin and evolution of the solar system. In particular, the study of carbonaceous meteorites, which contain bona fide prebiotic organic matter is likely to remain our most important means of determining how chemical evolution led to the origin of life.

Bibliography


Cross-references

- Asteroid and Comet Impacts
- Astrobiology
- Cosmic Molecular Clouds
- Origin of Life
METHANE OXIDATION (AEROBIC)

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Synonyms
Methanotrophy

Definition
Methane oxidation is a microbial metabolic process for energy generation and carbon assimilation from methane that is carried out by specific groups of bacteria, the methanotrophs. Methane (CH₄) is oxidized with molecular oxygen (O₂) to carbon dioxide (CO₂).

Biochemical basis
Methanotrophy is the microbially mediated process of the oxidation of CH₄ with O₂ to methanol, formaldehyde, formate, and finally CO₂ (Figure 1) (Hanson and Hanson, 1996; Madigan et al., 2003; Bowman, 2006). The process is performed by a specialized group of bacteria (qv), the methanotrophs (CH₄ oxidizing bacteria). They are a subgroup of the methylotrophs, bacteria capable of utilizing single-carbon compounds (Bowman, 2006). Methane is both the energy source (electron donor) and the sole or partial carbon source for methanotrophs. Aerobic oxidation of methane requires specific enzymes, most importantly methane monoxygenase (MMO), which catalyzes the first step in the reaction: the oxidation of methane with molecular oxygen to methanol and water. Two forms of this enzyme are known: particulate methane monoxygenase (pMMO), which is ubiquitous in methanotrophs and contains copper, and the iron-containing soluble methane monoxygenase (sMMO) (Hanson and Hanson, 1996). The reaction mediated by MMO requires additional electrons, which are supplied by cellular redox carriers such as cytochrome C (for pMMO) or NADH (sMMO). Energy is conserved in the subsequent stepwise oxidations of methanol, formaldehyde, and formate. In these reactions, electrons are donated back to a membrane-bound electron transport chain (see Chapter Aerobic Metabolism) via a pyrroloquinoline quinone cofactor to cytochrome C (methanol dehydrogenase) or NAD (in formaldehyde oxidation systems and formate dehydrogenase). Electron flow through the membrane ultimately produces a proton motive force that is converted to the cellular energy carrier ATP by the ATPase enzyme complex. O₂ is the terminal electron acceptor (see Chapter Aerobic Metabolism) (Figure 1) (Madigan et al., 2003). Thus, aerobic methane oxidation can be considered a special case of aerobic oxidation systems.
respiration. Differences between methanotrophs are thought to exist regarding the enzymes used, especially with regard to formaldehyde oxidation, where different systems are known (Lidstrom, 2006).

Methanotrophs

Most known methanotrophs fall into two well-defined phylogenetic groups belonging to the gamma- (Type I methanotrophs) and alpha-proteobacteria (Type II) that are also distinguished by morphological traits and differences in carbon assimilation (Hanson and Hanson, 1996; Madigan et al., 2003; Bowman, 2006). Both types share the characteristic extensive intracytoplasmic membrane system (ICM), which is the site of methane oxidation. However, in Type I methanotrophs, the ICM occurs as disk-shaped membrane stacks within the cell, while in Type II methanotrophs, the ICM is located around the periphery of the cell. Both groups are strict aerobes and obligate methylolectrophs. The presence of soluble methane monooxygenase (sMMO) seems to be mostly limited to Type II methanotrophs (Hanson and Hanson, 1996; Bowman, 2006). Type I and II methanotrophs employ different carbon assimilation strategies, although both incorporate methane carbon at the level of formaldehyde (Figure 1).

Type I methanotrophs use the ribulose monophosphate pathway and obtain all their carbon from methane. Type II methanotrophs use the serine pathway, which in addition to formaldehyde incorporation further involves fixation of one molecule of CO₂ per molecule of formaldehyde assimilated. The former pathway is energetically more favorable, requiring only 1/3 ATP per molecule formaldehyde fixed, instead of 1 ATP and reducing power for the serine pathway. This is in accordance with the observed higher growth rates in Type I methanotrophs (Madigan et al., 2003). Most methanotrophs can also grow using other single-carbon compounds, e.g., methanol, which is the first intermediate of methane oxidation.

Within the Type I methanotrophs, the genera Methylophilus, Methylobacter, Methylocystis, and Methylococcus, and Methylocaldum have been described. The latter two genera are often distinguished as “type X methanotrophs,” as they are phylogenetically and morphologically distinct from the other Type I genera. The cultivated type II methanotrophs belong to the genera Methylosinus and Methylocystis (Bowman, 2006). Environmental clone libraries suggest the existence of additional, yet uncultured, genera. Recently, acidophilic methanotrophs belonging to the Verrucomicrobia phylum have been isolated, showing for the first time that not all methanotrophs belong to the proteobacteria and that methanotrophs are phylogenetically more diverse than previously thought (Dunfield et al., 2007; Pol et al., 2007). The genome sequence of one isolate revealed the presence of pMMO genes (pmoA), but lacked homologs to known methanol monooxygenases and formaldehyde oxidation genes, indicating a methane oxidation system that deviates from those known from Type I and II methanotrophs (Dunfield et al., 2007).

Habitats

Although current isolates require high methane partial pressures for growth, some methanotrophs can apparently utilize methane with high affinity at low partial pressures, down to atmospheric levels (Hanson and Hanson, 1996). Therefore, they are ubiquitous in soil and water. Increased populations and activity occur whenever increased methane concentrations, whether from biogenic or other sources, meet oxic conditions. Typical habitats are thus closely tied to sites with methanogenesis (see Chapters Methane, Origin; Methanogens), e.g., rice paddies, swamps, aquatic and terrestrial sediments, landfill coverings, and the oxycline of stratified water bodies (Hanson and Hanson, 1996). In stratified systems, (e.g., in stratified water bodies or sediments) aerobic methane oxidation will often be located within a relatively narrow band within the oxycline, where methanotrophs can become highly enriched. Methanotrophs also occur as endosymbionts, e.g., of marine mussels found at cold seeps (qv) that use methane released to supply their hosts with carbon and energy (Hanson and Hanson, 1996; Bowman, 2006).

Methods to study aerobic methane oxidation and methanotrophs

Methane oxidation rates in environmental samples are frequently determined by incubation experiments. Samples are placed in a gas-tight container and the consumption of methane over time is determined in the headspace. Alternatively, 14C labeled methane can be used to quantify the conversion to CO₂ and incorporation into the bacterial biomass. Field methods include the use of push–pull tests (Urmann et al., 2005), or modeling of rates based on methane gradients.

Methanotrophs can be cultured from almost any environment using a nitrate mineral salts medium and a CH₄/air atmosphere (Madigan et al., 2003; Bowman, 2006). Since they are relatively easily cultured, viable count methods can be used but will severely underestimate the actual population, due to the prevalence of viable but nonculturable cells. Since they are phylogenetically well defined, methanotrophs can be detected and quantified using ribosomal RNA as a genetic marker, e.g., using specific oligonucleotide probes (e.g., for fluorescent in-situ hybridization) or primers for polymerase chain reaction based detection (PCR) (McDonald et al., 2008). In addition, the gene sequences of functional genes, most frequently the gene encoding pMMO (pmoA), but also genes for sMMO (mnoX) and methanol dehydrogenase (mxaF), are frequently used as molecular markers for...
PCR-based detection (Hanson and Hanson, 1996; Bowman, 2006; McDonald et al., 2008). A hybridization microarray was developed for rapid phylogenetic typing of PCR amplified pmoA (Bodrossy et al., 2003). Type I and II methanotrophs also contain biomarkers (see Chapter Biomarkers (Organic, Compound-Specific Isotopes)), e.g., specific lipids and phospholipid fatty acids that are useful in tracing community changes (Hanson and Hanson, 1996; Bowman, 2006; Schubert et al., 2006; Blumenberg et al., 2007).

Geochemical impact
Locally, methanotrophs can be an important basis of the food web, if sufficiently large fluxes of methane are converted to biomass and passed on down the food chain. Since biogenic methane (see Chapters Methane, Origin; Methanogens) has a strongly 13C depleted carbon isotope signature, carbon flow originating from oxidized methane can be studied using isotope measurements (see Chapter Isotopes (Methods)).

The primary importance of aerobic methane oxidation for the global carbon cycle (qv) is that it represents (together with anaerobic methane oxidation (qv) and photochemical methane oxidation in the atmosphere) a major sink for this radiatively active and therefore, important greenhouse gas. Globally, photochemical methane oxidation is by far the dominant process (94%), but microbial oxidation is an important factor to take into account when projecting global warming effects. Obtaining global estimates of microbial aerobic methane oxidation is not trivial due to high spatial and temporal variability and the difficulty in performing rate measurements in environments where methanogenesis and methanotrophy occur simultaneously. The total sink function of soils has been estimated at 29 Tg year\(^{-1}\), with an uncertainty range of 7–100 Tg year\(^{-1}\) (Smith et al., 2000). In rice paddies, methane oxidation can occur in the rhizosphere and at the soil surface, and is estimated to remove 80% of the methane produced in these systems, but there is uncertainty that CH\(_4\) bypass would possibly occur through vascular plants’ parenchyma. Methane oxidation rates in soil depend on various factors, e.g., soil bulk density, water content, temperature, and pH (Hanson and Hanson, 1996). The process is also often limited by the rate of methane diffusion into the oxic zone. Further, ammonium is a competing substrate for MMO that is not coupled to energy generation and therefore acts as a poison to methanotrophs at higher concentrations (Madigan et al., 2003). Thus, the methanotroph activity in soil is subject to considerable variability and sensitive to land use changes, and is one factor to be considered for mitigation strategies to reduce atmospheric methane concentrations (Hanson and Hanson, 1996).

Lakes were long considered a minor source of methane, a view that has somewhat changed based on observation of significant methane fluxes from reservoirs (St. Louis et al., 2000). In permanently or temporally stratified water bodies such as lakes, reservoirs, or the Black Sea, a large proportion of the methane is oxidized by aerobic methanotrophs in the chemocline (Schubert et al., 2006; Blumenberg et al., 2007).

While the open ocean is not considered a major source of methane, and methane oxidation activity is generally low, cold seeps (qv) release large amounts of methane from sediments and hydrocarbon deposits (including gas hydrates (qv)), creating high local fluxes of methane (see Chapter Methane, Origin). This creates a situation where symbiotic as well as free-living methane oxidizers are the basis of large oceanic food webs. However, in anoxic marine sediments anaerobic methane oxidation (qv) coupled to sulfate reduction is the most important process.

Summary
Aerobic oxidation of methane is performed by specific bacteria, the methanotrophs, which use a specific metabolic pathway for carbon assimilation and energy generation from methane. Most known methanotrophs belong to the Proteobacteria, but recent discoveries of novel methanotroph Verrucomicrobia indicated that the diversity of methanotrophs is not yet fully known. Aerobic methane oxidation is one of the major sinks for the greenhouse gas methane, along with anaerobic methane oxidation (qv) and photochemical oxidation in the atmosphere. It is, thus, an important process for the global carbon cycle (qv) and an important element in the regulation of the atmospheric concentration of methane.

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Methane, Origin

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Definition

Methane is a colorless and odorless gas, with the chemical formula CH₄. Due to its radiative force, it is a strong greenhouse gas and contributes to the warming of the earth. It is formed in the environment by methanogenesis. The main natural sources are wetlands and termites (30% of total emissions), while anthropogenic sources include rice fields, cattle farming, and energy production (70% of total emissions).

General aspects

Methane is a colorless and odorless gas and the major component (97% vol.) of natural gas. Methane has a boiling point of −161 °C at a pressure of one atmosphere. As a gas, it is flammable only over a narrow range of concentrations (5–15%) in air. It is mainly produced by microorganisms (methanogens) under anoxic conditions in a process called methanogenesis. These conditions exist under water-covered soils, such as rice paddies, tundra, swamps, and marshes, but also in freshwater and marine sediments. Other anoxic environments include ruminant stomachs and termite guts (see Table 1).

Methanogenesis is the final step in the anaerobic degradation of organic carbon, with methane being formed as a waste product. Methanogenesis is performed by strict anaerobes called methanogens, organisms belonging to the Archaea (a domain of life alongside Bacteria and Eukaryota). Methanogens are rather cosmopolitan in respect to environmental conditions (i.e., temperature, pH, salinity; as long as the environment is anoxic) and represent the largest and most diverse group in the Archaea domain (Megonigal et al., 2004; c.f. Boone et al., 1993). Methanogens are able to grow at temperatures between 4°C and 100°C, at pH values from 3 to 9, and at salinities ranging from brines to freshwater. Despite this huge taxonomic diversity, the substrates that can be used by methanogens are rather limited—hydrogen, acetate, formate, some alcohols, and methylated compounds (Zinder, 1993). The most important of these are hydrogen and acetate, which give rise to two different kinds of methanogenesis: (1) the fermentation of acetate to CO₂ and CH₄ and (2) the reduction of CO₂ with H₂.

Garcia et al. (2000) showed that over 70% of methanogens use the latter pathway, which is known as hydrogenotrophic methanogenesis or carbon dioxide/hydrogen reduction. Hydrogen in this reaction is used both as an energy source and electron donor, whereas CO₂ is the electron acceptor and the carbon source. This reaction yields the most energy of all methanogenic processes—much more than acetate fermentation, which yields the least (see Table 2). Since hydrogen occurs only in very low concentrations in natural environments, it is the limiting substrate in CO₂/H₂ reduction. Only 10% of the methanogenic genera, i.e., *Methanosarcina* and *Methanosphaera*, are able to use the other pathway, called acetate fermentation or acetoclastic

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**Cross-references**

Aerobic Metabolism  
Anaerobic Oxidation of Methane with Sulfate  
Bacteria  
Biogeochemical Cycles  
Carbon Cycle  
Cold Seeps  
Isotopes (Methods)  
Methane, Origin  
Methanogens

**METHANE, ORIGIN**

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**Definition**

Methane is a colorless and odorless gas, with the chemical formula CH₄. Due to its radiative force, it is a strong greenhouse gas and contributes to the warming of the earth. It is formed in the environment by methanogenesis. The main natural sources are wetlands and termites (30% of total emissions), while anthropogenic sources include rice fields, cattle farming, and energy production (70% of total emissions).

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**Methane, Origin, Table 1 Methane properties**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>CH₄</td>
</tr>
<tr>
<td>Mass</td>
<td>16.0425 g/mol</td>
</tr>
<tr>
<td>Melting point</td>
<td>−182.5 °C</td>
</tr>
<tr>
<td>Boiling point</td>
<td>−161.6 °C</td>
</tr>
<tr>
<td>Solubility in water</td>
<td>3.5 mg/100 ml</td>
</tr>
</tbody>
</table>
methanogenesis (Garcia et al., 2000). Despite its limited taxonomic distribution, most likely related to the small amount of energy gained from this process (see Table 2), acetate fermentation seems to be the dominant pathway for methane production in the environment.

**Isotopic composition**

One of the diagnostic features of methane often used in microbiological, geological, and biogeochemical investigations is its carbon and, to a lesser extent, hydrogen isotopic composition. Due to its biogenic or thermogenic formation, the $^{13}$C composition is strongly depleted (i.e., more negative values, see Equation 1).

$$\delta^{13}C = \left( \frac{[^{13}C/^{12}C \text{ sample}}{[^{13}C/^{12}C \text{standard}}} - 1 \right) \times 1,000 \text{ vs. VPDB}$$  \hspace{1cm} (1)

(Vienna Pee Dee Belemnite)

**Methane, Origin, Table 2** Reaction and standard changes in Gibbs free energies for methanogenesis (Adapted from Garcia et al., 2000)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$\Delta G^{\circ}$ (kJ/mol CH$_4$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$</td>
<td>$-135.6$</td>
</tr>
<tr>
<td>4Formate $\rightarrow$ CH$_4$ + 3CO$_2$ + 2H$_2$O</td>
<td>$-130.1$</td>
</tr>
<tr>
<td>2Ethanol + CO$_2$ $\rightarrow$ CH$_4$ + 2Acetate</td>
<td>$-116.3$</td>
</tr>
<tr>
<td>Methanol + H$_2$ $\rightarrow$ CH$_4$ + 2H$_2$O</td>
<td>$-112.5$</td>
</tr>
<tr>
<td>4Methylamine + 2H$_2$O $\rightarrow$ 3CH$_4$ + CO$_2$ + 4NH$_4^+$</td>
<td>$-75.0$</td>
</tr>
<tr>
<td>4 2-Propanol + CO$_2$ $\rightarrow$ CH$_4$ + 4Acetone + 2H$_2$O</td>
<td>$-36.5$</td>
</tr>
<tr>
<td>Acetate $\rightarrow$ CH$_4$ + CO$_2$</td>
<td>$-31.0$</td>
</tr>
</tbody>
</table>

This is related to a kinetic isotope effect where $^{12}$C carbon is preferentially used by microorganisms when methane is formed (Whiticar, 1999). This isotopic effect is not as pronounced in the case of methane formed by heating, or with higher pressures during gas formation from a source rock. In Figure 1, mean values of carbon isotopic compositions are shown for various organic and inorganic materials. Whereas carbonates formed in seawater have values around 0‰ VPDB, and atmospheric carbon dioxide shows a global value of around $-8\%$ VPDB, methane shows highly depleted values between $-25\%$ and $-110\%$ VPDB. This significant feature (sometimes in combination with hydrogen isotopes) has been used in numerous studies to reveal, for instance, the origin of methane (Whiticar et al., 1986) or methane oxidation processes in old carbonate rocks, sediments, or water columns of lakes and oceans (Boetius et al., 2000; Peckmann and Thiel, 2004; Schubert et al., 2006). Not only can the formation process (i.e., biological or thermodynamically controlled) be derived, but also changes that occur during its usage and transformation, and therefore the origin/source of methane be determined. Whenever microorganisms use methane as an energy or food source, they preferentially take up the light $^{12}$C carbon. This leads to very light carbon isotopic compositions of, for instance, the cellular lipid structure, as can be seen in the process of anaerobic methane oxidation (see Chapter Anaerobic Oxidation of Methane with Sulfate). In contrast, the remaining methane will be enriched in $^{13}$C, leading to the heavy isotopic values of, for instance, methane in water columns or sediments. Fractionation factors—the relation between the isotopic composition of the original methane and the methane after transformation—provide a tool for quantifying the amount of methane that has reacted.

**Methane, Origin, Figure 1** Carbon isotopic composition of various organic and inorganic materials, and recent marine sediments. Note that methane has the widest range of all compounds. Atmospheric methane has a global carbon isotopic value of about $-47\%$ versus VPDB.
Fractionation factors are also important during methane formation. Whiticar et al. (1986) showed that different microbial formation processes, such as carbon dioxide reduction or fermentation, lead to different isotopic compositions of the methane formed. This is related to the bonds that need to be broken during the methane formation process.

Whereas during acetate fermentation (Equation 2) the methyl group stays intact and therefore only a small overall isotopic fractionation occurs, during hydrogenotrophic methanogenesis (Equation 3) the bond between the oxygen and carbon of the carbon dioxide has to be broken, leading to a much bigger isotope effect.

\[
\begin{align*}
\text{CH}_3\text{COOH} & \rightarrow \text{CH}_4 + \text{CO}_2 & (2) \\
\text{CO}_2 + 4\text{H}_2 & \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} & (3)
\end{align*}
\]

The $\delta^{13}$CH$_4$ from different sources/environments covers a fairly wide range due to variations in methanogenic substrates and mechanisms (Tyler, 1991). Additionally, fractionation associated with oxidation leads to a rather wide $\delta^{13}$C range. In general, CH$_4$ from geological or thermogenic sources is isotopically heavier ($-30\%$ to $-50\%$ VPDB) than that from biological sources ($-40\%$ to $-80\%$ VPDB). Also, the CH$_4$ produced by decomposition of either C3 or C4 plant material leads to a distinctive isotopic composition, since the original material is formed via different photosynthesis systems known to fractionate carbon in a different way.

Atmospheric methane has a relatively stable carbon isotopic value globally – around $-47\%$ VPDB (see compilation in Stevens and Wahlen, 2000).

**Relevance as a greenhouse gas**

Methane is the shortest hydrocarbon and the most abundant organic compound in the atmosphere. Due to its ability to absorb on the infrared band, it is a potent greenhouse gas. Taking into account its residence time in the atmosphere (about 10 years), its concentration, and its specific absorbance wavelength, it is about 23 times more potent as a greenhouse gas than CO$_2$ (IPCC, 2001).

Methane mixing ratios in the atmosphere have doubled over the last 200 years, increasing by about 1% per year from 1978 and reaching a value of 1.7 ppm in 1990 (Figure 2a). Since then, the yearly increase has slowed down and values of 1.8 ppm are measured today (Figure 2b).

During the past 15 years, the annual growth rate of tropospheric methane has shown striking changes over 2- to 3-year periods, varying from $+1\%$ year$^{-1}$ to slightly negative values ($-0.2\%$ year$^{-1}$). These fluctuations are superimposed on an overall slowdown of the CH$_4$ growth rate since the 1980s. The general reduction in atmospheric methane growth seems to be related not to a change in source strength, but to source stabilization and the methane budget approaching a steady state (Dlugokencky et al., 2003).

**Methane sinks**

The strongest sink for methane is oxidation by OH radicals in the troposphere (Badr et al., 1992). Approximately, 94% of methane is lost due to oxidation here and in the stratosphere. Due to photochemical reactions, the above-mentioned reaction produces H$_2$O, CO, and CO$_2$, and hence influences concentrations of ozone, hydroxyl radicals, and CO in the troposphere. Another sink term that needs to be taken into account is soils, in which approximately 6% of methane is oxidized by methanotrophic bacteria.

Recently, the focus has been on oxidation processes that take place in aquatic systems. So far, these have not been included in the source/sink budgets, presumably since none of the methane reaches the atmosphere. However, it is mentioned here as it is an important sink that prevents methane from reaching the atmosphere, and therefore from acting as a greenhouse gas. In aquatic systems, two main processes lead to a loss of methane. One is oxidation by methanotrophic bacteria under anoxic conditions in the uppermost sediments and overlying water column (see Chapter Anaerobic Oxidation of Methane with Sulfate). The other possibility, and probably the major pathway, is oxidation by methanotrophic archaebae under anoxic conditions in deeper sediments and water columns lacking oxygen (see Chapter Anaerobic Oxidation of Methane with Sulfate). These two main degradation processes largely restrict the emission or ebullition (transport by bubbles) of methane to the atmosphere from aquatic systems. Normally, 95–99% of the methane found in deeper sediments is oxidized either directly in the sediments or later in the water column and does not, therefore, reach the atmosphere.

**Methane sources**

In general, methane sources can be divided into abiotic (20%) and biogenic sources (80%). Abiotic sources include fossil carbon sources, such as coal mining, industrial waste treatment, and combustion processes (e.g., aircraft and automobile exhausts), as well as sea floor and volcanic emissions. Biomass burning during deforestation and for heating purposes also falls into this category. However, most methane is produced by biological processes: the major sources are freshwater wetlands and rice paddies, but ruminants and the guts of termites are also important contributors.

The total methane budget may also be divided into emissions from natural or anthropogenic sources (Figure 3). The major sources are discussed in detail below. In considering the various contributors, it should always be borne in mind that estimates for individual sources still vary widely, although ongoing research is seeking to constrain the budget (c.f. Table 7.6 in Denman et al., 2007).

**Wetlands**

Wetlands, covering about $5.3 \times 10^{12}$ m$^2$ globally (Matthews and Fung, 1987), are one of the most important
natural sources of atmospheric methane, with an estimated contribution of 115 Tg year\(^{-1}\) (Cicerone and Oremland, 1988). Peat-rich bogs in the northern hemisphere (50–70°N) were mentioned in particular, accounting for 60% of total wetland methane emissions, with tropical and subtropical swamps releasing only 25% of the total (Matthews and Fung, 1987; Fung et al., 1991). The rest was attributed to fens, marshes, and floodplains. However, the high level of emissions from northern wetlands (based on only a few methane emission measurements) was later scaled down, using a process-based methane emission model (Cao et al., 1996). In northern wetlands, according to this publication, CH\(_4\) flux rates per square meter are low (40 mg CH\(_4\) m\(^{-2}\)day\(^{-1}\)), but the area of wetland is large. Therefore, northern wetlands and moist/dry tundra together contribute 25% to global emissions. The second term includes temperate wetlands with a higher mean flux rate (150 mg CH\(_4\) m\(^{-2}\)day\(^{-1}\)) but a much smaller area, leading to a contribution of 19%. Since tropical wetlands – mainly swamps – have an even higher mean daily flux (199 mg CH\(_4\) m\(^{-2}\)), they account for 56% of total wetland emissions despite covering a much smaller area than northern wetlands.

The carbon isotopic composition of methane emitted from wetlands varies from –86‰ to –31‰ VBDP (compilation by Bréas et al., 2001). This rather wide range is, of course, a function of the formation process (methanogenesis by acetate fermentation or CO\(_2\)/H\(_2\))
reduction), the original degradation material forming the substrate for methanogenesis (C3 or C4 plants), and more or less strong methane oxidation, leaving behind isotopically heavy methane.

Termites
Termites feed on all kinds of food, including wood, grass, leaf litter, and agricultural crops. It is very difficult to estimate the amount of methane produced anaerobically in the guts of termites, since it is highly variable between species and colonies. The total amount, mainly produced in tropical savannahs and forests, is estimated at 20 Tg CH₄ year⁻¹. The carbon isotopic composition of methane emitted from termites varies from −73% to −44% VBDP (Tyler et al., 1988). Interestingly, the δ¹³CH₄ of termite-emitted methane is independent of diet.

Rice fields
Rice is cultivated either on dry land, from which no methane is emitted, or – in the case of about 80% of global cultivation – on irrigated fields, leading to high methane emissions. Rice is cultivated between 50°N and 50°S on an area of 1.3 × 10⁶ km², mostly situated in Asia (90%) (Bréas et al., 2001).

Globally, methane emissions from rice fields are second to those from wetlands and represent the highest anthropogenic budget term (100 Tg CH₄ year⁻¹). On rice fields, a methane release rate between 0.4 and 0.8 g m⁻² day⁻¹ has been observed (Holzapfel-Pschorn and Seiler, 1986). Methane in rice fields is produced by methanogens which find an ideal environment in flooded soils, i.e., high organic carbon loads and anoxic conditions. The amount of methane produced has been shown to be influenced by several factors, such as soil properties, amount and type of fertilizer, irrigation, etc. The carbon isotopic composition of methane emitted from rice fields also varies widely due to the same variables as found in wetlands and lies between −50% and −68% VBDP (compilation in Bréas et al., 2001).

Enteric fermentation in animals
Methane is formed in the rumen of animals as a by-product of carbohydrate chain (e.g., cellulose) degradation. During digestion, ingested macromolecules are broken down into smaller molecules by enzymes. Subsequently, the fermentation of, for example, glucose leads to even smaller molecules, such as acetate, butyrate, H₂ and CO₂, etc. The H₂ produced, together with CO₂, is then immediately used by methanogens to form methane.

Global methane emissions mainly from cattle, buffalo, and sheep range between 65 and 100 Tg CH₄ year⁻¹ (Cicerone and Oremland, 1988) and are therefore comparable to rice cultivation and wetland emissions.

Biomass burning
Whereas the burning of biomass mainly produces CO₂, the subsequent smoldering phase – involving compounds with low combustion efficiency – mainly produces methane. About 85% of methane emissions related to biomass burning stem from tropical areas due to deforestation and fuel wood use. It is estimated that biomass burning accounts for 8–10% (i.e., 40–55 Tg CH₄ year⁻¹) of total methane emissions (Cicerone and Oremland, 1988; Hein et al., 1997).

Mining, gas drilling, natural geological sources
During coal mining and gas drilling, methane is produced not by biological processes but from catagenesis of coal or maturation of petroleum and subsequently released into the atmosphere. Cicerone and Oremland (1988) and...
Fung et al. (1991) estimated these emissions to be in the order of 75 Tg CH₄ year⁻¹.

Recently, Kvenvolden and Rogers (2005) estimated the amount of methane emitted from natural geological sources, i.e., not related to petroleum production or mining, to be in the order of 45 Tg CH₄ year⁻¹. Those geological sources include natural macro- and micro-seeps, mud volcanoes, and other miscellaneous sources such as gas hydrates, magmatic volcanoes, geothermal regions, and mid-ocean ridges.

Landfills

Globally, methane emitted from landfills following the breakdown of organic material via methanogenesis is estimated to contribute about 43 Tg CH₄ year⁻¹ (McGinnis et al., 2005).

Oceanic emissions

Methane production in the ocean is found basically in two different environments: (1) in anoxic microenvironments of surface waters, e.g., in fish intestines, plankton samples, marine snow, and fecal pellets and (2) in anoxic sediments due to the breakdown of organic material. The first of these is also known as the “Ocean Methane Paradox,” since methane production would not be possible normally in oxic ocean water and is therefore restricted to these microenvironments. Although only relatively small amounts of methane are formed by this mechanism, leading to supersaturation of surface waters, the huge area of the oceans is responsible for making this term globally significant. Methane produced in sediments might be released into the water column by diffusion or by methane seeps and vents, or it might be locked up in gas hydrates (see below).

Methane emissions from the various kinds of seeps that occur in the oceans – in shelf regions, slopes, or deep ocean locations – are estimated to be between 1 and 65 Tg CH₄ year⁻¹ (see compilation in Judd et al., 2002). The best investigated regions in this respect are the Santa Barbara Basin, where at Coal Oil Point an emission of 28 g CH₄ m⁻² year⁻¹ was estimated (Hornafius et al., 1999), and the Black Sea, where more than 2,000 seeps were detected in the area west of the Crimean Peninsula alone. The yearly atmospheric methane contribution from seeps in the Black Sea is estimated to range between 0.03 and 0.15 Tg (Dimitrov, 2002).

Since most of the methane from seeps or sediments is oxidized by either aerobic or anaerobic processes in the sediments or in the water column, total oceanic emissions today are estimated to be in the order of 10 Tg year⁻¹ (Cicerone and Oremland, 1988; Fung et al., 1991). It has to be taken into account that for methane released into the water column in the form of bubbles, the contribution to the atmosphere may be much higher, depending on the water depth. However, when bubbles travel through the water column, they exchange methane with the ambient water; consequently, most of the gas in the bubbles at the water surface is composed of nitrogen rather than methane (McGinnis et al., 2005).

Gas hydrates

Gas hydrates are nonstoichiometric solid structures composed of ice-like minerals that form at low temperatures and high pressures in the deep sea or at low temperatures in permafrost regions. They can be thought of as a hydrogen-bonded water framework called a host lattice that traps guest molecules (typically gases). In general, three types of gas hydrates can be distinguished based on the structure in which they crystallize. These are structure I, structure II, and structure H (which occurs more rarely). Hydrates contain gases such as hydrocarbons (including methane, hydrogen sulfide, and carbon dioxide) that glue themselves inside symmetrical cages of water molecules to form hydrate crystals (mainly found in type I structures). Smaller molecules, such as argon, krypton, and nitrogen, are found in smaller cages related to structure II.

Gas hydrates or clathrates are known to occur mainly on continental margins all over the world at depths of around 600 – 3,000 m, depending on the prevailing temperature gradient (see Figure 4). Since pipelines of offshore drilling platforms are also located in these depths, clogging of pipes due to the formation of gas hydrates is a well-recognized problem.

Gas hydrates are confined to the top few hundred meters of marine sediments. They are stable when the temperature is at or below the value T₃(P) for a three-phase equilibrium between clathrate, liquid water, and methane gas (Buffett and Archer, 2004). Experimental determinations of T₃(P) are shown in Figure 4 as a function of depth, assuming a hydrostatic increase in pressure. Superimposed on this figure is a schematic illustration of the temperature profile through the ocean and sediments. The zone of stability is limited by the intersection of the local temperature profile with T₃(P), although clathrate is confined to the sediments because the concentration of methane in the ocean is too low to form clathrate and, even if it did form, the buoyancy of clathrate in seawater would carry it out of the stability zone. Clathrate is unlikely to form below water depths of less than 600 m (except in the cold Arctic Ocean) because the bottom water is too warm for stability. As the depth of the water increases, and the temperature of the seafloor decreases, the zone of stability becomes thicker and capable of accommodating larger volumes of clathrate.

It must be understood that gas hydrates are by no means stable: these structures dynamically trap methane from, or release methane into, the ocean. This feature led Hovland and Judd (1988) to conclude that slides and craters on ocean shelves might be related to rapid hydrate decomposition.

Going back in time, gas hydrates have even been claimed to be responsible for larger environmental
catastrophes. Based on light carbon isotopic excursions in the late Paleocene (50 million years ago), Dickens et al. (1995) suggested that methane from gas hydrates might have been released into the atmosphere, leading to significant warming (4°C). One of the main features used to find gas hydrates on ocean margins today is the bottom-simulating reflector, which shows up using seismological methods. This is formed by the base of solid gas hydrate and free gas accumulating below in the sediments.

It has been estimated that the amount of carbon (and therefore, energy) stored in gas hydrates (10,000 Gt C) is at least twice as great as in all known oil, gas, and coal fields combined (Kvenvolden and Lorensen, 2001). This would be equal to 2,000 times the atmospheric methane inventory. However, recent estimates have brought the abundance of gas hydrates down from $1 \times 10^{17}$–$10^{18}$ m$^3$ to $(1–5) \times 10^{15}$ m$^3$, also reducing the energy stored in gas hydrates globally to 500–2,500 Gt C (Milkov et al., 2004). Buffett and Archer (2004) estimated that about 3,000 Gt C occurs in clathrate and 1,800 Gt C in methane bubbles. Estimates of the amount of gas hydrate-related methane actually emitted into the atmosphere reach 5 Tg CH$_4$ year$^{-1}$ (Wuebbles and Hayhoe, 2002).

**Freshwater emissions**

Recently, freshwater environments, including lakes and reservoirs for hydropower production, have become the focus of attention not only for CO$_2$, but also for methane emissions. Lakes kept in a natural state or with only minor influence by humans undergo normal aerobic degradation of organic material and mainly emit CO$_2$. However, when organic material production is high, natural lakes also turn anoxic either in the water column or in the sediments and release methane due to anaerobic degradation, i.e., methanogenesis. This occurs even more rapidly in systems that are highly eutrophicated, e.g., due to intensive agriculture or effluents from sewage treatment plants.

In contrast, reservoirs are normally man-made. By damming rivers, huge areas are flooded and the water is stored for energy production. If the flooded area is a forest, peatland, or grassland, then instead of being a sink for CO$_2$ due to photosynthesis – this area will become a source of greenhouse gases due to anaerobic decomposition of the submerged organic material. For example, in the boreal region of Canada, flooded peatlands represent a worst-case scenario, since they possess a large store of organic carbon held in peat, which can decompose and return to the atmosphere as greenhouse gases over a long period (Kelly et al., 1997). It makes a difference to the emission rate whether an area has only been flooded recently or was flooded decades ago. A newly formed reservoir will emit methane at a much higher rate, since fresh organic material is rapidly degraded. In contrast, reservoirs which are already tens of years old show lower methane emissions, since the older and more refractory organic material is harder to degrade.

Due to recent plans to trade or tax carbon dioxide emissions, “green” hydropower is under debate and it is therefore necessary to define the related CO$_2$ and methane emissions.

In a compilation (Table 1 in St. Louis et al., 2000), methane fluxes from temperate reservoirs varied from 1 to 260 mg m$^{-2}$ day$^{-1}$. Tropical reservoirs showed even higher fluxes, evolving from higher productivities, of 2–3,800 mg m$^{-2}$ day$^{-1}$ (St. Louis et al., 2000). Average fluxes were 20 mg m$^{-2}$ day$^{-1}$ CH$_4$ for temperate and 300 mg m$^{-2}$ day$^{-1}$ CH$_4$ for tropical reservoirs. Taking an area of $0.9 \times 10^6$ km$^2$ for temperate and $0.6 \times 10^6$ km$^2$ for tropical reservoirs, a global flux of 70 Tg can be estimated (St. Louis et al., 2000).

Emissions from natural lake systems have been recently evaluated by Bastviken et al. (2004). In these systems, three different pathways for methane emission are described: (1) ebullition, i.e., emission through bubbles, (2) diffusive flux, which mainly depends on surface methane concentrations and wind speed over surface waters, and (3) storage, i.e., methane released during lake
turnover. On average, these various pathways contribute 62%, 31%, and 7% to total emissions, respectively.

Using different regression equations for surface CH4 concentrations, ebullition, diffusive flux, and storage, together with data on lake area and lake numbers (Kalff, 2002), Bastviken et al. (2004) estimated methane emissions from lakes on a global basis. When compared to global nonanthropogenic emission values from Wuebbles and Hayhoe (2002), the estimated lake emissions of 8–48 Tg CH4 year–1 represent 6–16% or 2–10% of total emissions.

Taking the two systems (natural lakes and reservoirs) together results in global methane emissions between 78 and 118 Tg CH4 year–1. These figures are similar to emissions from wetlands, the largest natural contributor, and much higher than estimated oceanic emissions. Although these estimates might be on the high side, they clearly show that emissions from freshwaters have to be included in global methane budgets.

Summary
Methane is the end product of organic matter degradation under anoxic conditions. In freshwater environments methanogenesis is the main pathway due to the low sulfate concentrations, whereas in the marine environment methanogenesis becomes important only after sulfate, as an electron acceptor, has been used up completely. Methane is formed by microorganisms belonging to the Archaea known as methanogens. Methanogens are able to grow at temperatures between 4°C and 100°C, at pH values from 3 to 9, and at salinities ranging from brines to freshwater. Despite this huge taxonomic diversity, the substrates that can be used by methanogens are rather limited – hydrogen, acetate, formate, some alcohols, and methylated compounds. The two main pathways described in nature are hydrogenotrophic methanogenesis (also known as carbon dioxide/hydrogen reduction) which is mainly found in marine environments and acetate fermentation (acetoclastic methanogenesis) which is more dominant in freshwater environments. These two processes can be distinguished from each other by measurements of the carbon or hydrogen isotopic composition of the formed methane, since the fractionation factors between substrate (CO2 or acetate) and methane vary.

Methane is a potent greenhouse gas, ranking second after carbon dioxide as a contributor to global warming. Its concentration has doubled over the past 200 years, although the increase has slowed down over the past 20 years which is thought to be related to source stabilization and the methane budget approaching a steady state.

The main sources of natural emissions are wetlands, termites, and aquatic systems, making up 30% of total global emissions, while the anthropogenic sources, such as rice paddies, ruminants, energy production, and landfills, account for 70%. Gas hydrates occurring on continental slopes and permafrost regions also store high amounts of methane; however, most of this methane is oxidized to carbon dioxide before it reaches the atmosphere. Recently, lakes and especially reservoirs have been identified to be major contributors to the global methane budget questioning of hydropower as a “green energy” source.

The main sinks for methane are oxidation with OH in the troposphere (accounting for more than 90%), loss in the stratosphere, and oxidation by methanotrophs in soils.

Bibliography


Cross-references

An aerobic Oxidation of Methane with Sulfate
Archaea
Bacteria
Cap Carbonates
Carbonates
Cold Seeps
Methane Oxidation (Aerobic)

METHANOGENS

Please refer to “Methane, Origin” and “Archaea.”

MICROBIAL BIOMINERALIZATION

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Synonyms

Microbial biomineral formation

Definition

Microbial biomineralization describes the formation and deposition of minerals directly mediated or indirectly influenced by microorganisms (Mann, 2001; Weiner and Dove, 2003; Ehrlich, 1999). A huge variety of minerals results from individual biomineralization pathways linked to the phylogeny and metabolic activity of the microorganisms involved (Weiner and Dove, 2003;
Moreover, microbial biominerals may differ distinctly from their inorganically formed equivalents in shape, size, crystallinity, isotopic, and trace element composition (Bazylinski et al., 2007; Haferburg and Kothe, 2007; Takahashi et al., 2007; Weiner and Dove, 2003). A compilation of microbial biominerals and their source organisms is given in Table 1.

Two principal modes of microbial biomineralization processes occur, namely biologically induced mineralization (BIM) and biologically controlled mineralization (BCM, Lowenstam, 1981; Lowenstam and Weiner, 1989). These modes are introduced in the following.

### Biologically induced mineralization (BIM)

In BIM, the nucleation and growth of biominerals are extracellular processes triggered by the metabolic activity of the microorganism. Biomineralization takes place because of the changes in the chemical equilibrium of the surrounding environment and may also be linked to

### Microbial Biomineralization, Table 1

Overview of minerals formed by microbial biomineralization and the organism(s) involved

<table>
<thead>
<tr>
<th>Chemical formula</th>
<th>Mineral name</th>
<th>Involved microorganism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(OH)₃ (approx.)</td>
<td>Ferric/iron oxyhydroxide</td>
<td>Fe-oxidizing bacteria</td>
<td>Chan et al. (2009) and Yoshida et al. (2008)</td>
</tr>
<tr>
<td>2Fe(OH)₃ · Fe(OH)₂</td>
<td>(approx.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-FeOOH(OH)</td>
<td>Goethite</td>
<td>Gallionella ferruginea</td>
<td>Kukkadapu et al. (2004) and O’Loughlin (2008)</td>
</tr>
<tr>
<td>γ-FeOOH(OH)</td>
<td>Lepidocrocite</td>
<td>Marine bacteriophage, Bacillus subtilis</td>
<td>Daughney et al. (2004) and Châtellier et al. (2001)</td>
</tr>
<tr>
<td>5Fe₂O₃ · 9H₂O</td>
<td>Ferrihydrite</td>
<td>Gallionella ferruginea, Leptothrix ochracea, Bacillus subtilis</td>
<td>Hallberg and Ferris (2004) and Kennedy et al. (2004)</td>
</tr>
<tr>
<td>Fe₃O₄</td>
<td>Hematite</td>
<td>Gallionella ferruginea</td>
<td>Hallberg and Ferris (2004) and Kennedy et al. (2004)</td>
</tr>
<tr>
<td>γ-Fe₂O₃</td>
<td>Maghemite</td>
<td>thermophilic iron-reducing bacteria</td>
<td>Zhang et al. (1997) and Bharde et al. (2008)</td>
</tr>
<tr>
<td>FeCO₃</td>
<td>Siderite</td>
<td>Shewanella alga, thermophilic iron-reducing bacteria</td>
<td>Zhang et al. (1997) and Parmar et al. (2000)</td>
</tr>
<tr>
<td>FePO₄ · nH₂O</td>
<td>Hydrous ferric phosphate</td>
<td>Acidovorax sp.</td>
<td>Miot et al. (2009)</td>
</tr>
<tr>
<td>Fe₃(PO₄)₂ · 2H₂O</td>
<td>Vivianite</td>
<td>Shewanella putrefaciens, Desulfovibrio alaskensis (SRB), Alkaliphilus metaliredigens</td>
<td>Kukkadapu et al. (2004), Zegeye et al. (2007), and Roh et al. (2007)</td>
</tr>
<tr>
<td>FeS</td>
<td>Cubic FeS (Sphalerite-type)</td>
<td>Magnetotactic bacteria</td>
<td>Pósfai et al. (1998a, b)</td>
</tr>
<tr>
<td>FeS</td>
<td>Mackinawite (tetragonal FeS)</td>
<td>Magnetotactic bacteria, Desulfovibrio desulfuricans</td>
<td>Pósfai et al. (1998a, b) and Ivarson and Hallberg (1976)</td>
</tr>
<tr>
<td>Fe₃S₄</td>
<td>Greigite</td>
<td>Magnetotactic bacteria, Actinobacter sp., SRB</td>
<td>Bharde et al. (2008), Farina et al. (1990), Mann et al. (1990), Heywood et al. (1990), Reitner et al. (2005), and Faivre and Schüller (2008)</td>
</tr>
<tr>
<td>Fe₁₋ₓS</td>
<td>Pyrrhotite</td>
<td>Magnetotactic bacteria</td>
<td>Farina et al. (1990)</td>
</tr>
<tr>
<td>FeS₂</td>
<td>Pyrite</td>
<td>Magnetotactic bacteria, SRB</td>
<td>Mann et al. (1990), Bazylinski (1996), Folk (2005), Wilkin and Barnes (1997), and Donald and Southam (1999)</td>
</tr>
<tr>
<td>KFe₃(SO₄)₂(OH)₆</td>
<td>Jarosite</td>
<td>Sulfitobacillus thermosulfidooxidans, Acidithiobacillus ferronoxidans, Thiobacillus ferronoxidans</td>
<td>Egal et al. (2009)</td>
</tr>
<tr>
<td>Fe₈O₆S₄(OH)₆</td>
<td>Schwertmanite</td>
<td>Acidithiobacillus ferronoxidans</td>
<td>Zhang et al. (2002)</td>
</tr>
<tr>
<td>MnCO₃</td>
<td>Rhodochrosite</td>
<td>Leptothrix discophora</td>
<td>Tebo et al. (2004), Villalobos et al. (2003), and Brouwers et al. (2000)</td>
</tr>
<tr>
<td>Mn₂O₃</td>
<td>Manganese oxides</td>
<td>Pseudomonas putida, Leptothrix discophora, Bacillus sp.</td>
<td>Villalobos et al. (2003)</td>
</tr>
</tbody>
</table>

MICROBIAL BIOMINERALIZATION 587
particular metabolic products. The resulting biominerals typically show a poor crystallinity, are chemically heterogeneous, and often closely associated with the cell wall (Frankel and Bazylinski, 2003). An active and a passive mineralization process can be distinguished (Fortin and Beveridge, 2000; Southam, 2000). Active mineralization refers to mineralization by (a) the direct redox conversion of specific metal ions bound to the bacterial surface or (b) the excretion of metabolically produced ions and thereby forming minerals. The term passive mineralization is used when nonspecific binding of cations and the involvement of surrounding anions causes nucleation and growth of minerals. Passive mineralization can even be mediated by dead cells, due to the exposure of negatively charged surfaces acting as nucleation sites for metal cations (Urrutia and Beveridge, 1993). Particularly in BIM, extracellular polymeric substances (EPS) are involved in the mineralization process (Chan et al., 2009; Ercole et al., 2007). As an example of BIM, the precipitation of iron oxyhydroxides by the iron oxidizing bacterium Gallionella ferruginea is displayed in Figure 1.

**Biologically controlled mineralization (BCM)**

BCM implies that the organism actively controls the nucleation site, growth, morphology, and final location of the mineral (Banfield and Nealson, 1997; Bazylinski, 2003). An active and a passive mineralization process can be distinguished (Fortin and Beveridge, 2000; Southam, 2000). Active mineralization refers to mineralization by (a) the direct redox conversion of specific metal ions bound to the bacterial surface or (b) the excretion of metabolically produced ions and thereby forming minerals. The term passive mineralization is used when nonspecific binding of cations and the involvement of surrounding anions causes nucleation and growth of minerals. Passive mineralization can even be mediated by dead cells, due to the exposure of negatively charged surfaces acting as nucleation sites for metal cations (Urrutia and Beveridge, 1993). Particularly in BIM, extracellular polymeric substances (EPS) are involved in the mineralization process (Chan et al., 2009; Ercole et al., 2007). As an example of BIM, the precipitation of iron oxyhydroxides by the iron oxidizing bacterium Gallionella ferruginea is displayed in Figure 1.

**Microbial Biomineralization, Table 1 (Continued)**

<table>
<thead>
<tr>
<th>Chemical formula</th>
<th>Mineral name</th>
<th>Involved microorganism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au&lt;sup&gt;0&lt;/sup&gt;</td>
<td>Elemental gold</td>
<td>Bacillus sp., Rhodopseudomonas capsulata, Shewanella algae, SRB</td>
<td>Reith et al. (2009), He et al. (2007), Konishi et al. (2007), Konishi et al. (2006), and Lengke and Southam (2006)</td>
</tr>
<tr>
<td>CaCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Calcite</td>
<td>Communities of SRB and archaea, cyanobacteria, soil bacteria (Bacillus megaterium), Algae (e.g., Halimeda, Emiliania huxleyi)</td>
<td>Boetius et al. (2000), Reitner et al. (2005), Thompson and Ferris (1990), Lian et al. (2006), and de Vrind-de Jong and de Vrind (1997)</td>
</tr>
<tr>
<td>Aragonite</td>
<td>Cyanobacteria (Synechococcus leopoliensis), Nesterenkonia halobia, Halomonas eurithalina</td>
<td>Obst et al. (2009) and Rivadeneyra et al. (1998, 2000)</td>
<td></td>
</tr>
<tr>
<td>Vaterite</td>
<td>Nesterenkonia halobia</td>
<td>Kocuria, Myxococcus Xanthus, Bacillus sphaericus</td>
<td>Zamarreño et al. (2009), and Rodriguez-Navarro et al. (2007)</td>
</tr>
<tr>
<td>CaMg(CO&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Dolomite</td>
<td>Nesterenkonia halobia</td>
<td>Rivadeneyra et al. (2000)</td>
</tr>
<tr>
<td>SiO&lt;sub&gt;2&lt;/sub&gt; • nH&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>Amorphous silica</td>
<td>Calothrix, Fischerella sp., Shewanella oneidensis</td>
<td>Benning et al. (2004), Konhauser et al. (2001), and Furukawa and O’Reilly (2007)</td>
</tr>
<tr>
<td>SiO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Silica</td>
<td>Diatoms, radiolarians, Thiobacillus, Bacillus subtilis</td>
<td>de Vrind-de Jong and de Vrind (1997), Fortin and Beveridge (1997), and Urrutia and Beveridge (1993)</td>
</tr>
<tr>
<td>Ca&lt;sub&gt;3&lt;/sub&gt;(PO&lt;sub&gt;4&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;(OH)</td>
<td>Hydroxyapatite/calcium phosphate</td>
<td>Ramlibacter tataouinensis, Corynebacterium matrachotii, Streptococcus mutans, Streptococcus sanguis</td>
<td>Benzerara et al. (2004), Van Dijk et al. (1998), and Streckfuss et al. (1974)</td>
</tr>
<tr>
<td>MgNH&lt;sub&gt;4&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt; • 6H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>Struvite</td>
<td>Myxococcus xanthus, Pseudomonas, Flavobacterium, Acinetobacter, Yersinia, Corynebacterium, Azobacter</td>
<td>Da Silva et al. (2000) and Rivadeneyra et al. (1983)</td>
</tr>
</tbody>
</table>

For comprehensive reading, the following books are recommended: Sigel et al. (2008), Baeuerlein (2000), Banfield and Nealson (1997), Dove et al. (2003), Driessens and Verbeeck (1990), Lovely (2000), Lowenstam and Weiner (1989).
and Frankel, 2003). Although the modes of exerting crystallochemical control over the mineralization process may greatly vary across species, the common characteristic of BCM is that mineral formation takes place in a closed, isolated environment. While Weiner and Dove (2003) categorized BCM in extra-, inter- or intracellular mineralization, Mann (2001) differentiated between two key modes of BCM, namely matrix-mediated mineralization and boundary-organized mineralization. Generally speaking, extracellular mineralization corresponds to matrix-mediated mineralization, whereas inter- and intracellular mineral formation are equivalent to boundary-organized mineralization.

**Extracellular** BCM implies the production of a macromolecular matrix outside the cell. This matrix is typically composed of proteins, polysaccharides, or glycoproteins, forming a three dimensional framework, and the cell actively supplies cations to the matrix for an “on-site” nucleation and growth of the biomineral (Weiner and Dove, 2003).

**Intracellular** BCM takes place inside specific compartments within the cell, for instance vesicles or vacuoles. Thus, the organism is able to exactly regulate the chemical composition, morphological structure, and particle size of the mineral. The only bacteria known to perform intracellular BCM are magnetotactic bacteria, which use a vacuole-based system for the crystallization of magnetic biominerals (Table 1). Similar systems of intracellular BCM are only known from higher eukaryotic organisms, controlling for example, the bone and teeth formation in mammals (Kirschvink and Hagadorn, 2000). An example of microbial biominerals formed by intracellular BCM is shown in Figure 2.

### Bibliography


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**Microbial Biomineralization, Figure 2** Intracellular iron sulfide (greigite, Fe$_3$S$_4$) crystals formed by biologically controlled mineralization (BCM) within a magnetotactic bacterium. (Image courtesy of Joachim Reitner.)
Microbial diversity

Habitat

Microbial population

Microbial community

Guild

The composition of a microbial community and the abundance of its members.

Microbial diversity

The number of different species in a habitat; sometimes (falsely) used as a synonym for community structure.

Habitat

The physical location or dwelling place of a particular organism, where an individual microbial ectype can be found or isolated from.

Ecological niche

Different from the habitat (spatial), the niche defines the function of an organism.

Introduction

Microorganisms are an indispensable part of the biogeochemical cycles on Earth, have the longest history in evolution, and represent the largest genetic reservoir. Mostly invisible to the naked eye, microbes represent the largest source of biomass on Earth, with an estimated number of 4–6 × 10^{30} cells, and 350–550 Pg of C (1 Pg = 10^{15} g), and have colonized almost any niche available on Earth (Whitman et al., 1998). In their habitats, microorganisms usually do not exist as single, genetically identical populations, but live together with other microbial populations as microbial communities carrying out important functions of an ecosystem such as primary production and remineralization of biomass.

A hallmark of microbial life is its diversity. For a long time, only little was known about the magnitude of microbial diversity, as this was based on cultivation. Only about ~7,000 noneukaryotic species have been formally described (Euzeby, 1997) (http://www.bacterio.ict.fr/number.html), and insects were regarded as much more diverse than microorganisms. In microbiology, the term “diversity” is often used to refer to the number of different species in a given habitat, or, when based on molecular data, refers to the number of different sequences present. Diversity reflects the types of microbes present in a habitat, but “community structure” encompasses quantitative information on the different taxa and guilds (Liesack et al., 1997). Eventually, the analysis of community structure will allow for assessing the role and importance of microbes in their habitats and ecosystems. Presently, it is still methodologically difficult to obtain a complete view of microbial diversity and community structure.
fraction of microorganisms grows on standard media, and in some environments this has been estimated to be as low as 0.001–0.1%, e.g., in seawater (Amann et al., 1995).

**Molecular approaches:** The limitations of cultivation-based analysis to describe the diversity of microorganisms adequately were overcome by applying methods of molecular biology. Today, microorganisms are routinely classified by using the RNA of the small ribosomal subunit (small subunit ribosomal RNA – SSU rRNA), or its coding gene (SSU rRNA gene) as a molecular marker. In the 1970s, Carl Woese and coworkers used the sequences of the SSU rRNA to establish a phylogenetic framework for all life, from which the concept of the three domains of life – prokaryotes, archaea, and eukaryotes – emerged (Woese and Fox, 1977; Woese, 1987). Molecular classification of microorganisms was further developed by Norman Pace and his group to cultivation independent analysis of microbial communities in environmental samples by cloning the 16S rRNA gene directly from biomass (Pace et al., 1986). The basic idea is simple: rRNA sequences can be used as identifiers of microorganisms even if they were previously unknown; the sequence allows to establish an evolutionary relationship to other sequences of microorganisms in the databases. Directly extracting DNA from biomass, followed by cloning and sequencing of the (SSU rRNA) sequences of interest, facilitates to examine any environmental habitat. In August 2010, 418, 497 16S rRNA sequences were held in the dedicated database Ribosomal database project II (RDP; http://rdp.cme.msu.edu/), indicating how successful this approach has been. As the number of different microbial species can be very high in certain habitats, computational approaches have been developed to estimate diversity coverage of clone libraries (e.g., DOTUR) (Schloss and Handelsman, 2005). Cloning and sequencing approaches give an overview of microbial species diversity, but this approach is PCR based and therefore, may not adequately reflect the abundance of community members (von Wintzingerode et al., 1997). Analysis of community structure requires quantification of individual groups of microorganisms. Quantitative dot blot hybridization and fluorescent in situ hybridization (see Chapter Fluorescence In Situ Hybridization (FISH)), techniques are being used to determine abundance of uncultured prokaryotes using oligonucleotide probes directed against the SSU rRNA (Amann et al., 1995). Specific probes have been designed using SSU rDNA sequences obtained from the habitat studied and public databases; probeBase is an extensive repository of published rDNA-targeting oligonucleotide probe sequences available to the public (http://www.microbial-ecology.de/probebase/).

**How diverse is diversity?**

**Microbial diversity in natural environments:** By applying molecular techniques such as DNA reassociation kinetics, it was estimated that 30 g of soil might contain approximately 4,000 different microbial genomes, possibly representing as many as 13,000 different microbial species (Torsvik et al., 1990). Ever since, soils have been regarded as one of the habitats with the most diverse microbial communities; however, a recent comparative study suggests a higher phylogenetic diversity in sediments possibly linked to salinity as major determinant (Lozupone and Knight, 2007). Estimates from PCR-based 16S rRNA gene sequencing suggested up to 100,000 species per gram of soil (Schloss and Handelsman, 2006). However, these estimates were based on relatively low numbers of sequences (up to 1,000). A new DNA sequencing technology, massively parallel pyrosequencing (Margulies et al., 2005), has allowed to sample as many as 53,000 16S rRNA sequences from one soil sample (Roesch et al., 2007); estimates of diversity based on these sequences suggested that the number of unique sequences never exceeded 52,000.

**Function of microbial communities**

Ecosystems are individually functioning units including primary producers, consumers, and mineralizing organisms. Microbial communities are the most numerous organisms in any ecosystem, and they control the annual primary production including the recycling of carbon, sulfur, nitrogen, and iron. In a lake ecosystem for instance, photic zone, water column, and sediment harbor functionally different microbial communities. Light energy drives the primary production in the photic zone by a community of phototrophic microorganisms. Photosynthetically fixed carbon and carbon from other sources move through the ecosystem and fuel the community of pelagic chemoorganotrophic aerobes (or anaerobes) in the water column. Eventually, part of the carbon sinks down to the sediment, where a specifically adapted community consisting of several guilds of microorganisms degrades the organic matter in a concerted interdependent manner, resulting in the flow of carbon through the anaerobic microbial food chain (see Chapter Carbon (Organic, Degradation)). Several different guilds are involved in the degradation of polymers including polymer hydrolyzing, fermenting bacteria, fatty acid fermenting syntrophs, homoacteogens, and methanogens (Schink and Stams, 2006). As oxygen diffusion into the sediment is severely limited, often microoxic to anoxic conditions occur within a few millimeters depth of the sediment surface because of microbial respiration activity in the very top layer. Concomitantly, anaerobically respiring microorganisms oxidize carbon compounds and transfer electrons to inorganic electron acceptors such as nitrogen oxides, manganese oxides, ferric iron, and sulfate in a sequence that reflects the thermodynamic theory in many environments (Conrad, 1996). Once these electron acceptors are depleted, methanogenesis becomes the terminal electron accepting process, which is typically found in freshwater sediments rich in organic matter, in swamps, or in flooded soils such as rice paddies (Schink and Stams, 2006). The function of the major guilds involved in these processes is known in...
principle, mainly because representative microorganisms have been identified by enrichment and isolation techniques. The numerical importance as well as the actual physiological function of such guild representatives in the vast diversity of habitats is still elusive. Although 16S rRNA-based surveys with varying degrees of diversity sampling depths exist for a large number of habitats, mostly, sequences and derived phylogenetic identity alone do not reveal the physiological role these microorganisms have. The SSU rRNA is a very valuable phylogenetic marker; however, most guilds are not monophyletic, i.e., not all microorganisms falling into a coherent phylogenetic cluster have the same physiology. For example, within the Proteobacteria phototrophy is widespread, and thus, a close relative of a phototroph can be a nonphototrophic microorganism. Assignment of physiological characteristics based on close SSU rRNA relationship requires careful evaluation and often is not possible at all. The use of marker genes coding for key enzymes has allowed to target phylogenetically diverse but physiologically coherent guilds of microorganisms (Teske et al., 2003). For example, anaerobic respiratory processes carried out by methanogenic archaea (Friedrich, 2005) or sulphate-reducing (Wagner et al., 2005) prokaryotes can be traced in the environment by targeting the respective key genes.

Linking identity and function to microbial populations
Our understanding of microbial community functioning, mostly, is of principal nature for many ecosystems. Even for numerically abundant microbial species identified by molecular techniques, their ecophysiology and role in ecosystem functioning remain elusive. A major goal in current microbial ecology research is therefore to understand what uncultivated microorganisms do in their habitats. What are their metabolic properties, and which microbial species are responsible for defined processes in their habitat? An elegant concept for the elucidation of the ecophysiology of uncultivated microorganisms relies on the incorporation of isotopically labeled substrates into biomass and the subsequent identification of the microorganisms actively incorporating the label. This approach enables to link metabolic activity to active microorganisms by measuring label incorporation into biomarkers or even whole cells.

Microautoradiography in combination with FISH (MAR-FISH) can visualize cells actively incorporating radioactively labeled substrates in their natural habitats permitted that samples are suitable for FISH (Lee et al., 1999). Alternatively, oligonucleotide probe array technology detects radiolabel incorporation into RNA extracted from environmental samples (Adamczyk et al., 2003). The use of stable isotopes (13C, 15N, 18O) for labeling active microbial populations has led to a whole suite of approaches that rely on tracing the incorporation of labeled substrates into cell components such as lipids (Boschker et al., 1998), DNA (Radajelewski et al., 2000), RNA (Manefield et al., 2002), or even whole cells (Lechene et al., 2006). Tracing 13C incorporation into lipids is highly sensitive; however, phospholipids lack phyllogenetic resolution and require pure cultures for assigning individual lipids to species. Stable isotope probing (SIP) of nucleic acids represents a direct way to identify microbial populations active in a defined metabolic process. SIP is based on the incorporation of labeled substrate into the cellular biomarker nucleic acids, separation of labeled nucleic acids from unlabeled nucleic acids by density gradient centrifugation, and molecular identification of active populations carrying labeled DNA or RNA. By now, SIP has been used to identify the active players involved in carbon and nitrogen cycling, as well in bioremediation in a large variety of different habitats and has been extended even to metagenomic analysis of labeled DNA (for a recent overview see (Neufeld et al., 2007)).

Whereas the original SIP is based on physical separation of labeled nucleic acid from unlabeled nucleic acid, newer approaches target single cells. Raman confocal microscopy detects shifts in vibrational energy of chemical bonds induced by 13C labeling of cellular single microbial cells. In combination with FISH, Raman microscopy enables identification of 13C labeled population of active cells (Huang et al., 2007). A conceptually similar approach uses secondary ion mass spectrometry (SIMS) operating at lateral resolution of now down to 33 nm. NanoSIMS has high sensitivity allowing to detect shifts in isotopic signals at per mill rather than the atomic percent range (standard SIP and Raman SIP) and high isotope measurement precision (Lechene et al., 2006). Recently, it has been demonstrated that NanoSIMS analyses can be combined with 16S rRNA targeting oligonucleotide probes by employing halogen atoms as element label allowing in parallel phylogenetic identification and detection of metabolic activity of individual cells (Li et al., 2008).

Conclusions
Molecular biology based approaches have advanced our understanding of microbial diversity enormously over the last 20 years. However, we are just at the beginning of a new era in surveying microbial diversity: we will be witnessing the rapid accumulation of staggering amounts of sequence data not only for the single phylogenetic marker 16S rRNA, but also for whole metagenomes fueled by parallel DNA sequencing technology. New developments in linking phylogenetic identity to function, including renewed efforts in cultivation, will boost our understanding of the ecophysiology of the uncultivated vast majority of microorganisms. Eventually, this ongoing revolution of microbial ecology will allow to test general ecological theory using microbial systems (Prosser et al., 2007).
Bibliography


Cross-references

**Acetogens**

**Anaerobic Transformation Processes, Microbiology**

**Archaea**

**Bacteria**

**Carbon (Organic, Cycling)**

**Carbon (Organic, Degradation)**

**Chemolithotrophy**

**Fe(III)-Reducing Prokaryotes**

**Fluorescence In Situ Hybridization (FISH)**

**Metagenomics**

**Methanogens**

**Photosynthesis**

**Sulfate-Reducing Bacteria**

**Sulfur Cycle**
MICROBIAL DEGRADATION

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Synonyms
Mineralization; Nutrient cycling

Definition
Microbial. Processes driven by microorganisms.
Degradation. Use of chemical substances as carbon and energy source for metabolism, thereby breaking down larger molecules to smaller ones.

Introduction
Microorganisms are the basis for the process of mineralization by utilizing organic molecules for their catabolism, thereby returning the elements to the geobiological cycles. In catabolism, or energy conservation, energy-rich adenosine triphosphate (ATP) is formed (for general reading on microbial physiology: Madigan and Martinko, 2006). This is the basis for metabolic degradation performed by microorganisms in a great diversity. The degradation of organic material in terrestrial, limnological, and marine environments is thus based on the growth of microorganisms on organic compounds, which is termed chemoheterotrophic growth style, depending on the availability of organic nutrients for growth. The processes can be dependent on aerobic respiration, but many degradative processes, e.g., in sediments, are performed in the absence of oxygen under anaerobic conditions (Schink, 2002).

Aerobic and anaerobic respiration

The respiration with oxygen as the terminal electron acceptor is the process yielding the most ATP per mol substance oxidized and thus, aerobic respiration is the preferred route for degradation whenever enough oxygen is available. Under aerobic conditions, the degradation of organic matter is performed following general and ubiquitous pathways such as glycolysis; the pyruvate produced in the glycolytic chain enters the citrate cycle; and the reduction equivalents produced are then reoxidized by the respiratory chain coupled to the reduction of oxygen to water. All building blocks of living cells can be degraded that way, allowing growth of microorganisms. The carbon moieties are released as CO₂ into the atmosphere.

Under anaerobic conditions, anaerobic respiratory chains using alternative electron acceptors can ensue. For example, nitrate, nitrite, sulfate, sulfite, sulfur, iron, manganese or fumarate, or carbon dioxide in methanogenesis is then used to accept the electrons, thereby reducing these substances. Halogenated compounds can serve as electron acceptors in dehalorespiration.

All these respiratory chains depend on membrane-bound electron transport, and the reactions of electron transport are coupled to proton export across the cellular membrane to form a proton motive force. The proton motive force is then used to drive ATP formation via the enzyme ATP synthase, which can form 1 mol of ATP per 3 mol of protons carried back into the cytoplasm. The electrons for electron transport chains are derived from oxidation of the organic molecules of the nutrient source. If possible, the organic matter is fully oxidized to carbon dioxide. In this way, the organic matter is fully decomposed.

Fermentation

Another route, independent of respiratory chains, is possible for the fermentative degradation of organic matter. The fermentations performed by microorganisms can use oxidation of substrates to produce ATP and then reduce organic molecules for reoxidation of the reducing equivalents. While ATP yield in these systems is limited when compared to respiration, it still allows growth under conditions when no inorganic electron acceptor is available in sufficient amounts, or when iron as part of the electron carriers in the respiratory chain is not available and therefore, an electron transport chain cannot be synthetized.

In contrast to respiration, both aerobic and anaerobic, during fermentation the organic substrate is not fully oxidized to CO₂. Rather, energy conservation is dependent on the oxidation of an organic molecule in which an aldehyde may be formed as intermediate, which subsequently can be oxidized to form an acid. This exergonic reaction is coupled to the activation of an anorganic phosphate moiety which then can be transferred to ADP, thereby forming ATP. This process is called substrate-level phosphorylation in contrast to the electron transport phosphorylation occurring in respiratory chains. The oxidative reaction releases reduction equivalents, which cannot be transferred to an inorganic electron acceptor due to the lack of respiratory chains in fermenting microbes. Thus, the reduction of an organic compound is necessary to release the carrier for a new cycle of oxidation. The reduced organic substance is released into the environment. An alternative is to release the reduction equivalents as H₂.

The fermentation of sugars, which are present in large amounts due to photosynthesis products and constituents of plant cell wall material, can be performed using the Embden–Meyerhof pathway of glycolysis, the Entner–Doudoroff pathway, or modifications or parts thereof. Sugars with 5 C moieties, sugar acids, or carbonic acids can be converted by different pathways. Examples for sugar fermentation are homo- or hetero-fermentative lactic acid fermentation, alcoholic fermentation, propionate fermentation, butyrate or butanole fermentation, mixed acid fermentation, solvent fermentation, and many others. The names of the pathways indicate the products released in these different fermentative pathways.

Many bacteria can switch between fermentative and respiratory metabolism, depending on the availability of...
electron acceptors for respiratory chains. If they can switch between anaerobic and aerobic metabolism, they are called facultative aerobes, while anaerobes are usually oxygen sensitive and grow exclusively under anaerobic conditions. Within this group, the Gram-positive Clostridia have evolved many fermentation pathways including those to degrade fatty acids, lipids, amino acids and proteins, or nucleic acids under anaerobic conditions. Thus, degradation of all constituents of the cytosol of living organisms under anaerobic conditions can be achieved.

As no oxygen is necessary for the fermentative metabolism, most fermentations will proceed under anoxic conditions. However, aerotolerant microorganisms such as some lactic acid bacteria that are active in decomposition of plant litter can perform this reaction also under oxic conditions, although these anaerobes do not use oxygen as electron acceptor.

**Syntrophic degradation**
The reduced compounds released by fermenting microorganisms constitute good nutritional sources for organisms that can use respiratory systems. In turn, the use of the product will allow for the continuous production of this compound, as no restrictions for the fermenting bacteria will inhibit their metabolism. The concomitant occurrence of bacteria in an ecological niche in which both partners are dependent on each other and degradation of the substrate is only possible when both partners are present is termed syntrophy.

The basis is the metabolic activity of one organism which forms a substrate for the other. This constant removal of a metabolic end product is exemplified by the release of H₂ which is often a link in such syntrophic systems. Use of hydrogen by a second organism allows the first organism to constantly produce this compound. In pure culture, the partial pressure of H₂ reaches levels inhibitory to the metabolism of the producing organism which subsequently ceases growth. Since the syntrophic partner constantly removes hydrogen, no cessation of growth is observed due to product inhibition in this coculture. This syntrophy, depending on hydrogen transfer between two microbes present in the same environment, is of special importance, and the term interspecies hydrogen transfer has been coined for this syntrophic relationship. However, many different examples can be given for such syntrophic associations of microorganisms (e.g., Ueda and Beppu, 2007). Degradation of recalcitrant substrates, including, e.g., halogenated compounds, is often seen in syntrophic associations.

**Cometabolic degradation**
Recalcitrant substrates are not, or very slowly, degraded because their conversion is not easily or not at all coupled with energy conservation. Thus, the degradation of these compounds is costly for the cell, and the microorganisms do not profit directly from degradation. Instead, the bacteria have to use energy from a different, catabolic reaction to drive the unfavorable degradation process.

An example of immediate importance is lignin degradation, which is performed by white rot fungi. The brown rot fungi degrade lignin mainly in order to get access to the cellulose of plant cell wall which is a good nutrient source but not directly accessible in wood and timber. The recalcitrant nature of lignin is seen under anoxic conditions, where the degradation is not possible and lignin of plant origin is thus the basis for coal formation.

As cometabolic systems are costly, they are mostly linked to respiration. Degradation of plant alcaloids, which are also recalcitrant substrates, often occurs at higher rates in oxic conditions.

**Degradation of plant litter and soil formation**
Plant material is composed of the contents of the living cells and the cell wall materials. Within the cell, secondary plant metabolites are stored in the vacuole. Upon leaf fall or death of a plant, especially the secondary metabolites and the wall materials persist, while proteins and lipids are easily degradable in the unsaturated oxygen containing upper soil layers.

Plant cell walls are composed of cellulose, hemicellulose, pectin, polygalacturonans, and lignin. Even in herbaceous plants, a quarter of the secondary cell wall is lignin. This macromolecule can be degraded aerobically, while anoxic conditions lead to the formation of coal, since under these conditions lignin cannot be degraded. Wood rotting fungi, both brown rot and white rot fungi, are able to degrade lignin (Crawford, 1981). The enzymes involved in lignin degradation, peroxidases and laccases, are able to attack the bonds in the macromolecule linking the phenolic monomers to form the high molecular weight lignin. The monomers can then be degraded further and are metabolized to carbon dioxide. These processes have been studied in some detail at the molecular level (Gold and Alic, 1993). Whether bacteria are also able to degrade wood is still under discussion. Streptomycetes are able to perform some partial degradation. However, complete decomposition has yet to be shown with bacterial systems.

An interesting question in wood and lignin degradation is regarding the possible symbiosis of wood degrading fungi and bacterial populations at an oxic/anoxic border. Anaerobic bacteria able to convert lignin decomposition products and partially degrade these compounds have been described. The fermentation products of these organisms may serve as growth substrates for the lignin degrading fungi. Secondary metabolites of plants and microorganisms are often recalcitrant because they contain aromatic rings, heterocycles, and polyketides or are halogenated. Such compounds are not easily degraded, especially under anoxic conditions. If degradation does not lead to complete oxidation of carbon moieties to CO₂, the formation of humic acids and fulvic acids and thus the production of humus ensues. This process is the driving force for soil genesis. Under aerobic conditions,
enzymatic reactions known from lignin oxidation are often involved (Bumpus and Aust, 1987; Hammel, 1989).

Degradation of aromatic and halogenated compounds

Under aerobic conditions, the chemically very stable aromatic ring can be broken by activities of mono- and di-oxygenases. While monooxygenases introduce one of the oxygen atoms of molecular oxygen into the organic molecule forming a hydroxyl group at one site of the ring, dioxygenases incorporate both oxygen atoms which leads to decyclization and ring opening. The linear compound can then be further oxidized after activation and fueled into general metabolic pathways. The aerobic degradation of aromatic compounds can be used to clean oil spills or other anthropogenic contaminations, especially applicable for the residual contamination after a first mechanical cleansing has been performed. Inoculation of a beach for the residual contamination after a first mechanical cleansing could show visible removal of the remaining oil within 1 year.

Under anoxic conditions, the ring opening seems more difficult and it had long been argued that this would not be possible. However, bacterial enrichment cultures could show that an anaerobic degradation of aromatic compounds is possible usually via activation and conversion to benzoyl CoA followed by ring reduction. These energetically unfavorable reactions are feasible with a thermodynamically favorable electron acceptor, e.g., when nitrate is available in an anaerobic respiratory chain. Thus, bacteria related to the nitrogen fixing genus Azoarcus link the degradation of aromatic compounds with denitrification. Many gene clusters with different substrate specificities have been identified to perform anaerobic degradation of different organic molecules including aromatic amino acids, benzene and its derivatives, phenols, and heterocyclic compounds. The linearized structures again can be fueled into metabolic pathways via acetyl CoA or propionyl CoA. Anthropogenic aromatic compounds can be degraded by these same processes, thus allowing complete decomposition and natural attenuation. The introduction of bacteria able to anaerobically degrade aromatic compounds into aquifers with (anthropogenic) contaminations opens the road to bioremediation of contaminated groundwater resources. In tertiary oil recovery, introduction of bacteria into the well to partially degrade the high molecular crude oil fraction uses bacterial enzymatic activities to form molecules of lower molecular weight from the tar fraction that then can be recovered.

Many anthropogenic contaminations contain halogen atoms which generally decrease metabolic degradation of the compounds. However, halogenated compounds are also known from natural sources. These include antibiotics with chloride or bromide atoms, plant-derived alkyl halides, or deterrents incorporated into, e.g., marine slugs or tropical frogs. Since nature already has learned to cope with these halogenated compounds, anthropogenic halogenated substances can also be degraded by bacterial consortia, both aerobically and anaerobically (Abraham et al., 2002; Smidt and de Vos, 2004). Not only bacteria are known to be able to perform dehalogenation reactions, but also basidiomycete fungi can also degrade organohalogenes (de Jong and Field, 1997). While aerobic dehalogenation is a simple enzymatic reaction, anaerobic dehalorespiration is an interesting field of research. In this case, the dehalogenation is coupled with electron transfer and directly coupled to ATP formation in an anaerobic respiratory process.

Use of microbes in bioremediation

The properties discussed above make the use of microbes or microbial consortia useful for bioremediation of different contamination scenarios. These include aerobic conditions such as in soil, anoxic condition such as in aquifers or sediments, and all imaginable sources of contamination from oil spill to xenobiotics such as plant protection chemicals or heavy metals. Some recent reviews are included to facilitate further reading on microbial degradation (Holtze et al., 2008; Johnsen and Karlson, 2007; Demnerova et al., 2005; Pieper, 2005; Kim and Rhee, 2003) and bioremediation (Watanabe et al., 2002; Röling and van Versefied, 2002; Schwitzguebel et al., 2002).

Summary

The biogeochemical cycles of the macronutrients carbon, nitrogen, sulfur, and phosphorus are dependent on microbial degradation of organic matter. Organic matter originating from carbon dioxide fixation, which is in terrestrial system usually performed by photosynthetically active bacteria and plant, is degraded to form carbon dioxide, either directly in one metabolic reaction or by cocoliterates in which the intermediate is released and taken up by another microbe. In addition, the formation of methane from CO2 or acetate is possible. Thus, the carbon cycling involves CO2, CH4, and organic molecules. Nitrogen fixation from N2 to NH3 followed by fixation in organic amines constitutes one side of the cycle, while nitrification/denitrification releases the bound nitrogen into the atmosphere. The latter reaction sequences, i.e., oxidation of NH3 to NO3− or NO2− and nitrate or nitrite respiration leading to N2 formation, are dominant processes in the cycling of nitrogen. For the sulfur cycle, assimilation of sulfates and fixation into organic biomass, the oxidation of sulfur and reduction of sulfate or sulfite to elemental sulfur, or reduction of sulfur to H2S are the main processes. Sulphur is a constituent of the amino acids cysteine and methionine and of Fe/S clusters in redox enzymes. Phosphate is generally not altered; however, there have recently been reports on the use of phosphite in an unusual energy fixation process.

These elemental cycles in the biogeoosphere all involve steps of fixation within living biomass, and the degradation of dead biomass is an essential part in releasing the mineralized elements back into the cycle. Higher
organisms are not capable of using plant litter, e.g., directly and thus entirely dependent on microbial degradation processes to have sufficient minerals available for their nutrition. Properties of microbes for degradation can be useful in bioremediation approaches.

Bibliography

Cross-references
Aerobic Metabolism
Anaerobic Transformation Processes, Microbiology
Phosphorus, Phosphorites
Soils

MICROBIAL ECOLOGY OF SUBMARINE CAVES

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Definition
Essential concepts, current investigations, and discussion.
In recent years, the search for the limit of biodiversity in extreme and/or uncommon environments led to the investigation of peculiar ecosystems such as deep-sea, subterranean niches, Antarctic cores, hydrothermal vents, and extraterrestrial sites.
Submarine caves and cavities have also been explored, some were mainly exploited as popular attractions for scuba divers, but some were shown to be of great interest for geologists, biologists, and microbiologists.
Available scientific data on these ecosystems are not abundant, particularly as far as biological disciplines are concerned. Most of the published works have been related to specific submarine cavities known as Blue Holes and Black Holes, as well as to submarine caves showing inner sulfide emissions. A larger number of data are currently available regarding the microbiology of terrestrial caves, particularly on the microbial ecology of carbonate layers, sediments, bat guano, and flooding waters (Vlasceanu et al., 1997, 2000; Holmes et al., 2001; Engel et al., 2003; Northup et al., 2003; Sugita et al., 2005; Zhou et al., 2007), but those latter ecosystems are not part of the current review.

Blue holes and black holes
The so-called blue holes are underwater karst systems which can develop horizontally in a very extensive way and appear blue due to a combination of blue sky reflection with the white carbonate sand deposited in the cave (Smart et al., 1988; Mylroie et al., 1995; Schwabe et al., 1997; Marano-Briggs, 2000; Colantoni et al., 2003; Canganella et al., 2004). Most of these sites were found and described in Bahamas and Hawaii.
Other cavern systems are known as “black holes,” which are cave systems without known lateral passages and located in the interior regions of the larger Bahamian islands. Most black holes are located in the central to western side of the island of South Andros in the Bahamas (Figure 1), although one has been found on the northern transitional shore of the Grand Bahama Island. They are vertical cave systems which
develop from the surface downward and appear to have no direct link to the sea, except through rock fissures and local porosity (Schwabe, 1998; Schwabe and Herbert, 2004).

In both cave systems, as water exchange is severely restricted, physicochemical gradients are highly stable and even strict anaerobic conditions can develop with remarkable hydrogen sulfide concentrations (from 5 μM to 6 mM).
Submarine caves with sulfidic water springs
A unique marine ecosystem is represented by the submarine caves of Capo Palinuro (Salerno, Italy), of which 13 have inside sulfidic springs exhibiting temperatures up to 25°C that remarkably affect the surrounding ecosystem.

Preliminary investigations concerning the submarine speleology of the site have been carried out since the mid 1980s, but only after 1990 an extensive biological knowledge was achieved. Several ecological and geological studies were performed (Alvisi et al., 1994a, b; Mattison et al., 1998), particularly on the “Grotta Azzurra” (Figure 2). The cave may be separated into two topographically distinct regions: a weakly illuminated outer region and an inmost dark region (Snow Hall). The latter is characterized by the presence of sulfur springs that arise from fissures in the cave floor. Currently, no other submarine caves with sulfide springs have been reported.

Microbiological studies
The blue holes
Abundant microbial development has been observed in these cave systems, usually linked to temperature anomalies in the range of 36–41°C as recorded by investigators. Marano-Briggs (2000) reported temperatures up to 41°C in the Tarpon Blue Hole, supporting the theory that the mass population of anoxygenic phototrophic bacteria present at a specific water layer may dissipate excess light energy by heat. A dense layer of purple sulfur bacteria

Microbial Ecology of Submarine Caves, Figure 2 A drawing map of the “Grotta Azzurra” showing both entrances, halls, and freshwater circulation.
was indeed found at 4.5–5.5 m depth in the estuarine Tarpon Blue Hole and the dominant population was represented by *Chromatiaceae* species. These bacteria are capable either of photolithoautotrophic growth with sulfide or elemental sulfur under anoxic conditions in the light or of chemo-organotrophic growth under micro-oxygenic conditions in the dark. Their abundance in the site was consistent with their physiological traits, and among them a phototrophic bacterium in particular was identified as a novel strain of *Marichromatium purpuratum* that contained okenone instead of spirilloxanthin as its major carotenoid.

Recently, microbiological studies on a blue hole discovered in the Indian Ocean (Canganella et al., 2004) were preliminarily performed in order to investigate the distribution of microbial populations along the water column and in the bottom sediment of the cave. In Figure 3, the DGGE analysis carried out shows how bacterial populations are distributed among surface layers, mid- and deep-layers, indicating in particular a similar biodiversity between 30 and 50 m depth. The same was investigated by the Biolog system, particularly for anaerobic populations.

The molecular investigation showed that at surface there was a complex microbial community showing several peculiarities with respect to standard pelagic seawater. Characteristic species were *Thioalkalivibrio* and *Thioploca* within the Gamma-Proteobacteria and *Desulfosarcina* and other Delta-Proteobacteria; sequences within the Delta-Proteobacteria related to sponge symbionts were also found. Subsurface bacteria present in the water column appeared dominated by Alpha-Proteobacteria represented by sequences related to photoorganotrophs (*Rhodovibrio*-related), methanotrophs, and coral surface-related bacteria.

Near the bottom, although the Alpha-Proteobacteria were still well represented, the proportion of Delta-Proteobacteria increased. These Delta-Proteobacteria represent sulfate-reducing bacteria mainly belonging to the Desulfovibriaceae, or related to *Desulfonanicus*, *Syntephus*, and others related to marine and salt-marsh uncultured bacteria.

The black holes of the Bahamas

The water in these holes appears black in color due to the presence of a 1 m thick white microbial veil located within the upper third of the water column (18–19 m), at the boundary between the oxygenic low salinity upper water mass and the denser anoxic saline water layer. The boundary between the two water masses is characterized by sharp discontinuities in physicochemical gradients: salinity increased from 12 to 35 psu and temperature from 29°C to 36°C; pH decreased from 8.6 to 6.45 as well as dissolved O₂ from 6 mg/l to <1 mg/l. Morphological microscopic observations of collected samples showed mainly nonmotile spherical and motile rod-shaped purple sulfur bacteria. The dominant members of this warm (36°C), saline, and sulfide-rich layer have been identified as anoxic phototrophic bacteria belonging to the genera *Allochromatium* and *Thiocapsa* and showed population densities >10⁷ viable cells/ml. These bacteria grow well in the presence of sulfide and carbon dioxide in the light. During photoautotrophic growth sulfur globules are stored intracellularly as intermediate oxidation products. Moreover, the intracellular photosynthetic membranes are of the vesicular type, and bacteriochlorophyll *a* and carotenoids of the normal spirilloxanthin series are present (Herbert et al., 2005). Calculations made by these authors revealed that the layer of anoxic phototrophic bacteria in the South Andros black hole may have a biomass content of approximately 5.06 ton dry weight.
The marine caves of Capo Palinuro

The emergence of sulfidic water in some of the caves (particularly in the “Grotta Azzurra,” “Grotta Sulphurea,” and “Grotta di Cala Fetente”) gives rise to an unusual situation, characterized by abiotic as well as biotic fluctuations, represented by emissions from sulfidic springs and higher concentration of sulfur-utilizing bacteria following each outflowing event, respectively. Warm sulfidic water of reduced salinity enters the Snow Hall (Grotta Azzurra), from fissures in the bottom rocks, and rises above the more dense seawater to form a thermocline and chemocline as a visible boundary at a depth of about 9.5 m. The geochemistry of water samples from the Grotta Azzurra was extensively reported by Mattison et al. (1998): above and below the chemocline temperatures were 24.0 ± 0.2 and 22.8 ± 0.3, respectively; NaCl concentrations (%) were 2.6 ± 0.1 and 3.0 ± 0.1, respectively; pH values were 7.22 ± 0.02 and 8.15 ± 0.25, respectively.

Microbial Ecology of Submarine Caves, Figure 4 A phylogenetic tree based on 16S rRNA gene sequence comparison, including representative heterotrophic isolates from the “Grotta Azzurra.”
The present ecosystem is biologically unique particularly because of the following points: (1) the peculiar emissions represent the only available model in shallow waters resembling the deep-sea hydrothermal vents (Canganella, 2001); (2) alike what happens in usual submarine caves, the development of micro- and macrofauna increases with the decrease of irradiation; (3) the freshwater flow is placed above the seawater and, inside the cave, an interface between the warm/anaerobic upper zone (H₂S-enriched) and the cold, sulfurless lower zone can be observed; (4) giant forms, particularly among Cnidaria and Porifera, occur mainly along the aerobic/anaerobic interface (Alvisi et al., 1994a, b).

During the last decade both, “Grotta Azzurra” and “Grotta Sulfurea” have been investigated. Some preliminary data on the lithotrophic microflora were published (Mattison et al., 1998), and other studies were performed in order to (1) investigate the microscopic structure of bacterial mat and (2) to understand both the taxonomy and physiology of heterotrophic bacteria inside the “Grotta Azzurra” (Canganella and Bianconi, 1999, 2003). The 16S rRNA genes analysis (Figure 4) showed that heterotrophic isolates were closely related to the genera Escherichia, Citrobacter, Vibrio, and Bacillus.

In addition to the analysis of the heterotrophic microflora, recently a preliminary molecular investigation was performed to evaluate the biodiversity of both eubacterial and archaeal populations (Canganella F. and Bianconi G. 2009).

Some bacterial sequences were closely related to the sulfur-oxidizing bacterial group, involving the genus Beggiatoa and Thiothrix. Showing that these microorganisms may well represent a major microbial community in the bacterial mat of the cave. Other bacterial sequences were phylogenetically linked to the Delta-proteobacterial group, particularly to sulfate-reducing bacteria. Archaeal sequences were partially related to methanogen archaeal group, but the phylogenetic distance was still remarkable; so the involvement of a novel archaeal group can be considered. Other archaeal sequences were related to MG-1, which is a common uncultivable archaeal group in marine environment.

As regards the microscopic structure of bacterial mat inside the cave and sediments collected nearby a sulfidic spring along the main entrance of the “Grotta Azzurra,” the distribution of various morphological forms is shown in Figures 5a–c. Filamentous microbial forms were abundant and morphologically similar to Beggiatoa, Desulfinema, Leucothrix, or Thiothrix species; morphology was, however, not enough to identify such organisms. Several cells showing either coccoid or rod morphologies were also observed in samples collected inside as well as outside the mat.

Conclusions

As regards the blue and black holes, it has been shown that the dominant anoxigenic phototrophic bacteria in the microbial layers are member of the Chromatiaceae (Imhoff et al., 1998). The presence of dense phototrophic populations of purple sulfur bacteria particularly explains why the South Andros black hole appears black and not blue. Moreover, the sulfide profile data obtained in this site showed that sulfide concentration was low (30 μM) suggesting that biomass production by the purple sulfur bacteria was sulfide rather than light limited.

Another important point to consider is that the dense microbial phototrophic population probably plays a significant role in enhancing carbonate CO₂-mediated dissolution operated by heterotrophic bacteria. The effects of microbiological activity on degradation of limestone deposits have been described by several authors (Ehrlich, 1996; Golubic and Schneider, 1979; Paine et al., 1993) and similar observations were reported for the Bahamas cave systems from Smart et al. (1988).

Recent studies have shown that biotic oxidation of sulfide into sulfuric acid can occur in anoxic marine ecosystems due to chemoautotrophic sulfur-oxidizing bacteria such as Beggiatoa (Mattison et al., 1998; Vlasceanu et al., 1997, 2000). This biological process may also be involved in carbonate dissolution for the South Andros black hole but the role of sulfur-oxidizing bacteria needs
to be evaluated by further investigations. The same consideration may be valid for the blue hole in the Indian Ocean. In this site the occurrence of highly toxic sulfide and low oxygen content caused the death of the macrobenthos in deep waters, where a chemolithoautotrophic microbial community settled on the cave walls as mucilaginous and hardened short filaments of bacterial origin whose study is still in progress.

In the “Grotta Azzurra” cave of Capo Palinuro, fluids emitted from active springs with maximum temperatures of 25°C and enriched in dissolved sulfur species (H$_2$S, S$_2$O$_3^{2-}$) allowed the development of a significant micro- and macro-fauna, resembling in some way what occurs around hydrothermal vent sites (Jeanthon, 2000; Canganella, 2001).

Morphological/physiological observations were previously carried out by Mattison et al. (1998) on microbial mats in the “Grotta Azzurra” of Capo Palinuro. In recent studies both sampling and isolation were carried out regardless of the chemocline. Future investigations on the distribution of microbial populations will eventually lead to discrimination between more thermostolerant and more mesophilic species living above or below the chemocline; the same can be true for noncultivable species.

The characterized heterotrophic isolates partially represent the microbial ecology of the site, showing its biological complexity at a phylogenetic level. Moreover, the wide resistance of isolates to temperature, salinity and pH may be associated with the specific ecological system, an hypothesis that certainly deserves further investigations.

In general terms, similar environmental traits can be described for the blue hole in the Indian Ocean and the submarine caves of Palinuro Cape; first of all, the presence of elevated concentrations of hydrogen sulfide, ranging from 5 μM to 200 mM. A chemocline in the water column may be clearly observed in the blue hole of the Indian Ocean or in the blue hole of the Bahamas, whereas in the submarine caves of Palinuro Cape the environmental borderline inside the habitat is represented by the threshold between upper anoxic, sulfide-rich freshwater and lower seawater.

As regards the autoctonous microflora, the blue hole of the Indian Ocean showed the presence of filamentous forms as described in the Palinuro caves but mucilaginous forms on the rocky side walls were not found in the latter; moreover sulfate-reducers and sulfide-oxidizers were also detected in both systems.

Some microbiological features of both systems are also shared with other sulfide-rich environments, such as the presence of anaerobic methane oxidizers, filamentous bacteria, anaerobic phototrophs, and archaeal strains. However, the incomplete data set on the inhabitant microflora, the geological diversity of the systems described earlier, and their different geographical locations make a microbiological comparative study difficult. With no doubts the combination of different approaches and techniques may be a powerful tool to investigate further such astonishing ecosystems by a multiphasic approach.

As a matter of fact, further investigations on the microbial diversity in sulfide-rich, either marine or freshwater, caves will be based on traditional techniques of isolation coupled to simultaneous molecular investigations.

Bibliography


MICROBIAL MATS

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Synonyms
Algal mats; Bacterial mats; Biomats; Thick biofilms

Definition
Microbial mats are often centimeter-thick multilayered structures of microorganisms, mainly bacteria, archaea, fungi, and sometimes these mats are enriched with protozoa. Microbial biofilms are in contrast much thinner (10–100 µm) than microbial mats and have a different architecture (Characklis and Wilder, 1989). Microbial mats grow at interfaces between different types of material, mostly on moist surfaces, but some of them are also found in dry environments like deserts (e.g., Stal and Caumette, 1994; Characklis and Wilder, 1989). They colonize environments located in high altitude, they are common in the Deep Biosphere, in subterranean environments with temperatures from −40°C to +120°C. There are also common as endosymbiotic structures, for example, in animals. They are very common in extreme environments like hydrothermal vents, cold seeps, and alkaline and hypersaline lakes (Van Dover, 2000; Pedersen, 2006; Arp et al., 2003; Riding and Awramik, 2000).

Geobiological implications
A microbial mat consists of several layers dominated by specific types of microorganism (Figures 1 and 2). Although the composition of individual mats varies depending on the environment, as a rule the by-products of each group of microorganisms serve as energy source for other groups. Different types of microorganisms dominate different layers based on their comparative advantage (Canfield and Des Marais, 1993; Allison et al., 2000). Ecological relationships between different groups are a combination of competition and cooperation. The metabolic capabilities of bacteria and archaea are generally depend on their phylogeny. In a wet environment where sunlight is available, the upper layers are generally dominated by aerobic consortia like cyanobacteria (Figure 1), while the lowest layers are normally dominated by anaerobic microorganisms such as sulfate-reducing bacteria, methanogenic and/or methanotrophic archaea, or general archaea. Marine mats may grow to a few centimeters in thickness, of which only the top few millimeters are oxygenated. Microbial mats are held together and bound to their substrates by sugar- and protein-rich extracellular polymeric substances (EPS), which they secrete (Decho et al., 2005; Wingender et al., 1999). In many cases, some of these microorganisms form filaments (threads), which tangle and thus increase the microbial...
colonies structural strength, especially if the filaments have sheaths. Microbial mats are the earliest complex form of life on Earth. There is a fossil record from 3,500 million years ago, and they have always been the most important members and maintainers of earth ecosystems (Allwood et al., 2006; van Kranendonk, 2007). Early Precambrian microbial mats are the first evolved highly complexly organized multicellular organisms. Microbial mats occasionally mineralize if the ionic strength in the aquatic environment allows it. The mineralized structures are known as stromatolites and thrombolites and they represent the most important organo-sedimentary fabrics since the early beginning of microbial life on earth (Burne and Moore, 1987; Riding, 2000). Originally, microbial mats first occurred at hydrothermal vents for energy. Since the late Precambrian microbial mats have also played an important role at cold hydrocarbon seeps, so-called Cold Seeps. Most of the Cold Seeps are methane vents coupled with hydrogen sulfur microbial metabolism (Reitner et al., 2005) (Figure 2). However, phototrophic microorganisms such as cyanobacteria, unicellular algae (e.g., diatoms), and related consortia are the most abundant microorganisms in the formation of microbial mats (e.g., Arp et al., 2010). Within subterranean environments iron-oxidizing microbial consortia show a strong ability to form thick microbial mats (Kurz et al., 2010).

Microbial mats are the context within which the first multicellular organisms evolved. Sponges are, for example, highly evolved microbial mats with a mutual relationship with eukaryote choanoflagellates. May be the Vendobionta, large Ediacaran organisms with characteristic quilt bauplan are part of well-developed microbial mats and may themselves be large colonies of various microbial communities (Seilacher, 1999; Schieber et al., 2007; Steiner and Reitner, 2001).

Conclusion
Microbial mats are normally cm-thick microbial communities often related with microbially induced mineral precipitates (calcite, aragonite, iron-oxides etc.). Characteristic for these mats is a multilayered structure which represents various microbial communities with different metabolic activities. In phototrophic microbial mats (e.g., cyanobacteria) dominate the uppermost layers whereas within the deep layers of the mats anaerobic microbes dominate like sulphate reducing bacteria and archaea. Microbial mats are complex multicellular systems which exhibit some accordance to basic metazoans like sponges. It is speculated that microbial mats are a model for the basic metazoan bauplan.

Bibliography
Cross-references

Anaerobic Oxidation of Methane with Sulfate
Archaeon
Bacteria
Biofilms and Fossilization
Calcite Precipitation, Microbially Induced
Chemolithotrophy
Cold Seeps
Cyanobacteria
Diatoms
Ediacaran Biota
Extracellular Polymeric Substances (EPS)
Mat-Related Sedimentary Structures
Microbial Biomineralization
Microbial Communities, Structure, and Function
Microbial Degradation
Microbial-Metal Binding
Microbialites, Modern
Mud Mounds
Ores, Microbial Precipitation and Oxidation
Protozoa (Heterotroph, Eukaryotic)

MICROBIAL SILIFICATION – BACTERIA (OR PASSIVE)

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Definition

The encrustation, impregnation, and/or replacement of microbial tissue by silica minerals, usually opal-A.

Overview

Silica precipitation is an important geological process in many modern geothermal systems where venting of super-saturated solutions leads to the formation of finely laminated siliceous sinters around geyser and hot spring vents and on their discharge aprons. The spring waters, which typically originate from deep, hot reservoirs, at equilibrium with quartz, commonly contain dissolved silica concentrations significantly higher than the solubility of amorphous silica at 100°C (Fournier, 1985). Therefore, when these fluids are discharged at the surface, decompressional degassing, rapid cooling to ambient temperatures, evaporation, mixing, and changes in solution pH collectively cause the solution to suddenly become supersaturated with respect to amorphous silica (also known as opal-A). In such supersaturated solutions, the discharged monomeric silica, Si(OH)4, polymerizes, initially to oligomers (e.g., dimmers, trimers, and tetramers), and eventually to polymeric species with spherical diameters of 1–5 nm, as the silanol groups (–Si-OH-) of each oligomer condense and dehydrate to produce the siloxane (–Si–O–Si–) cores of larger polymers. The polymers grow in size through Ostwald ripening such that a bimodal
composition of monomers and particles of colloidal dimensions (>5 nm) are generated. The latter stay in suspension due to the external silanol groups exhibiting a residual negative surface charge, they coagulate via cation bridging and nucleate homogeneously, or they precipitate heterogeneously on a solid substratum (Ihler, 1979).

Siliceous sinters commonly contain numerous mineralized microorganisms (e.g., bacteria, algae, fungi) in varying stages of preservation (Jones et al., 1997; Konhauser et al., 2001). Examination under the scanning or transmission electron microscopes (SEM/TEM) shows that their preservation typically involved the precipitation of spheroidal, amorphous silica grains on (i.e., encrustation) or within (i.e., impregnation, replacement) the walls and/or sheaths of the microbes. The manner in which a particular microbe is preserved depends upon the balance between the timing of opal-A precipitation/impregnation and decay of the soft tissues of the microbe (Figure 1). In many cases, a microbe merely acts as a template as opal-A is precipitated around its outer surface. Such encrustation begins with the attachment of preformed silica colloids, on the order of only 10 s of nanometers in diameter, to the cell surfaces (Figure 2a). Those silica particles then grow in size, and may reach micrometer diameters in size. If opal-A precipitation is sustained, those particles invariably coalesce until the individual precipitates are no longer distinguishable, resulting at times in entire colonies being cemented together in a siliceous matrix (Figures 2b and 3a–d).

Replacement, which involves impregnation of the organic tissue by opal-A, can lead to superb preservation of the microbes (Figure 3e). Jones et al. (2001, their Fig. 8), for instance, illustrated silicified specimens of *Calothrix* in which the sheath and outer splayed lamina are still evident. Septa may also be preserved in this way (Figure 3h). If encrustation is the sole process, then many silicified filamentous microbes will be evident only as hollow tubes (Figure 3g and i). Subsequent filling of that tube with opal-A will produce a solid opal-A rod, with the size of the original lumen/filamentous microbe only being apparent if there is a discontinuity preserved (e.g., Figure 3f). Irrespective of the nuances, the silicification of microbes must take place quickly if they are to be preserved, and certainly before the microbes undergo any degree of desiccation and degradation (Jones et al., 2001). This notion is supported by the fact that the three-dimensional form of most microbes is still apparent following silicification (Figure 3f–l). Indeed, some microbes are coated with silica while they are still viable (Phoenix et al., 2000) and it seems that silicification must take place in a matter of days in order for the three-dimensional morphology to be preserved.

Plant material bathed in the polymerizing solutions may be encrusted, impregnated, and/or replaced with opal-A similar to microbes. In many of the hot spring systems of New Zealand, for example, grass stems, pieces of wood, and leaves are heavily encrusted with opal-A but their tissues have usually not been replaced. In fact, the grass is commonly still growing even though encased by opal-A. In contrast, wood and leaves on the discharge apron around Geysir, Iceland that have been superbly preserved through replacement have not been encrusted with opal-A. Similar comparisons apply to some of the microbes. The factors that dictate encrustation as opposed to replacement are currently not understood as there are no obvious correlations between preservation style and the chemical attributes of the associated waters.

![Microbial Silicification – Bacteria (or Passive), Figure 1](image-url) Schematic longitudinal cross-section of a sheathed filamentous microbe showing morphological variations that can develop as a result of silicification. Modified form Jones et al. (2001, their Fig. 10).
Despite the fact that many silicified microbes appear to be superbly preserved, only a limited number of features are usually apparent. Even in the best-preserved specimens, features are generally limited to overall morphology, filament diameter, presence/absence of branching, presence/absence of a sheath, and/or size of cells. Identification in terms of extant taxa is usually difficult because the limited numbers of morphological features apparent in the silicified forms are insufficient unless they are particularly distinctive in some way. This issue is compounded by the fact that many extant taxa are defined by their DNA characteristics and/or the conditions under which they were cultured in a laboratory setting. In most cases, therefore, the identification of silicified microbes must be done with caution.

The role that the microbes (and some plant material) play in their own silicification has been a matter of debate. Being soft-bodied organisms they do not “actively” mediate amorphous silica formation in the manner of diatoms, radiolarians, and sponges. In contrast, bacteria, algae, and fungi facilitate silica precipitation by creating a suitable microenvironment and/or template for opal-A precipitation. For example, they may produce organic ligands that promote the mineralization process (Lalonde et al., 2005), or they may change the pH immediately around the cell through their metabolic activity, and in this regard, induce supersaturation with respect to opal-A (Amores and Warren, 2007). At other times, the microbes may simply act as solid surfaces for silica precipitation and exert no direct influence over the process (Walter et al., 1972).

Experimental evidence seems to indicate that the microbial role in the silicification processes is incidental and not limited to any particular taxa. First, microbes have little affinity for monomeric silica, even at high bacterial densities and low pH conditions where most organic functional groups are fully protonated and neutrally charged (Fein et al., 2002). Second, at supersaturated conditions, there appears to be very little difference in the rates or magnitude of silica precipitated between microbial and microbial-free systems (Yee et al., 2003; Benning et al., 2004). Third, silicification occurs on dead cells, and continues autocatalytically and abiogenically for some time after their death due to the high reactivity of the newly formed silica. This notion is supported by microscopic examination of thermal spring deposits where it has been shown that the silica precipitated in the porous spaces between filaments has the same basic motif as the silica precipitated on the original filaments (Jones et al., 1998). From these observations, it can be inferred that at high silica levels there is such a strong chemical driving force for silica polymerization, homogeneous nucleation, and ultimately silica precipitation that there is no obvious need for microbial catalysis.

Despite the observations above, the fact that there are species-specific patterns of silicification infers that some microbes may influence silicification in some way. The initial coating of opal-A spheres around microbes in Iodine Pool in the Waimangu geothermal area, for example, are consistent in size and highly organized in terms of their distribution (Jones et al., 1998). In contrast, the outer layer of opal-A beads is larger, variable in diameter, and follows no set distribution pattern. Thus, it seems that some aspect of the microbes may have influenced the initial phase of opal-A precipitation but not later phases. This seems feasible given that the initial layer of opal-A precipitates would have effectively encased the microbes and thereby isolated their surfaces from the water. Furthermore, different microbes exhibit different degrees of silicification. This is not surprising

**Figure 2** Transmission electron micrographs (TEM) of cyanobacteria from a geyser outflow channel at Strokkur, Iceland. (a) Cell showing abundant silica spheres on, and within the surrounding sheath material. (b) Colony of unidentified cyanobacteria that are completely encrusted by silica matrix. Remnants of cytoplasm are still evident inside some cells. Scale bars = 3 μm.
Microbial Silicification – Bacteria (or Passive), Figure 3 (Continued)
given that the actual mechanisms of silicification rely, in part, on the microbes providing reactive surface ligands that adsorb silica from solution, and accordingly, reduce the activation energy barriers to heterogeneous nucleation (Konhauser et al., 2004). This means that cell surface charge may have a fundamental control on the initial silica sorption process.

At present there appears to be three different mechanisms by which microbes bind dissolved/colloidal silica: (1) hydrogen bonding, (2) cation bridging, and (3) electrostatic interactions (Figure 4). In the first instance, Phoenix et al. (2002) showed that the polysaccharide-rich sheath of Calothrix is electrically neutral at pH 7, and opal-A precipitation occurs through hydrogen bonding between the hydroxy groups associated with the sugars and the hydroxyl ions of the dissolved silica. Second, the highly anionic nature of most bacteria, such as Bacillus subtilis, limits opal-A from occurring on the cell wall, likely as a result of electrostatic charge repulsion between the anionic ligands of the organic functional groups and the negatively-charged silica species (Phoenix et al., 2003). Therefore, in order for silica to sorb, some form of cation bridge (e.g., Fe$^{3+}$, Al$^{3+}$) is necessitated (Fein et al., 2002). Third, some species, such as Aquifexsp., produce protein-rich biofilms that contain sufficient cationic amino groups (NH$_4^+$) to electrostatically react with dissolved silica (Lalonde et al., 2005).

Given that a wide assortment of microbes live in environments where opal-A is being precipitated, it is important to determine if those species survive the mineralization process. In this regard, Phoenix et al. (2000) tested cell viability during biomineralization, and observed that experimental encrustation of Calothrix by silica was not notably detrimental to the organisms. Even after 12 days of incubation in a 600 mg l$^{-1}$ silica solution, during which many of the filaments developed extensive mineral crusts up to 5 μm thick, the cells still fluoresced, they continued to generate oxygen and the mineralized colonies exhibited comparable rates of photosynthesis to the non-mineralized colonies. Interestingly, silica precipitation only occurred upon the outer surface of sheath material from viable cyanobacterial cells – no viable cells exhibited internal or cell wall mineralization. This contrasts with dead and lysed cells where mineralization of the cell wall and cytoplasm had occurred. In a more recent study with the thermophilic chemolithoautotroph Sulforhodobacter azorense, Lalonde et al. (2005) showed that the bacterium, when grown as a H$_2$-oxidizer, reacted to increasing silica concentrations by producing excess protein that appears linked to biofilm production. Since silicification was not observed directly on the cell surface, they proposed that S. azorense may prevent cellular silicification to some degree by providing abundant reactive sites in the biofilm matrix and regulating biofilm production appropriately, with potential contributions from metabolic effects. Thus, it appears that some microbes can thrive in silica-rich environments because they form protective layers that isolate the cells from the potentially damaging effects of silicification.

**Summary**

Microbial silicification includes the encrustation, impregnation, and replacement of cells by amorphous silica. Most microbes play a passive role in the mineralization process by providing organic surfaces that facilitate the binding of silica polymers and colloids from solution. These sorption reactions can occur by either (1) hydrogen bonding between dissolved silica and cell hydroxy functional groups, (2) cation bridging between anionic cell functional groups and the silica, or (3) direct electrostatic interaction of silica with cell surface ammonium groups.
Microbial Silicification – Bacteria (or Passive), Figure 4  Three mechanisms by which microorganisms silicify: (a) hydrogen bonding between silica polymers with hydroxy groups in sheaths of Calothrix; (b) cation bridging between negatively-charged cell walls in Bacillus subtilis and silica; and (c) electrostatic interactions between silica and positively-charged amino groups in the biofilm of Sulfurihydrogenibium azorense. Reproduced from Konhauser (2007) with permission of Wiley-Blackwell.

Bibliography


**Cross-references**

Cherts  
Cyanobacteria  
Diatoms  
Hydrothermal Environments, Terrestrial  
Microbial Biomineralization  
Microbial Surface Reactivity  
Radiolaria

**MICROBIAL SURFACE REACTIVITY**

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**Definition**

The chemical charge on a cell surface caused by the deprotonation/protonation of organic functional groups.

**Overview**

Similar to an aqueous organic acid, bacterial cell surfaces can either bind or release protons (H⁺) into solution depending upon the solution pH. In the case of the latter, this deprotonation process, which accurately mimics a number of the functional groups associated with cell surfaces, leads to the formation of an organic anion, or ligand. Unlike an aqueous organic acid, which exhibits proton buffering over a narrow pH range (±1–2 units), bacterial cell walls have continuous buffering capacity over a wide range of pH values which span most geologic settings (pH 2–10). Ultimately, most surface reactivity of bacteria with its external environment results from the enhanced electronegativity of the cell wall as these functional groups deprotonate. In its most simplistic form, deprotonation can be expressed by the following equilibrium reaction:

\[ B - A H^0 \leftrightarrow B - A^- + H^+ \]  

(1)

where B denotes a bacterium to which each protonated ligand type, A, is attached. The distribution of protonated and deprotonated sites can be quantified with the corresponding mass action equation:

\[
K_a = \frac{[B - A^-][H^+]}{[B - AH^0]}, \tag{2}
\]

where \( K_a \) is the dissociation constant; \([B−A^-]\) and \([B−AH^0]\) represent the concentration of exposed deprotonated and protonated ligands on the bacterium, respectively (in mol l⁻¹); and \([H^+]\) represents the activity of protons in solution. Each functional group has its own \( K_a \), and based on equation (2), the pH at which \([B−A^-]\) and \([B−AH]\) are equivalent is known as the pKₐ value, where \( pK_a = -\log_{10}K_a \). At pH < pKₐ surfaces are protonated and at pH > pKₐ, they are deprotonated.

The ionization of functional groups provides an electrical charge ([L]ₜ) represented in mol mg⁻¹, which in the laboratory can be calculated as a function of pH from the difference between total base or acid added to a suspension of organic material and the equilibrium H⁺ and OH⁻ ion activities (Reaction 3):

\[
[L]_T = \frac{C_a - C_b + [OH^-] - [H^+]}{S} \tag{3}
\]
The components, $C_a$ and $C_b$, are the concentrations of acid and base added, respectively; $n_{OH}$ and $n_{H^+}$ are the number of moles of $OH^-$ and $H^+$ in the solution at the measured pH, respectively; while "S" is the quantity of the solid (usually expressed in dry weight, i.e., mg). When such calculations are done over an entire pH range, an acid–base titration curve is created that shows the pH range over which some functional groups are chemically active and how the net surface charge of the organic surface varies with pH (Figure 1). A large difference in the total base or acid added and the free $H^+$ ion activity indicates significant pH buffering and a high concentration of surface functional groups. On the titration curve this is shown by a steep slope. A small difference in the total base or acid added and the free $H^+$ ion activity indicates weak pH buffering and a low concentration of surface functional groups. This translates into a gentle slope on a titration curve. In terms of microbial biomass, carboxyl groups account for the buffering capacity from pH 2–6 (Reaction 4), phosphate groups from pH 5–8 (Reaction 5), amino groups at pH > 8 (Reaction 6), and hydroxy groups deprotonate at pH values above 10 (Reaction 7) (see Konhauser, 2007).

$$R - COOH^0 + OH^- \rightarrow R - COO^- + H_2O$$ \hspace{1cm} (4)

$$R - PO_4H_2^0 + OH^- \rightarrow R - PO_4H^- + H_2O$$ \hspace{1cm} (5)

$$R - NH_3^+ + OH^- \rightarrow R - NH_2 + H_2O$$ \hspace{1cm} (6)

$$R - OH^0 + OH^- \rightarrow R - O^- + H_2O$$ \hspace{1cm} (7)

As a consequence of the deprotonation reactions, the microbial surface develops an electrical charge. Therefore, when subjected to an electric field, microorganisms move in the direction, and at a rate, commensurate with the polarity and density of the overall surface charge. This process is termed electrophoresis, and the electrophoretic mobility of a microbial suspension can be quantified by measuring their velocity in an electric field. This measurement can, in turn, be used to calculate the zeta potential ($\zeta$), using the Smoluchowski equation below (Hunter, 2001):

$$\zeta = \frac{\eta \mu}{\varepsilon_0 \varepsilon}$$ \hspace{1cm} (8)

The component “$\mu$” is the electrophoretic mobility of a particle; $\varepsilon_0$ and $\varepsilon$ are the relative dielectric constants of the vacuum and solution, respectively; and $\eta$ is the viscosity of the solution. The zeta potential reflects the electrical potential at the interfacial region (viewed as a shear plane) separating an inner layer, where cations are held tightly in place and move with the bacterium, and an outer layer where ions are mobile (Wilson et al., 2001). The greater the absolute value of the $\zeta$ potential, the greater the charge...
density on the surface (Blake et al., 1994). The electrophoretic mobility (and zeta potential) is pH dependent because the activity of protons in solution controls the ionization reactions of functional groups at the microbial surface (Ahimou et al., 2001) (Figure 1). This leads us to the concept of isoelectric point, which is defined as the pH value where the net surface charge of a solid is zero. The isoelectric point can be estimated from acid–base titrations, but it can also be directly measured with electrophoretic mobility experiments because at the isoelectric point microbes do not exhibit motion in an electric field. The isoelectric point of bacterial cell walls is typically between pH 2–4 (Harden and Harris, 1953). This means that at low pH, when the surface functional groups are fully protonated, bacteria are either neutral or positively-charged, the latter being the result of a cell possessing abundant amino groups. Meanwhile, at the growth pH of most bacteria, cells inherently display a net negative charge and the magnitude of negativity increases with higher pH values. As a result, under low pH conditions, most bacterial surfaces behave hydrophobically, but with an increasingly hydrophilic nature with increasing pH.

One of the major challenges faced by the researchers today is how to translate acid–base titration data in terms of cell wall biochemistry. Ascribing $pK_a$ values from a titration curve to specific functional groups can be problematic because there is often significant variation in $pK_a$ values for the same functional group. This occurs because the magnitude of the dissociation constant is controlled by the structure of the molecule to which it is attached (see Martell and Smith, 1977 for details). Consequently, a single carboxyl group in two different organic acids will have different $pK_a$ values, as will an organic acid with multiple carboxylic groups. As might then be expected, the $pK_a$ for a carboxyl group on one microbial species versus another could yield widely different values simply because of subtle conformational variations within the wall macromolecules. Furthermore, titration experiments are only able to resolve those groups that contribute significant amounts of protons to solution. Minor groups are simply undetectable with the resolution of current techniques. It is thus important to keep in mind that the model-derived binding sites do not directly represent the functional groups of the cell surface; their identity can only be inferred by comparison of the functional group $pK_a$ values with $pK_a$ values of model compounds. Unequivocal identification of the types of functional groups responsible for acid–base buffering may be obtained by spectroscopic techniques, such as Fourier transform infrared spectroscopy (FTIR), calorimetry or gas/liquid chromatography of cell wall extracts. For instance, Yee et al. (2004) and Jiang et al. (2004) used infrared spectroscopy to examine the pH dependence of deprotonation of bacterial cell-wall functional groups. Although not all pH conditions were studied, it was clear that the vibrational frequencies of carboxyl type groups increased with increasing pH. Calorimetric measurements of protonation reactions on bacterial surfaces indicate these reactions are exothermic with relatively small site-specific entropies (Gorman-Lewis et al., 2006). This provides more evidence that (1) hydrogen bonding between protonated and deprotonated sites likely stabilizes the cell wall, and (2) bacterial cell wall functionalities are similar to multifunctional organic acids rather than simple organic acids.

Summary

Microbial surface reactivity results from the protonation and deprotonation of the organic moieties of microbial cell surfaces including carboxyl, phosphate, amino, and potentially hydroxyl groups. These groups behave similarly to multifunctional organic acids – they hydrogen bond with each other which leads to negative charge on the cell wall as pH increases. At most environmentally relevant pH values, microbial cell surfaces will possess a net negative charge, and therefore, will interact with positively charged cations or mineral surfaces.

Bibliography


Cross-references

Metalloenzymes
Metals, Acquisition by Marine Bacteria
Microbial Biomineralization
Microbial-Metal Binding
Organomineralization
Siderophores
MICROBIALITES, MODERN

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Synonyms
“Cryptalgal sedimentary rock” (Aitken, 1967); Microbolite (Riding, 1991)

Definition
Microbialite: “Organosedimentary deposits that have accreted as a result of a benthic microbial community trapping and binding sediment and/or forming the locus of mineral precipitation” (Burne and Moore, 1987), or “Benthic microbial deposits” (Riding, 1991).

Introduction
In this encyclopedia, microbialite is discussed under two chapters: (1) modern and (2) fossil. This part discusses modern microbialites, with emphasis on processes of formation. The fossil extension and classification of microbial deposits (fossil forms being more diverse) are treated in the “fossil microbialite” section of the encyclopedia (see Chapter Microbialites, Stromatolites, and Thrombolites). Microbialites are composed of trapped, bound, and/or precipitated sediment, and exhibit a range of mineralogies (Figure 1). This chapter focuses on carbonate microbialites, which are the most widespread and the most studied.

Microbialites are “rocks” that are produced, induced, or influenced by benthic microbial communities, primarily bacteria and sometimes also microeukaryotes, such as diatoms. These microbial communities are organized in mats or biofilms (e.g., Stolz, 2000), consisting of microscopic organisms enveloped in an organic matrix of extracellular polymeric substances (EPS; Decho, 1990; Bhaskar and Bhosle, 2005; see Chapter Extracellular Polymeric Substances (EPS)). Because the microbial mat is dominantly organic, it is rarely preserved in the fossil record. In some unusual cases, exceptional taphonomic conditions can preserve intact cells and even the EPS structure (e.g., silification or preserved remnants of EPS matrices, Golubic and Hofmann, 1976; Barbieri et al., 2004; Barbieri and Cavalazzi, 2005; Altermann et al., 2006; Benzerara et al., 2006; Kumar and Pandey, 2008; Lepot et al., 2008). More commonly, the organic matter decomposes to carbon dioxide (CO₂) or methane (CH₄), although in some cases incomplete degradation can lead to organic matter enrichment of the sediment. Evidence of the original microbial mat or biofilm is generally indirect (e.g., specific sedimentary (bio)structure, micromorphology, organic biomarkers, and stable isotopic fractionation). Microbial mats can help preserve sedimentary structures in the fossil record (“microbially-induced sedimentary structures” – MISS; Noffke et al., 2003). The most spectacular examples are carbonate microbialites. Examples of modern microbialites in the following section are followed by a discussion of processes of formation.

Types of modern microbialite
Microbialite descriptions incorporate a range of scales, from macrometers to micro/nanometers (e.g., Shapiro, 2000). Megastructure relates to the geographical extension of a microbial deposit and overall deposit morphology (e.g., ridges, biostrome, and bioherm). Macrostructure characterizes the morphology of an individual buildup (e.g., domal, branching, knobby). Mesostructure refers to structures visible with the naked eye, such as lamination. Microstructure is observed with a microscope, such as light, confocal, scanning electron (SEM), or transmission electron microscope (TEM).

Microbialite classification is based on mesostructure with four common types (Figure 2): laminated microbialites classified as stromatolites, thrombolites are clotted, leiolites are structureless, and dendrolites are dendritic (Burne and Moore, 1987; Riding, 1991, 2000; Dupraz and Strasser, 1999, 2002). All of these structures exhibit a wide range of microstructures including micropeloidal, densely micritic, or agglutinated microfabrics (Riding, 1991; Dupraz and Strasser, 1999). Three types of modern microbialites are described in the following section; dendrolites have not yet been found in modern environments. For further description of microbialite classification and microstructure see Chapter Microbialites, Stromatolites, and Thrombolites.

Stromatolite
The term stromatolite is derived from the Greek “stroma,” meaning mattress or stratum and “lithos,” meaning rock. The term was first introduced by Kalkowsky in 1908 to refer to “laminated benthic microbial deposits” (see also Riding, 1999; Chapter Microbialites, Stromatolites, and Thrombolites). The first modern stromatolites were found in the Shark Bay, Western Australia in 1954 in the hypersaline water of Hamelin Pool. Until this date, scientists believed that stromatolites were extinct, found only in the fossil record. Although the biogenicity of 3.5 Ga stromatolites has been questioned (Buick et al., 1981; Lowe, 1994; Grotzinger and Knoll, 1999; Lindsay et al., 2003), all modern microbialites called stromatolites are associated with microbial communities that induce, or serve as organic substrate for, precipitation (Riding, 2000). Modern stromatolites are found in many different locations including marine, hypersaline, freshwater, and even continental environments.

Open marine stromatolites
The Bahamas hosts the only known examples of open marine stromatolites. These structures are found at a variety of locations on the margins of Exuma Sound including Schooner Cays, Lee Stocking Island, Stocking Island Highborne Cay, and Darby Island (Dravis, 1983;
Nomenclature and chemical compositions of minerals produced by biologically-related mineralization processes. (Modified from Weiner and Dove, 2003; data from Lowenstam and Weiner, 1989; Simkiss and Wilbur, 1989; Mann, 2001; Weiner and Addadi, 2002.)

**Carbonates**
- Calcite - CaCO₃
- Mg-Calcite - (Mg,Caₓ₋₁)CO₃
- Aragonite - CaCO₃
- Vaterite - CaCO₃
- Monohydrocalcite - CaCO₃ • H₂O
- Protodolomite - MgCa(CO₃)₂
- Hydrocerussite - Pb₃(CO₃)₂(OH)₂
- Amorphous forms - CaCO₃ • H₂O or CaCO₃

**Phosphates**
- Octacalcium phosphate - Ca₈H₂(PO₄)₆
- Brushite - CaHPO₄ • 2H₂O
- Francolite - Ca₁₀(PO₄)₆F₂
- Carbonated-hydroxylapatite (dahlite) - Ca₅(PO₄,CO₃)₃(OH)
- Whittlockite - Ca₅H₂(Mg,Fe)₂+2(PO₄)₄
- Struvite - Mg(NH₄)(PO₄) • 6H₂O
- Vivianite - Fe₂+2(PO₄)₂ • 8H₂O
- Amorphous Calcium Phosphate (at least 6 forms) variable
- Amorphous Calcium Pyrophosphate - Ca₃P₂O₇ • 2H₂O

**Sulfates**
- Gypsum - CaSO₄ • 2H₂O
- Barite - BaSO₄
- Celestite - SrSO₄
- Jarosite - KFe₃⁺(SO₄)₂(OH)₆

**Sulfides**
- Pyrite - FeS₂
- Hydrotellure - FeS • nH₂O
- Sphalerite - ZnS
- Galena - PbS
- Hydrotellure FeS • nH₂O
- Sphalerite ZnS
- Wurtzite ZnS
- Greigite Fe₃S₄
- Mackinawite (Fe,Ni)ₓSₙ₋₁
- Amorphous Pyromorphic Fe₁₋ₓS (x = 0–0.17)
- Acanthite Ag₂S

**Arsenates**
- Orpiment - As₂S₃

**Hydrated Silica**
- Amorphous Silica - SiO₂ • nH₂O

**Chlorides**
- Atacamite - Cu₂Cl(OH)₃

**Fluorides**
- Fluorite - CaF₂
- Hieratite - K₂SiF₆

**Oxides**
- Magnetite - Fe₃O₄
- Amorphous Iron Oxide - Fe₂O₃
- Amorphous Mn Oxide - Mn₃O₄
- Amorphous Ilmenite Fe²⁺TiO₃

**Metals**
- Sulfur - S

**Hydroxides and Hydrous Oxides**
- Goethite - α - FeOOH
- Ferricyanide - 5Fe₂O₃ • 9H₂O
- Lepidocrocite γ - FeOOH
- Ferricyanide - 5Fe₂O₃ • 9H₂O
- Todorokite - (Mn²⁺CaMg)Mn₄⁴⁺O₇•H₂O
- Birnessite - Na₃Mn₄O₂⁷ • 9H₂O

**Organic Crystals** following Lowenstam & Weiner (1989)
- Whewellite - CaC₂O₄ • H₂O
- Weddelite - CaC₂O₄ • (2+χ)H₂O
- Mn Oxalate - Mn₃C₂O₄ • 2H₂O
- Earlandite - Ca₃(C₂H₃O₂)₂ • 4H₂O
- Glushinskite - MgC₂O₄ • 4H₂O
- Manganese Oxalate - Mn₂C₂O₄ • 2H₂O
- Sodium urate - C₂H₇N₂NaO₃
- Uric Acid - C₅H₄N₂O₅
- Ca tartrate - C₄H₄CaO₆
- Ca malate - C₄H₇CaO₆
- Paraffin Hydrocarbon
- Guanine - C₃H₃(NH₂)N₄O
Microbialites, Modern, Figure 2 Descriptive terminology for microbialite macro-, meso, and microfabrics. (From Kennard and James, 1986; Riding, 1991; Schmid, 1996; Dupraz and Strasser, 1999; Shapiro, 2000.)
Dill et al., 1986; Reid et al., 1995). These stromatolites are coarse grained structures, composed of ooid sand; they form in intertidal and subtidal environments, exposed to high wave energy and strong tidal currents (e.g., Gebelein, 1976; Reid et al., 2000). Many of the stromatolites exhibit well-developed laminae (Figure 3; e.g., Reid et al., 2000). The lamination results from iterative growth of different types of microbial mats on the top of the build-ups (Figure 4). Each microbial mat forms a distinct mineral product, which can be recognized in thin section and SEM (Figure 5; Reid et al., 2000). The first mat type (1) is dominated by the filamentous cyanobacterium Schizothrix, which traps and binds ooids. The stickiness of the mat EPS results in the trapping of particles, which are subsequently bound by the upward growth of the cyanobacterial filaments. Other microorganisms (e.g., diatoms that produce copious amounts of EPS) can also participate in trapping grains. The second mat community (2) consists of a thin biofilm with abundant heterotrophic bacteria. Precipitation of aragonite within this biofilm forms a micritic lamina, which caps the underlying ooids. The third mat type (3) is characterized by an abundance of the endolithic coccoid cyanobacterium Solentia spp. Solentia tunnels through grains, leaving bore holes filled with EPS. Aragonite precipitation within the EPS results in micritization of the grains and, when tunnels cross between grains, welds the grains together. This process forms a well-cemented layer (MacIntyre et al., 2000; Reid and MacIntyre, 2000; Reid et al., 2000). The in situ precipitation of carbonate, which leads to early lithification of the stromatolite-forming mats, is a result of prokaryotic activity (Visscher et al., 2000; Reid et al., 2003a).

Dominance of prokaryotic communities on the surfaces of modern marine stromatolites, is favored by the selective environmental pressure of periodic burial by sand (Andres and Reid, 2006; Kromkamp et al., 2007). In contrast to eukaryotes, which are typically killed when buried for periods of weeks to months, prokaryotes such as cyanobacteria are able to survive and recover from long burial events (Kromkamp et al., 2007). This attribute may contribute to the success of stromatolites in modern and past environments. Eukaryotic colonization (macroalgae: e.g., Batophora), during week- to month-long periods of exposure disrupts the lamination resulting from the cycling of prokaryotic mats, producing intervals of crude to no lamination within the stromatolite head.

It should be noted that trapping and binding alone does not form a stromatolite. The key factor in allowing development of a lithified laminated buildup is a hiatus in sediment accretion and corresponding development and lithification of a microbial biofilm. During long hiastral periods (i.e., several weeks), surfaces infested with the coccoid bacterium Solentia forms thicker, well-cemented lamina of fused ooid grains. Precipitation of aragonitic needles associated with the activity of sulfate-reducing bacteria is a key feature in carbonate precipitation within Bahamian stromatolites (Visscher et al., 2000). Precipitation resulting from anaerobic heterotrophic activity is supported by isotopic data (Andres et al., 2005).
Upward migration and/or new colonization

Type 1 mat (see Fig. 5)

Type 2 mat (see Fig. 5)

Type 3 mat (see Fig. 5)

EPS-rich biofilm formation and aragonite precipitation through biological activity

Trapping and binding by the microbial communities

Microbialites, Modern, Figure 4 (Continued)
Factors leading to the cycling of surface communities responsible for lamination in Bahamian stromatolites are currently under investigation (Andres and Reid, 2006). Both biological and environmental factors may be important (Figure 6; Seong-Joo et al., 2000; Reid et al., 2003a). Biological factors include microbial ecological interactions, and production and consumption of organic and inorganic compounds. Extrinsic factors include temperature, light, nutrients, and hydrodynamics, which could affect the stickiness of EPS and/or sediment supply (Figure 6).

Hypersaline and saline stromatolites
In contrast to open marine environments, where modern stromatolites are rare, living stromatolites are more common in hypersaline environments. This is because microbial communities are able to cope with high salinity, whereas most euksaryotes, such as macroalgae and burrowing/grazing invertebrates, which compete with or ingest cyanobacteria and disrupt lamination, are largely excluded (Fischer, 1965; Awramik, 1971, 1982, 1992; Walter and Heys, 1985; Riding, 2006).

Stromatolites in Shark Bay, Western Australia are the most famous stromatolites thriving in hypersaline water. They are found in the Hamelin Pool Marine Nature Reserve of the Shark Bay UNESCO World Heritage Site. Ranging from 55–70 ppt throughout the year (Playford, 1990), Hamelin Pool has approximately double normal marine salinity and hosts abundant and diverse stromatolites. Shark Bay stromatolites were discovered in 1954 by Johnstone, Playford, and Chase of the West Australian Petroleum Pty. Ltd (Playford and Cockbain, 1976). They were the first modern stromatolites discovered with sizes and shapes comparable to fossil counterparts (Logan, 1961; Logan et al., 1974; Playford and Cockbain, 1976; Playford, 1979; Bauld et al., 1979; Bauld, 1981; Burns et al., 2004). Like Bahamian stromatolites, Shark Bay structures are formed by both (1) microbial trapping and binding and (2) microbial precipitation. Both processes are important in the intertidal zone, forming coarse grained sandy stromatolites. Microbial precipitation is the primary accretionary mechanism in the subtidal zone of Shark Bay, forming muddy, micritic stromatolites (Reid et al., 2003b).

As a result of the dominant sandy textures of Shark Bay stromatolites and open marine stromatolites in the Bahamas, some authors have proposed that these stromatolites are not appropriate analogs for fossil stromatolites, which are generally display fine-grained, micritic microstructures (Awramik and Riding, 1988). Despite their overall coarse-grained texture, these modern stromatolites have micritic laminae formed as a result of microbially-induced precipitation (Reid et al., 2000; Visscher et al., 2000). Moreover, the ecological model developed for Bahamian stromatolites, in which lamination results from the cycling of prokaryotic communities on the stromatolite surface, is likely applicable to the growth of Shark Bay stromatolites. Indeed, a model of iterative accretion of laminae, which record both microbial and environmental fingerprints and progressively shape the emergent morphology, may serve as a conceptual model for the growth of fossil stromatolites.

In addition to Shark Bay, living stromatolites are also present in many other saline and hypersaline environments. Several saline lakes in Australia harbor stromatolites, including Lake Thetis, near Cervantes, lakes on Rottnest Island (Reitner et al., 1996), and Lake Clifton near Mandurah (e.g., Grey et al., 1990). Cyanobacterial stromatolitic domes have been described in the intertidal zone of Bermuda (e.g., Sharp, 1969; Golubic and Folke, 1978) and small crudely laminated knobs have been found in a hypersaline lake on Bonaire Island in the Netherlands Antilles (Golubic and Folke, 1978). Various islands in the Bahamas also have hypersaline lakes with well-developed microbial mats and stromatolites, including Storr’s Lake on San Salvador. Storr’s lake harbors alternating stromatolitic and thrombolitic buildups (Mann and Hoffman, 1984; McNeese, 1988; Neumann et al., 1988; Mann and Nelson, 1989; Pentecost, 1989; Zabielski, 1991).

Fresh water and continental stromatolites
Numerous examples of freshwater laminated microbialite have been published. One of the most widespread examples of these microbialites is travertines. Tufa stromatolites (meteogene travertine following Pentecost (2005)) are freshwater fluviatile tufas, which locally exhibit thick laminated crusts formed by calcified cyanobacteria (Pentecost, 1978; Riding, 1991b, 2000). Most of the precipitation of carbonate in these travertines results from physicochemical
Microbialites, Modern, Figure 5 Micrographs illustrating the main features of the surface mats responsible for the formation of modern marine stromatolites. Stromatolite lamination results from the iterative growth at the surface of the build up of three mat types: (1) Type 1 mats are dominated by filamentous cyanobacteria, which trap carbonate sand grains through EPS stickiness (A1-A4); (2) Type 2 mats consist of a continuous biofilm, draping the stromatolite surface (white arrows in B1-B3); the biofilm is composed of extracellular polymeric substances (EPS, B3) containing numerous heterotrophic bacteria. This biofilm rapidly lithifies as a result of precipitation of aragonite needles within the biofilm (B4); (3) Type 3 mats are characterized by endolith-infested ooid grains (yellow arrows in C1-C2), which appear gray and are fused together, below a surface biofilm (A1-B1-C1) binocular micrographs, (A2-B2-C2) petrographic micrographs, (A3-B3-C3) high vacuum scanning electron microscope images (SEM; chemical drying), and (A4-C3-C4) cryo-SEM (frozen samples) images.
Degassing of CO₂ through resurgence, cascades, or waterfalls. Photosynthetic uptake of CO₂ by cyanobacteria can, however, be responsible for the precipitation of calcium carbonate in slow running and low DIC streams (Verrecchia et al., 1995; Merz-Preiss and Riding, 1999; Arp et al., 2001). Relatively low DIC and high Ca²⁺ concentration is required for photosynthesis to influence carbonate alkalinity and enable precipitation (Arp et al., 2001, 2003).

Fresh water stromatolites are also present in Antarctic lakes, which are permanently ice-covered (Parker et al., 1981). These lakes are seasonally fed by glaciers, which modify salinity from fresh-to-saline while oxygen levels vary from anoxic to supersaturated. The stromatolites are formed through sediment trapping and binding as well as carbonate precipitation in cyanobacterial-dominated (e.g., *Phormidium*, *Oscillatoria*) microbial mats. Other non-marine environments with stromatolites include alkaline lakes, such as the Caldera lakes of Niuafo’ou Island (Tonga), with spectacular laminated buildups (Kazmierczak and Kempe, 2006).

Laminated deposits, referred to as terrestrial stromatolites (Verrecchia et al., 1995; Riding, 2000), are observed in “non-water-saturated” environments. These deposits have also been called lichen stromatolites (Klappa, 1979) and subaerial stromatolites (Riding, 1991b). They consist of laminar calcretes formed by microbial activity or in close association with microbial communities. Determining between biogenic and non-biogenic calcretes is often difficult as carbonate precipitation may be caused by physicochemical evaporation (e.g., Read, 1976), and/or cyanobacterial activity (e.g., Verrecchia et al., 1995).

**Thrombolite**

The term thrombolite was introduced by Aitken (1967) for microbial “structures related to stromatolites, but lacking lamination and characterized by a macroscopic clotted fabric”. Since this initial definition, thrombolites and stromatolites have been recognized as two distinct microbialite structures (Kennard and James, 1986). The key microstructure of thrombolites is mesoclots, which produce the clotted fabric (Shapiro, 2000). The mesoclots consist of polymorphic (simple spheroids to polylobate), millimeter to centimeter-sized, aggregates which display a variety of forms, from simple spheroids to polylobate masses (e.g., Shapiro, 2000). Clotted mesostructures have been described for many fossil thrombolites, which are mostly composed of fine-grained carbonate. Therefore, many authors define mesoclots as mostly composed of micrite or micropeloids (Kennard, and James, 1986; Dupraz and Strasser, 1999; Shapiro, 2000). However, modern thrombolites, as well examples from the Miocene, show agglutinated, clotted fabrics (Riding, 2000). The mesoclots of modern thrombolites thus consist of micrite, micropeloids, or agglutinated particles (Figure 2).
The clotted fabric of thrombolites can have a variety of origins. The fabric can be attributed “to the in situ calcification of coccoid or coccoid-dominated microbial communities” (Kennard and James, 1986). Other mechanisms involve bioturbation and bioerosion, and thrombolitic mesostructures within large Bahamian stromatolites are often due to disruption of the lamination by eukaryotic colonization (e.g., Batophora sp. and other algae).

In some cases, environmental conditions may trigger changes between thrombolite and stromatolitic fabrics. In Storr’s Lake (San Salvador, Bahamas), well-developed thrombolites alternate with stromatolitic layers (McNeese, 1988; Mann and Nelson, 1989). This alternation might be triggered by lake-level fluctuations and associated turbidity variability. This mechanism results in a succession of phototrophic-dominated (stromatolites) to heterotrophic-dominated communities (thrombolites) as surface communities, as modeled by Dupraz et al. (2006).

Many other modern lakes host thrombolite deposits: Lake Clifton, Lake Tethys, Lake Richmond and different lakes on Rottnest Island (Australia), Kelly and Pavillon lakes (British Columbia, Canada), lakes on Bonaire Island (Netherlands Antilles, southern Caribbean; Kobluk and Crawford, 1990), Lago Sarmiento (Patagonia, Chile), Poza Azul lake (Cuatro Cienegas, Mexico), Yellow Stone National Park (USA), Green Lake (Fayetteville, New York, USA). In addition, travertines showing thrombolitic features have been referred to as “Tufa Thrombolite” (Riding, 2000).

**Leiolite**

The term leiolite comes from the Greek word “leios,” meaning uniform or smooth and “lithos,” (meaning rock) and was originally applied to late Miocene (Messinian) deposits in Spain (Figure 2; Braga et al., 1995). Leiolite is characterized by relatively structureless, aphanitic, mesostructure, lacking lamination or clots, and can be a synonym for “cryptomicrobial” as used by Kennard and James (1986). Most authors tend to classify microbialites as either stromatolites or thrombolites, despite the fact that many of these deposits lack the defining mesostructures and would more appropriately be termed leiolite (see Riding, 2000). No examples of modern leiolite have been published. However, examples could include non-laminated, non-thrombolitic portions of Bahamian or Shark Bay stromatolites, carbonate crust formation in hypersaline lakes (Dupraz et al., 2004), and biological stabilization of sand dune by chasmolithic microorganisms (Hillgaertner et al., 2001).

Other types of microbialites

Microbial processes or the presence of microbes are often intimately related to sedimentary processes in modern carbonate systems, although the resulting deposits may not be considered as true microbialite and classified as stromatolite, thrombolite, or leiolite. These microbial carbonate deposits include metogene and thermogene travertine (Pentecost, 2005), where microbial influence creates specific microfabrics. Spectacular examples of travertines can be observed in many alkaline lakes, forming carbonate towers (e.g., Pyramid Lake, Nevada (Benson, 1994)), Lake Can, Turkey (Kempe et al., 1991), and Mono Lake, California (e.g., Bischoff et al., 1993; Riding, 2000). Carbonate chimneys are also formed in hydrothermal systems, where microbial activity is important (e.g., “Lost City” Hydrothermal Field; Kelley et al., 2001; Ludwig et al., 2006).

Other deposits that are difficult to classify include microbial calcrite – a terrestrial carbonate the formation of which is strongly influenced by bacteria and fungi (Jones and Wilson, 1986; Wright et al., 1988; Verrecchia and Verrecchia, 1994; Verrecchia et al., 1995). In addition, in continental environments, the formation of needle fiber calcite (NFC) is related to fungi activity (Verrecchia and Verrecchia, 1994; Cailleau et al., 2004, 2005). Mondmilch or Moonmilk – a soft paste-like or powdery cave deposit – includes important microbial populations, which influence precipitation (Thrailkill, 1976; Canaveras et al., 2006). Microbial mats are also involved in the formation of oncoids, which are unattached spherical stromatolites (e.g., Bathurst, 1966), and microbial activity may play a role in the formation of “whitings” (Thompson, and Ferris, 1990; Robbins, and Blackwelder, 1992).

**Processes of microbialite formation**

Early lithification is a key process in the formation of modern carbonate microbialites and is critical for the preservation of these structures in the fossil record. Lithification processes start at the time of deposition and continue during burial (diagenesis). Lithification consists of in situ precipitation of minerals, which progressively cement trapped grains (if present), and preserve the biosedimentary structures. Two closely coupled components are fundamental in carbonate precipitation within microbial mats: (1) the alkalinity engine and (2) the organic matrix in which this mineral is forming.

**The alkalinity engine**

The precipitation of carbonate minerals depends on the availability of carbonate ions, specific cations (e.g., Ca$^{2+}$, Mg$^{2+}$), and suitable nucleation sites. Carbonate minerals may precipitate when a solution is saturated with respect to that mineral. The degree of saturation is calculated via the saturation index of the specific mineral:

$$SI = \log(\text{IAP}/K_{ps})$$

IAP is the “ion activity product” and $K_{ps}$ is the solubility product of the corresponding mineral (solid phase). In the case of CaCO$_3$, both the carbonate alkalinity and the activity of the available cations are taken into account.
when calculating the saturation index (Stumm and Morgan, 1996):

\[ S_{ICaCO_3} = \log\left(\frac{\{Ca^{2+}\} \cdot \{CO_3^{2-}\}}{K_{sp}}\right). \]  

The activity of a given chemical compound corresponds to the concentration of that compound multiplied by the activity coefficient. The solubility products for aragonite and calcite are $10^{-6.19}$ and $10^{-6.37}$, respectively, at 25°C, 1 bar atmospheric pressure and 35 PSU salinity (Zeebe and Wolf-Gladrow, 2001). A solution is supersaturated with respect to a given mineral when SI is greater than 0, meaning that $K_{sp}$ is smaller than IAP. Experimental evidence showed that a supersaturation must be greater than 0.8 in order to have spontaneous precipitation of CaCO₃ (Kempe and Kazmierczak, 1994). Arp et al. (2001) used SI greater than 1 (i.e., a 10-fold supersaturation) as a prerequisite for carbonate precipitation in non-marine environment.

Carbonate ion activity, $\{CO_3^{2-}\}$, depends on the carbonate equilibrium. CO₂ dissolves in water to form carbonic acid (H₂CO₃). The amount of dissolved CO₂ is proportional to the partial pressure of CO₂ (pCO₂) in the gas phase in contact with the liquid (i.e., Henry’s Law). This value varies with pressure and temperature. Low temperature and high pressure allow for more CO₂ dissolution in water. Carbonic acid is weak acid that will only partially dissociate in an aqueous solution to produce H⁺ ion and the conjugate base. It will deprotonate as follows (with pKₐ values for fresh water):

\[ \text{H}_2\text{CO}_3^* = \text{HCO}_3^- + \text{H}^+ \]
\[ K_{a1} = 4.3 \times 10^{-7}; \quad \text{p}K_{a1} = 6.36. \]  

The two pKₐ values vary as a function of the salinity. For seawater conditions, pKₐ₁ and pKₐ₂ are 5.9 and 8.9, respectively (at 25°C, 1 bar atmospheric pressure and 35 PSU salinity; Zeebe and Wolf-Gladrow, 2001).

Availability of carbonate ions is a prerequisite for precipitation of carbonate minerals. The amount of bicarbonate and carbonate ions in solution denotes carbonate alkalinity, which is a part of total alkalinity, including borate, hydroxide, phosphate, and silicate. In marine and most of freshwater environments, phosphate and silicate are minor components, and the carbonate equilibrium typically drives global alkalinity. Various processes can have an impact of carbonate alkalinity, indirectly promoting precipitation or dissolution of carbonate mineral. The sum of processes that creates alkalinity represents the “alkalinity engine.” This engine is intrinsically driven when alkalinity is affected by microbial communities, or extrinsically driven when physico-chemical processes of the macro-environment cause alkalinity shifts (Figure 7).

### Intrinsic alkalinity engine (metabolism)

The way microbial communities acquire their energy and their source of carbon (i.e., through metabolism) can have a strong impact on local carbonate alkalinity. In a simplified view of the microbial carbon cycle, the production of organic matter through photosynthesis (oxygenic or anoxygenic) is coupled with the oxidation of organic carbon by aerobic (oxygen as oxidant) or
anaerobic (SO$_4^{2-}$, NO$_3^-$, Fe$^{3+}$, etc. as oxidant) respiration (Figure 8). Most microbial metabolites (products of metabolism) are efficiently recycled within the mats, completely closing the cycles of major elements. Many microbial mats function as light-driven engines, fueled only by sunlight.

The biogeochemical niche of microorganisms can be described through three overarching metabolic characteristics related to energy generation and biomass acquisition: the energy source, the electron donor used for energy generation, and the carbon source for biomass production. Each of these three properties have two possibilities: potential energy sources are light (photo-) or chemical redox reactions (chemo-), electron donors can be organic (organo-) or inorganic (litho-), and biomass can be derived from CO$_2$ fixation (autotrophy) or from carbon that is already fixed (heterotrophy). For example, a cyanobacterium derives energy from light (photo) uses water as electron donor (litho) and fixes CO$_2$ for biomass (auto-); it is hence designated as a photolithoautotroph (trophos means “to feed”). A typical aerobic bacterium that uses organic carbon is a chemoorganoheterotroph, etc.

Different types of metabolism impact carbonate precipitation and dissolution by taking up (autotrophy) or releasing (respiration) CO$_2$ and through production or consumption of organic acids. Changes in organic acid concentrations modify alkalinity and pH of the mats (Ehrlich, 1996; Visscher and Stolz, 2005). Mechanisms
that can promote precipitation of carbonate minerals include the following:

1. **Oxygenic photosynthesis** (producing O₂) by cyanobacteria (photolithoautotrophy). Uptake of CO₂ during photosynthetic activity leads to precipitation of carbonate in the sheath or in the direct vicinity of the cyanobacteria (Pentecost and Riding, 1986; Freytet and Verrecchia, 1998, 1999; Merz-Preiss and Riding, 1999; Riding, 2000) (Figure 9). The increase in alkalinity is attributed to an exchange of HCO₃⁻ and OH⁻ through the cell membrane (Figure 9). Photosynthesis is an important mechanism for microbialite formation in freshwater environment (Arp et al., 2001), where it may help overcome the kinetic barrier for CaCO₃ precipitation, even in highly supersaturated freshwater settings (Shiraiishi et al., 2008).

2. **Aerobic respiration** (chemoorganoheterotrophy) generally dissolves calcium carbonate through production of CO₂. However, precipitation can occur in a well-buffered alkaline environment when CO₂ produces carbonate ions. Carbonate minerals can also be formed when aerobic respiration consumes strong organic acids and produce CO₂ that will be degassed. This process is proposed for carbonate precipitation in tropical soil associated to the oxalate-carbonate cycle (Braissant et al., 2004). In the absence of O₂, some bacteria can generate energy through fermentation, which process uses the same compound (e.g., organic carbon, inorganic sulfur) both as electron donor and acceptor. Most fermentation of organic carbon will lead to dissolution of carbonate (Visscher and Stolz, 2005).

3. **Anaerobic respiration**: certain bacteria can oxidize organic matter using respiratory chains that do not use O₂ as final electron acceptor (also chemoorganoheterotrophy). These bacteria can use, e.g., sulfate (sulfate-reduction), nitrate (nitrate-reduction), iron (iron-reduction), manganese (manganese reduction), or even HCO₃⁻. These types of metabolism can promote precipitation of carbonate through various processes (e.g., Visscher and Stolz, 2005): consumption of organic acid, production of base (e.g., NH₃), removing precipitation inhibition (removing of SO₄²⁻), etc. Sulfate reduction is the main process in marine environment due to high concentration of SO₄²⁻ in seawater.

4. **Chemolithoautotrophy**: Some bacteria do not oxidize organic matter to generate energy. They will instead use the chemical energy (chemo) produced by the oxidation of inorganic (litho) electron donors such as H₂, CO, Fe³⁺, NH₄⁺ and HS⁻ in order to fix CO₂ (autotrophy). Sulfide (in sediments) and ammonium (in the water column) oxidation are the most abundant chemolithoautotrophic electron donor in marine environment. Aerobic sulfide oxidation and ammonium oxidation is likely to induce dissolution of carbonate minerals, whereas anaerobic sulfide oxidation (e.g., using nitrate as electron acceptor) may induce precipitation (Visscher and Stolz, 2005). However, most of these types of metabolism are all autotrophic, i.e., fixing CO₂. The uptake of CO₂ can increase carbonate alkalinity and induce precipitation of carbonates in similar way that physicochemical degassing of CO₂ does.

### Extrinsic alkalinity engine

The determination of alkalinity-driving forces that form microbialites is generally a complex task. In many cases, a combination of intrinsic and extrinsic factors is responsible for precipitation. There are however, a few examples where the macroenvironment acts as a major player in microbial mat lithification. The two important physicochemical processes that can lead to carbonate precipitation in microbial mats are water evaporation and CO₂ degassing. Even in these cases, however, the microbial communities of the mats can serve as substrates for physicochemical carbonate precipitation and affect mineral mineralogy or morphology.

In the marine environment, solar evaporation of water may lead to the formation of evaporitic minerals (e.g., Warren, 2006). Although a variety of carbonate minerals can be produced through this mechanism (e.g., calcite, aragonite, Mg-calcite, dolomite, and magnesite), evaporites largely consist of halite and gypsum. Examples of microbial mats associated with gypsum deposits have been described (e.g., Babel, 2004). Moreover, bacteria trapped in gypsum crystals are able to survive for millions of year (Vreeland et al., 2000, 2007). The specific role of microbes in gypsum nucleation is, however, not clear as the evaporation processes generally obscure microbial signatures. For reviews on evaporites, see Yechieli, and Wood (2002) or Warren (2006).

Precipitation of carbonate can also result from degassing of CO₂ and forms travertines. Travertines represent chemically-mediated continental carbonate deposits that form around sinter sources along streams and lakes (Pentecost, 2005). The precipitated minerals, consisting of aragonite or calcite, are mainly related to the release of CO₂ from the system, resulting in supersaturation with respect to calcium carbonate. This can be illustrated by the buffering equation of the carbonate:

\[
Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O
\]  

Removal of CO₂ through degassing will drive this equation to the right, promoting precipitation of CaCO₃. Similarly, increasing dissolved CO₂ drive the equation to the left, resulting in dissolution of calcium carbonate.

Travertines can be classified based on the source of the CO₂ that is degassed (Pentecost, 2005). “Meteogene travertines” are formed by degassing of meteoric carbon dioxide, whereas “thermogene travertines” are formed by the degassing of hydrothermal CO₂. In meteogene travertines, the CO₂ can originate from the atmosphere or from the soil-zone. In thermogene travertines, the bulk of the CO₂
Microbialites, Modern, Figure 9 Role of photosynthesis in the precipitation of carbonate minerals. (a) Photomicrograph of Microcoleus spp., a filamentous cyanobacterium showing the trichoma (chains of cells) embedded in a sheath. (b) Longitudinal section through a freshwater travertine (Sarine River, Switzerland) showing precipitation of CaCO₃ in the sheath of the filamentous cyanobacteria Oscillatoria sp. and Phormidium sp. (c) Perpendicular section through the same travertine. (d) Model of photosynthetically-driven carbonate precipitation. CO₂ dissolve in water, forming bicarbonate ions (the most common form of DIC between pH 5 and 9). The bicarbonate is taken up by cells of the trichoma; the enzyme carbonic anhydrase inside the cells produces CO₂ (used for photosynthesis) and OH⁻. The release of hydroxyl ions outside the trichoma increases the alkalinity in the surrounding area of the cyanobacteria and induces precipitation of CaCO₃ (provided Ca²⁺ ions are available).
**EPS binding capacity**
Inhibition of precipitation

Production of EPS by cyanobacteria and other microbes

Alveolar EPS matrix with carboxyl group

Adsorption of Ca$^{2+}$ (and other cations)

Main acidic groups that can bind cations (e.g., Ca$^{2+}$, Mg$^{2+}$) within EPS

**Main acidic groups**

- HCO$_3^-$
- CO$_3^{2-}$

**EPS alteration leading to precipitation of carbonates**

**Biologically-induced mineralization**

- EPS Degradation
  - Microbial degradation of EPS liberating calcium and inorganic carbon (increasing the carbonate mineral saturation index)
  - Various types of mineralogy and morphology
- EPS binding capacity
  - Inhibition of precipitation

**Biologically-influenced mineralization**

- Extrinsic Supersaturation of EPS
  - Forcing of mineralization on the EPS matrix.
  - Organic matrix and kinetic of precipitation can control mineral morphology.
- Intrinsic Supersaturation of EPS:
  - Steric Hindrance
  - Changes in availability of functional groups (steric hindrance).
  - Cation binding capacity does not reflect abundance of functional groups

**EPS alteration**

- Alteration of the EPS matrix can end this inhibition, allowing carbonate precipitation to commence.
- EPS alteration is achieved via two main pathways: (1) EPS degradation, (2a) extrinsic, and (2b) intrinsic supersaturation of the EPS binding capacity (modified from Dupraz and Visscher, 2005).

**Microbialites, Modern, Figure 10**
Mechanistic role of the organic EPS matrix in microbial mat lithification. The exopolymeric substances are produced by various groups of bacteria (especially cyanobacteria). EPS can bind large amounts of cations (i.e., Ca$^{2+}$), reducing the calcium carbonate saturation index, inhibiting precipitation. Alteration of the EPS matrix can end this inhibition, allowing carbonate precipitation to commence. EPS alteration is achieved via two main pathways: (1) EPS degradation, (2a) extrinsic, and (2b) intrinsic supersaturation of the EPS binding capacity (modified from Dupraz and Visscher, 2005).
originates from thermal processes. These hydrothermal systems are commonly associated with regions of recent volcanic or tectonic activity. It is important to note that the term travertine has variable usage in the literature. Many authors use travertine only for hot water deposits, using “tufa” for cold-water deposits (Pedley, 2000). Other authors use tufa for actively-forming deposits and travertine for non-active, fossil forms (see Chapter Tufa, Freshwater).

Although carbonate precipitation in travertines and tufas is driven by abiotic processes, precipitation often initiates on organic substrates, e.g., leaves, woods, algae, or microbial mats. In these cases, mineralogy and morphology are strongly influenced by the organic matrix (e.g., Fouke et al., 2000; Farmer, 2000; Pentecost, 2005; Turner and Jones, 2005). Microbial mats at hot springs, for example, can therefore have a profound impact on thermogene travertine by providing templates for mineralization, even though precipitation is due to vigorous CO2 degassing (Farmer, 2000; Fouke et al., 2000). Travertine formation is thus an example of biologically-influenced mineralization, since the carbonate precipitation is not due to biological activity, but microorganisms indirectly affect the characteristics of the resulting minerals.

The organic matrix (EPS)

Microbial communities generally produce extracellular polymeric substances (EPS; see Chapter Extracellular Polymeric Substances (EPS)). This organic matrix is an important part of the microbial mat, preventing desiccation, retaining essential nutrients, protecting against UV radiation, and providing water channels for transport of metabolites and signaling compounds (Decho, 1990, 2000). Cyanobacteria are often considered to be the major producers of EPS. However, many other bacteria can produce EPS matrix, including aerobic and anaerobic (chemoorganoheterotrophs, anoxygenic phototrophs, and chemolithoautotrophs (De Philippis et al., 1998, 2001; Stal, 2000, 2003; Richert et al., 2005). Anaerobic bacteria, especially sulfate-reducing bacteria, which play a key role in carbon metabolism in marine microbial mats (Thode-Andersen, and Jorgensen, 1989; Canfield and DesMarais, 1991; Visscher et al., 1991), are known to produce large amounts of EPS (Bosak and Newman, 2005; Braissant et al., 2007).

The EPS matrix is generally the location where the carbonate minerals nucleate and grow (Dupraz et al., 2004). EPS contains negatively-charged acidic groups (e.g., carboxyl, sulfhydril, amine, hydroxyl groups), which can bind a large amount of mono- and divalent cations, notably Ca\(^{2+}\) (Braissant et al., 2007). This binding capacity may inhibit the precipitation of carbonate minerals by depleting calcium from the surrounding microenvironment (Figure 10). The saturation index of calcite depends on the carbonate alkalinity and the availability in calcium. Even if carbonate ions are in solution, the lack of free Ca\(^{2+}\) may inhibit precipitation (Dupraz and Visscher, 2005). Therefore, the physicochemical properties of the polymer matrix, such as the acidity or composition of the functional groups associated with the EPS, are key factors in the formation of modern microbialites.

In order to precipitate calcium carbonate in microbial mats, the Ca\(^{2+}\)-binding capacity of the EPS has to be reduced. Various mechanisms have been proposed (e.g., Dupraz and Visscher, 2005), which can be grouped into two main processes (Figure 10): (1) degradation of the EPS matrix and (2) intrinsic or extrinsic supersaturation of cation-binding sites (e.g., Dupraz and Visscher, 2005). As EPS is a sugar polymer, it serves as metabolic substrate for (chemooorganoheterotrophic bacteria. Depolymerization (hydrolysis) of EPS, possibly by fungi and heterotrophs, including spirochetes (Harwood and Canale-Parola, 1984; Weaver and Hicks, 1995), must occur before sugar monomers are available. Microbial degradation of EPS liberates the calcium and produces inorganic carbon (under alkaline conditions, the dominant species is carbonate ion). Alternatively, if the amount of Ca exceeds the number of binding sites in the EPS, free calcium will be present and available for precipitation. These conditions can be achieved through extrinsic factors (the environment) through continuous input of Ca. It can also result from the steric hindrance of acidic groups within the EPS. Although EPS may indeed possess abundant functional groups capable of binding Ca\(^{2+}\) or Mg\(^{2+}\) ions, these groups may be “sterically-inhibited” (i.e., blocked) via molecular-scale interactions into a structurally complex EPS (Figure 10).

Summary

Microbialites are “organosedimentary deposits that accrete as a result of a benthic microbial community, which traps and binds sediment and/or forms the locus of mineral precipitation” (Kennard and James, 1986). Living examples of these structures can be found in many modern environments including marine, hypersaline, freshwater, and even continental settings. Microbialites are classified based on mesostructure as stromatolites (laminated), thrombolites (clotted), and leiolites (structureless). Among the most spectacular examples are modern stromatolites, which were first discovered in a hypersaline lagoon of Shark Bay in 1954. The only known examples of stromatolites presently forming in open marine environments are in the Bahamas. Lamination in these coarse-grained structures results from the iterative growth of three different types of microbial mats on the surfaces of the build-ups. These microbial communities are responsible for successive “trapping and binding” of sediment (type 1) and in situ precipitation of calcium carbonate (types 2 and 3). Both processes are essential for stromatolites formation. Modern thrombolites and leiolites are less well-studied than modern stromatolites.

Early lithification is a key process in the formation of modern microbialites. Lithification consists of in situ precipitation of minerals, which preserves the biosedimentary structures. Two key and closely coupled components
involved in carbonate precipitation within microbial mats are (1) the alkalinity engine and (2) the organic matrix. The alkalinity engine, which effectively changes the saturation index, is intrinsically driven when microbial metabolism is the dominant process causing carbonate precipitation. The alkalinity engine is extrinsically driven when the macroenvironment is responsible for microbial mat lithification. In both the cases, the organic matrix of extracellular polymeric substances (EPS), which embed the microbial communities, is the physical location where carbonate minerals nucleate and grow. Fresh EPS contains negatively-charged acidic groups, which bind large amount of cations (e.g., Ca\(^{2+}\)), inhibiting CaCO\(_3\) precipitation. In order to precipitate carbonate, this inhibition has to be reduced by degradation of the EPS or by oversaturation of the cation-binding capacity. Microbial activity is ultimately responsible for EPS production and degradation leading mineral precipitation.

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Microbialites are in place benthic sediments produced by the interaction of microbial growth and metabolism, cell surface properties, and extracellular polymeric substances (EPS) with mineral precipitation and binding detrital sediment and/or forming the accreted as a result of a benthic microbial community trapping. The early lithification that is essential for the accretion and preservation of benthic microbial carbonates is both biologically mediated and environmentally dependent. Consequently, microbialite history reflects not only microbial mat evolution, but also long-term changes in seawater and atmospheric chemistry that have influenced microbial metabolism and seawater carbonate saturation state.

Microbialites are in place benthic sediments produced by microbial processes. The term “microbialite” has been most widely used to describe carbonate stromatolites, thrombolites, and similar structures that occur as domes and columns in the shallow waters of lakes and seas, but it can also apply to many additional authigenic accumulations in which microbes are locally conspicuous, such as

Microbialites are biomineralizations of organic matter in sediments and rocks. They are formed by the interaction of microorganisms with their environment, leading to the precipitation of minerals such as calcium carbonate, which can form the characteristic layers or laminations of microbialites. These structures are important for understanding the role of microorganisms in the Earth's history and the evolution of life.

Cross-references

- Biofilms
- Extracellular Polymeric Substances (EPS)
- Microbial Biomineralization
- Microbial Mats
- Microbialites, Stromatolites, and Thrombolites
- Organomineralization
- Sediment Diagenesis – Biologically Controlled
- Tidal Flats
- Tufa, Freshwater

MICROBIALITES, STROMATOLITES, AND THROMBOLITES

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Microbialite Definition

Microbialites are “organismsedimentary deposits that have accreted as a result of a benthic microbial community trapping and binding detrital sediment and/or forming the locus of mineral precipitation” (Burne and Moore, 1987, pp. 241–242).
some tufa, travertine, speleothem and spring, seep, and vent deposits. A series of terms and definitions were proposed between 1967 and 1987 in attempts to distinguish benthic sediments formed by microbial sediment trapping and/or precipitation. The first term, cryptalgal, was proposed by Aitken (1967, p. 1163) for rocks or rock structures “believed to originate through the sediment-binding and/or carbonate precipitating activities of non-skeletal algae.” The second was redefinition of stromatolite by Awramik and Margulis’ (1974) as “organosedimentary structures produced by sediment trapping, binding and/or precipitation as a result of growth and metabolic activity of organisms, primarily blue-green algae.” Both Aitken (1967, p. 1163) and Awramik and Margulis (1974) used blue-green algae to indicate cyanobacteria, and therefore Kennard and James (1986, p. 496) replaced cryptalgal by cryptomicrobial. The third term was microbialite (Burne and Moore, 1987), which effectively repeated Awramik and Margulis’ (1974) definition of stromatolite, thereby broadening its scope. The primary focus of all these definitions was stromatolites and thrombolites.

Research during the 1900s revealed many details concerning the nature and history of stromatolites, but it also led to nomenclatural uncertainty as it became necessary to accommodate newly recognized deposits, such as thrombolites. Aitken (1967, p. 1164) sought to clarify use of “algal” in carbonate rock descriptions by distinguishing “rocks composed of the remains of skeletal calcareous algae . . . from those formed by noncalcareous blue-green . . . and green . . . algae.” He termed the latter cryptalgal, defined as rocks or rock structures “believed to originate through the sediment-binding and/or carbonate-precipitating activities of nonskeletal algae” (Aitken, 1967, p. 1163). Aitken (1967, Fig. 1) regarded both thrombolites and stromatolites as cryptalgal deposits, distinguishing by their respective clotted and laminated macrofabrics. However, Awramik and Margulis’ (1974) proposed a broader definition of stromatolite as “megascopic organosedimentary structures produced by sediment trapping, binding and/or precipitation as a result of growth and metabolic activity of organisms, primarily blue-green algae.” This therefore subsumed thrombolite as a category within stromatolite.

As a result of these and other developments, by the 1980s stromatolites could variously be regarded as (1) microbial and laminated (e.g., Kalkowsky, 1908; Hofmann, 1969; Krumbein, 1983), (2) microbial but not necessarily laminated (Awramik and Margulis, 1974), and (3) laminated but not necessarily organic (Semikhatov et al., 1979) (see Riding, 1999, p. 324). Since the wording of Burne and Moore’s (1987, pp. 241–242) definition of microbialite closely followed that of Awramik and Margulis’ (1974) definition of stromatolite, it therefore required return to a narrower definition of stromatolite, as layered or laminated structures. This relatively narrow definition was intended by Kalkowsky (1908) and is implied by his term stromatolith; stromat, Greek for “to spread out,” suggests a layer. Burne and Moore’s (1987) new term, microbialite, encompassed more diverse benthic microbial deposits. The outcome of these developments was that stromatolite and thrombolite could be considered as equal categories within microbialite, mirroring Aitken’s (1967, Fig. 1) classification of stromatolites and thrombolites within cryptalgal carbonates. Subsequently, these and other varieties of microbialite have commonly been distinguished by their dominant internal macrofabric: stromatolite (laminated), thrombolite (clotted), dendrolite (dendritic), and leiolite (aphanitic) (Riding, 2000, pp. 189–195) (Figure 1).

Given the importance of stromatolites in the development of these concepts, this orderly arrangement of terms under the microbialite umbrella relied heavily on stromatolites being microbial structures. However, as soon as Kalkowsky’s (1908) article was published, researchers began to point out similarities between stromatolites and abiogenic precipitates (Reis, 1908; Bucher, 1918), and this chorus has continued ever since. Logan et al. (1964, p. 69) suggested recognition of “inorganic stromatolites”; Hofmann (1973, Fig. 5) showed the difficulty of distinguishing stromatolites from other laminites; and Semikhatov et al. (1979, p. 994) proposed a descriptive rather than purely genetic definition of stromatolite. Reports of essentially abiogenic stromatolites have persisted, especially in the Proterozoic (Grotzinger and Read, 1983; Grotzinger, 1989a; Pope et al., 2000). Attempts to separate abiogenic and biogenic stromatolites are obstructed by hybrid stromatolites (see below, Stromatolites) consisting of millimetric alternations of abiogenic crust and lithified microbial mat (Riding, 2008). Consequently, although microbial mat models seem to apply well to all or most thrombolites, dendrolites and leiolites, they do not account for all stromatolite-like deposits, particularly in the Precambrian. The implications of this complication for stromatolite interpretation as well terminology have still to be worked out.

Microbialites in space and time

Microbes occupy a very broad range of environments, including waters of widely differing chemistry and composition, and their involvement in sedimentation is equally varied. Most microbialites are carbonate (e.g., aragonite, calcite, dolomite) in composition, but siliceous, phosphatic, iron, manganese, and sulfate examples also occur. The microbes in microbialites are dominantly bacteria, including cyanobacteria, together with small algae. From the perspective of biocalcification, microbialite carbonates are bioinduced. Bacteria in general exert relatively weak control over the organic site and mineralogy of calcification, and present-day microbial carbonates appear to be most widespread in environments where precipitation is inorganically favored. This environmental dependence means that where rapid cooling and degassing strongly favor mineral precipitation irrespective of organic intervention, as in hot water springs and vents, the overall sedimentary contribution of microbes may be

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relatively minor. In such cases, they influence the fabric more than the process of deposition. In contrast, where waters are less saturated for minerals, microbial activity can play a major role in sediment precipitation. These differing roles are reflected in the form and fabrics of the resulting deposits.

Sensitivity to environmental influence is also reflected in the secular distribution of microbialites. Without ruling out the influence of other factors – such as competition with other organisms – marine microbial carbonate abundance through time significantly reflects fluctuation in carbonate saturation state (Fischer, 1965; Grotzinger, 1990; Riding and Liang, 2005). Microbial carbonates can therefore be relatively sensitive proxies for seawater carbonate chemistry, and for atmospheric composition, since cyanobacterial sheath calcification is promoted by increased bicarbonate uptake (Merz, 1992) when CO₂ levels decline (Riding, 2006).

Scope
The term microbialite, with its emphasis on benthic microbial deposits, encompasses a wide range of sediments; what does it not cover? “Benthic” would seem to exclude both allochthonous and interstitial microbial sediments.

If so, then biogenic “whiting” deposits produced by photosynthetically induced nucleation of small CaCO₃ crystals in the water column are not microbialites, and neither are diagenetic bacterially induced precipitates such as cements and concretions. However, these somewhat artificial distinctions could be blurred, as in some soil crust and beachrock deposits. On the other hand, “microbial” must exclude abiogenic stromatolites, identified in both Precambrian and Phanerozoic sediments (Grotzinger and Read, 1983; Pope et al., 2000). This is a complication, especially since microbialite (Burne and Moore, 1987) is based on a preexisting definition of stromatolite proposed by Awramik and Margulis (1974), and arose from a need for a general term to encompass stromatolite, thrombolite, and related deposits. Such difficulties of definition should be resolved by increased understanding of both present-day and ancient examples of these important and geologically widespread deposits.

**Stromatolites**

**Definition**
Stromatolites are laminated benthic microbial deposits (Riding, 1991).
Introduction

Kalkowsky (1908) proposed the term stromatolite (Stromatolith) for columns and domes in early Triassic playa lake oolites, but similar structures were already long known from examples such as those in the late Cambrian of New York State that Steele (1825, pp. 17–18, pl. 2) described as “calcareous concretions” and Hall (1883) named Cryptozoom (Figure 2). The search for present-day analogs of these ancient stromatolites led from freshwater tufa (Walcott, 1914; Roddy, 1915) and marsh deposits (Black, 1933) to marginal marine domes (Ginsburg et al., 1954) and columns (Logan, 1961). These discoveries strongly supported Kalkowsky’s (1908) inference that stromatolites are essentially microbial deposits. But optimism that they could provide appropriate analogs for all ancient marine stromatolites was tempered by studies of Precambrian examples that include significant abiogenic precipitated components (e.g., Serebryakov, 1976, p. 633; Grotzinger and Knoll, 1999, p. 314). Some definitions of stromatolite have therefore encompassed both microbial and abiogenic layered/laminated authigenic crusts that characteristically form domical and columnar morphologies.

Definitions of stromatolite range from that of Kalkowsky (1908) to that of Semikhvatov et al. (1979, p. 993): “a stromatolite is ... an attached, laminated, lithified, sedimentary growth structure, accretionary away from a point or limited surface of initiation. Although characteristically of microbial origin and calcareous composition, it may be of any origin, composition, shape, size, or age.” Understanding of Kalkowsky’s view has been hindered by a definition incorrectly attributed to him by Krumbein (1983, p. 499): “stromatolites are organogenic, laminated, calcareous rock structures, the origin of which is clearly related to microscopic life, which in itself must not be fossilised.” Kalkowsky (1908) did not write this (see Riding, 1999, p. 323), but it has been repeated as if it were a literal translation from his paper (e.g., Ginsburg, 1991, p. 25; Feldmann and McKenzie, 1998, p. 201; Grotzinger and Knoll, 1999, p. 316; McLoughlin et al., 2008, p. 96). Compounding this mistake, the somewhat awkward wording (use of “must not” rather than “need not”) has been cited as “paradoxical,” and “confusion” to be avoided, and also as an example of the deficiencies of genetic definitions (Grotzinger and Knoll, 1999, p. 316; McLoughlin et al., 2008, p. 96). In his 1908 paper, Ernst Kalkowsky did not provide a specific definition of stromatolite, but he did repeatedly emphasize that it is a laminated organic structure. He thought that the life forms involved were “niedrig organisierte planzliche Organismen” (simple plantlike organisms, Kalkowsky, 1908, p. 125). It is therefore reasonable to conclude that Kalkowsky essentially regarded stromatolites as laminated microbial deposits (Riding, 1999).

However, at the same time that support was growing for Kalkowsky’s (1908) biogenic interpretation of stromatolites, the contrary view was also being expressed. Reis (1908) interpreted stromatolites as inorganic precipitates, Bucher (1918) compared them with hot spring sinter. Semikhvatov et al. (1979, p. 994) were also concerned by the similarities between microbial stromatolites and “morphologically similar structures of nonmicrobial origin” such as those in caves and hot springs, and therefore suggested their descriptive definition. Subsequent research has shown that some stromatolite-like deposits are essentially abiogenic (e.g., Grotzinger and Read, 1983; Grotzinger and James, 2000, p. 9). Pope et al. (2000, p. 1139) contrasted microbial and abiogenic stromatolites, and considered “isopachous stromatolites to have been dominated by chemogenic precipitation in the absence of microbial mats, and the growth of peloidal stromatolites to have been controlled by sedimentation in the presence of microbial mats.”

Fabric criteria can be used to distinguish abiogenic evenly layered sparry crust from fine-grained lithified microbial mats with uneven layering. In well-preserved examples, it should therefore be possible to tell abiogenic and biogenic stromatolites apart. However, as noted under microbialite, the millimetric interlayering of these two fabrics that can occur in hybrid stromatolites (Riding, 2008) makes such straightforward separation impossible. Consequently, stromatolites are regarded here, essentially, as laminated benthic microbial deposits, but they can contain abiogenic precipitates and be intimately inter-layered with them. In the marine realm, there is a strong secular perspective to this. Many Phanerozoic and Neoproterozoic stromatolites are probably essentially lithified microbial mats, whereas many older examples probably contain at least some precipitated abiogenic crust.

Stromatolite types

Stromatolite micro- and macrofabrics commonly intergrade with those of dendrolites and thrombolites (Figure 3). Their formation can involve up to three main processes: microbial precipitation, inorganic precipitation, and grain trapping (Figure 4). The main types represent
Lithified microbial mat, sparry crust, and combinations of these ("hybrid stromatolite").

Lithified microbial mat stromatolites exhibit a variety of intergradational fabrics. Two main types are fine-grained and coarse agglutinated. They form diverse stratiform, domical and columnar structures and, overall, tend to show relatively uneven to discontinuous layers with relatively poor inheritance and can include abundant fenestrae. Fine-grained stromatolites appear mainly to be products of synsedimentary microbial precipitation and are dominated by fine-grained (micrite, microspar) and filamentous fabrics. Fine-grained microfabrics are typically clotted and peloidal and are probably largely produced by heterotrophic bacterial calcification (e.g., dissimilatory sulfate reduction) of EPS and other cell products. Filamentous fabrics tend to be more crudely layered and grade to thrombolitic. They have been termed porostromate (Pia, 1927, pp. 36–40), skeletal (Riding, 1977), and calcimicrobial (James and Gravestock, 1990). Coarse agglutinated stromatolites/thrombolites are produced by trapping sandy sediment by uncalcified EPS and erect filaments that can include microalgae. Present-day examples include some Shark Bay and Lee Stocking Island columns. They often have crudely layered macrofabrics (Logan, 1961) and have been termed thrombolitic stromatolites (Aitken, 1967).

Sparry crust (Riding, 2008) can form stromatolite-like abiogenic precipitates (Grotzinger and Rothman, 1996) characterized by even, often isopachous, laterally persistent layers with good inheritance (Pope et al., 2000). They have been most widely recognized in the Paleoproterozoic and Mesoproterozoic (Grotzinger and Knoll, 1999, Fig. 6a, b), with microdigitate forms occupying peritidal environments (Grotzinger and Read, 1983) and isopachous laminite (Jackson, 1989) relatively deeper water facies.
Hybrid crust stromatolites (Riding, 2008) typically consist of light-dark, often millimetric, alternations of sparry and fine-grained crust. These build stromatolites with well-developed even, although not usually isopachous, layering that is laterally quite persistent with generally good inheritance, as in some Conophyton (e.g., Walter, 1972, p. 86) (Figure 5). This layering is therefore intermediate in regularity between that of stromatolite-like sparry crust and fine-grained crust stromatolites. Hybrid crust appears to be a major component of Palaeoproterozoic (e.g., Sami and James, 1996) and Mesoproterozoic stromatolites (e.g., Petrov and Semikhatov, 2001), which can include very large domical and conical examples. Laminated freshwater cyanobacterial mats in shallow lakes of Andros Island show similarities to hybrid stromatolite, with fine-grained incipiently lithified cyanobacterial mats alternating with laminar fenestrae and elongate open voids (Monty, 1976, Fig. 4). If this structure were early lithified, it could resemble some Conophyton and Baicalia fabrics, as Bertrand-Sarfati (1976) suggested.

Intertidal mats and wrinkle structures
At the present day, intertidal and supratidal sediments are commonly colonized by cyanobacteria dominated mats in siliciclastic (e.g., Cameron et al., 1985; Stal et al., 1985), evaporitic (e.g., Gerdes et al., 2000), and carbonate (Black, 1933; Logan et al., 1974) environments (Figure 6). Where these mats are cohesive, but insufficiently early lithified, they do not show significant accretion but nonetheless stabilize layers of sediment. These microbialites that were un lithified during their formation can be preserved in place, and also imprinted and disrupted, e.g., by desiccation and water movement. They and their incorporated sediment may be cracked, curled, and folded, and this synsedimentary deformation can be preserved after burial. Such patterned surfaces in
Mesoproterozoic limestones were named *Kinneyia* by Walcott (1914, p. 107, pl. 11, Fig. 3) and subsequently linked to "Runzelmarken" (wrinkle marks, Häntzschel and Reineck, 1968) and other mat-related structures (Hagadorn and Bottjer, 1997). They have also been termed microbially induced sedimentary structures (MISS) (Noffke et al., 1996). The exact origins of these bedding plane structures are probably complex and varied (Porada et al., 2008) but they have been widely linked to deformation of microbial mats and have been used to infer the presence of mats in the Proterozoic (Horodyski, 1982) and Archaean (Noffke et al., 2006).

**Siliciclastic stromatolites**

In contrast to wrinkle structures, siliciclastic stromatolites can possess large domical morphologies and considerable synoptic relief. They are much scarcer than carbonate stromatolites, and are only known from mixed siliciclastic-carbonate environments that provide both siliciclastic grains and the early lithification required to maintain their support (Martin et al., 1993). Most examples are Palaeozoic, e.g., Ordovician (Davis, 1968), Devonian (Draganits and Noffke, 2004), Carboniferous (Bertrand-Sarfati, 1994), and Permian (Harwood, 1990), but diverse examples also occur in the late Miocene of South-east Spain (Martin et al., 1993; Braga and Martin, 2000). Based on the proportion of siliciclastic grains they contained, Martin et al. (1993) recognized three compositional types: “carbonate,” <10%; “siliciclastic-carbonate,” 10–50%; and “sandstone,” >50%. They are typically metric, locally decametric, domes with both stromatolitic and thrombotic macrofabrics, associated with marginal marine beach (Braga and Martin, 2000), fan-delta and conglomeratic debris-flow deposits (Martin et al., 1993), and also with oolitic stromatolites–thrombolites (Braga et al., 1995). Sediment within the more siliciclastic domes includes abundant quartz, mica, and lithic fragment sand, and occasional quartz and metamorphic rock granules and pebbles.

**Thrombolites**

**Definition**

Thrombolites (Greek: *thrombos*, clot; *lithos*, stone) are "cryptalgal structures related to stromatolites, but lacking lamination and characterized by a macroscopic clotted fabric" (Aitken, 1967, p. 1164).

**Introduction**

Aitken’s (1967) seemingly straightforward definition of thrombolite contained the seeds of more confusion than might have been anticipated. Since stromatolites are generally regarded as internally laminated; it could be expected that thrombolites are internally clotted. However, stromatolitic laminae are internal features of microbial carbonate, whereas "clot" could be used to describe both the external shape and internal structure of microbial carbonate (Figure 1). Aitken’s (1967) descriptions of Cambro-Ordovician examples, and his emphasis that thrombolite clotted fabric is macroscopic, have directed most researchers to regard “clots” as centimetric patches of microbial carbonate within interstitial sediment. In this view, a thrombolite dome consists of numerous such clots within lighter colored interstitial detrital matrix. Consequently, the dome has an overall macroclotted fabric, but the individual clots themselves are not necessarily internally composed of smaller clots, although they can be. This contrasts with stromatolitic domes in which each individual stromatolite is internally laminated and its shape is described separately, e.g., as domical, columnar, digitate, etc.
Aitken’s (1967) concept of clots as patches of microbial carbonate within matrix is appropriate where they are irregularly rounded, but in digitate thrombolites the patches elongate into decimetric branches (e.g., Armella, 1994, Fig. 9) and use of “clot” to describe these is awkward. If some of Aitken’s (1967) thrombolite domes contain dendritic fabrics, as seems likely, then why did he stress their clotted macrofabric? A likely explanation is that, in domes composed of radial branches, any section other than a vertical one through the dome center (e.g., Armella, 1994, Fig. 9) tends to show clot-like rounded outlines of oblique sections of branches. If this is correct, then to be consistent the branches should also be regarded as clots. This approach was followed by Armella (1994) and Kennard (1994) (although they used thromboid as an equivalent term to clot) to describe rounded lobate patches and elongate columns alike. There seems little doubt that this is what Aitken (1967) intended, and Shapiro (2000, p. 166) recognized that he used clot to describe the thrombolite columns themselves. Nonetheless, in describing similar branched Cambro-Ordovician thrombolites (Favosamaceria), Shapiro (2000) and Shapiro and Awramik (2006) broadened “clot” to refer to both millimetric clots within the branches and centimetric clots embedded in interstitial sediment. And there is a further complication. Aitken (1967) suggested that poorly laminated columns at Shark Bay are “thrombolitic stromatolites.” As a result, in addition to describing Cambro-Ordovician domes with well-defined fine-grained clots, thrombolite has been applied to agglutinated present-day microbial columns in which the clots are much less distinct patchy fabrics.

These complications of usage still require clarification. At present, it is safe to say that “clot” (and the equivalent terms mesoclot and thromboid) has not been used consistently in thrombolite studies. It has been applied to millimetric patches within microbial carbonate, to centimetric lobate patches and also extended columns of microbial carbonate surrounded by detrital carbonate sediment, to transverse sections of these columns (here termed pseudoclots), and to diffuse patches of trapped sand, as well as to secondarily enhanced clots. Given these complications, it is understandable that earlier workers often referred to thrombolites as “unlaminated stromatolites” (e.g., Aitken, 1967, p. 1166; Pratt, 1982a; Schopf and Klein, 1992, p. 1202). Nonetheless, thrombolites can generally be regarded as benthic microbial carbonates with macroclotted fabric.

Thrombolite types
Two main types of thrombolite are calcified microbe and coarse agglutinated (Riding, 2000, pp. 192–193). Both intergrade with microbial mat stromatolites and primarily reflect the presence of major components that form irregular aggregates rather than thin layers. Nonetheless, the two types have quite distinct origins:

(i) Calcified microbe thrombolites include the classic Cambro-Ordovician examples described by Aitken (1967) and also by Pratt and James (1982) and Kennard and James (1986). Aitken (1967) noted that burrows and trilobite fragments are common in these thrombolites, and examples of similar age often show mottled interiors and stromatolitic outer coatings (Figure 7). They consist of clots composed of framework whose most recognizable components are calcified microfossils, such as Girvanella filaments and Renalcis botryoids. The clots may be irregular centimetric amoeboid forms (Figure 8) or extend vertically into elongate (Armella, 1994, Fig. 9) meandriform and laterally amalgamated (Pratt and...
branches named *Favosamaceria* by Shapiro and Awramik (2006). These clots are surrounded by detrital sediment infill. Progression from irregular clots to elongate branches has confused descriptive terminology. Shapiro (2000) suggested that in branched forms the clots typical of thrombolites are to be found within the branches, although Aitken (1967) seems to have regarded the columns as clots. James and Gravestock (1990) used “calcimicrobe” (calcified microbial microfossil) to refer to filamentous and botryoidal fossils that are common in Cambrian reefs. These typically form dendrolite (Figure 9) and thrombolite (Figure 10) fabrics and include calcified cyanobacteria such as *Angusticellularia*, *Botomaella*, and *Girvanella*, and also *Epiphyton* and *Renalcis*, whose affinities are less certain. Calcified microbe thrombolites are widespread in shallow marine environments during the Neoproterozoic and early Palaeozoic.

(ii) Coarse agglutinated thrombolites are largely composed of fenestral microbially trapped sandy sediment within finer-grained microbially lithified matrix. They are closely associated with coarse agglutinated stromatolites (Figures 3 and 4) that Aitken (1967, p. 1171) described as “thrombolitic-stromatolites.” They are only known in the late Neogene and their development appears to be linked to the rise of algal-cyanobacterial mats able to trap coarse sediment.

Coarse-grained thrombolites in the late Miocene of South-east Spain are closely associated with stromatolite fabrics in composite domes and columns up to 1.5 m high and 4 m across (Martin et al., 1993; Braga et al., 1995; Feldmann and McKenzie, 1997). They include oolitic examples in which clotted fabric is produced by irregular fenestrae up to 15 mm in size (Riding et al., 1991a, p. 123). Braga et al. (1995, Fig. 8, pp. 358–359) attributed the formation of these thrombolite fabrics to “a complex of irregular agglutination, microbial calcification, skeletal encrustation, and erosional processes.”

Logan (1961) regarded Shark Bay columns as stromatolites but recognized that the lamination is often poor, describing some as “crudely laminated with laminae of 1–10 mm. in thickness” (p. 526, pl. 1, Fig. 4). Playford and Cockbain (1976, p. 403) observed that “Hamelin Pool stromatolites range from un laminated (thrombolites) to finely laminated; most show only crudely developed lamination.” Walter (1972, p. 64) attributed “the crude lamination of many Shark Bay stromatolites” to the thick irregular mats and coarse sediment. Logan et al. (1974, p. 154) related carbonate fabric to mat type, with thick irregular pustular mat dominated by *Entophysalis* in particular producing “un laminated to poorly laminated” fenestral fabric, and Gebelein (1974) suggested that clotted fabric could reflect degradation of organic material. Similar Bahamian subtidal columns (Dravis, 1983) are coarse-grained and crudely laminated (Dill et al., 1986) and it has been suggested that abundant algae, including diatoms in these mats contribute to the coarse texture and poor lamination typical of both Shark Bay (Awramik and Riding, 1988) and Bahamian (Riding et al., 1991b) columns.

Mesozoic-Cenozoic thrombolites: Thrombolites are also common in the mid-late Jurassic (e.g., Leinfelder et al., 1993; Parcell, 2002; Kopaska-Merkel, 2003; Mancini et al., 2004), often in association with sponges and stromatolites in deeper water (e.g., Jansa et al., 1988; Leinfelder et al., 1994, p. 37; Dromart et al., 1994) and with corals in shallow-water (e.g., Bertling and Insalaco, 1998; Dupraz and Strasser, 1999; Olivier et al., 2003; Olivier et al., 2006; Helm and Schülke, 1998, 2006). They exhibit a variety of forms, including
arborescent and pendant that commonly occur as thick crusts on frame-building invertebrates, and are also closely associated with a variety of problematic encrusting organisms such as *Bacinella*, *Lithocodium*, and *Shamovella/Tubiphytes*, as well as annelids and foraminifers (Leinfelder et al., 1993, Fig. 6; Olivier et al., 2003, Fig. 4). Similar – but more often stromatolitic – crusts occur in late Neogene, including present-day, scleractinian reefs (Riding et al., 1991c; Montaggioni and Camoin, 1993; Leinfelder et al., 1993, pp. 222–224), and provide important clues to the formation of microbial carbonate microfabrics (Reitner, 1993; Reitner et al., 2000). In addition, the macrofabrics of these Mesozoic-Cenozoic examples broadly resemble those of early Palaeozoic calcified microbe thrombolites, but with significantly different skeletal components. Their microfabrics are fine-grained, with peloids and microclotted micrite (Olivier et al., 2006, Fig. 9), similar to those of many lithified microbial mat stromatolites.

Tufa thrombolite and stromatolite (Riding, 2000, pp. 191, 194) forms in calcifying lakes and streams as a result of cyanobacterial calcification that includes sheath impregnation and encrustation, together with calcification of associated microbes. It was comparisons with freshwater tufa that first led to recognition of links between stromatolites and cyanobacteria (Walcott, 1914; Roddy, 1915). These complex fabrics show considerable variety due to local differences in organisms and the extent of biocalcification and external encrustation. There are similarities between tufa stromatolites and hybrid crust stromatolites (Riding, 2008, p. 88) (Figure 4).

Kennard and James (1986, p. 498) noted that, although “poorly laminated and partially clotted microbial structures” occur on some present-day hypersaline marine shorelines (Laguna Mormona, Shark Bay) and in some lakes of various salinities (Great Salt Lake, Lake Clifton, Green Lake), in these examples “individual microbial clots are generally poorly defined” and “form an irregular, botryoidal-like, mesoscopic fabric that is unlike any of the fabrics observed by us in Lower Palaeozoic thrombolites.” However, Moore and Burne (1994, pp. 23) considered that thrombolites in brackish to normal marine Lake Clifton, Western Australia, do closely resemble some early Palaeozoic examples in mesoclote shape and “the interframework of infilled fenestrae” and they pointed out that in Lake Clifton filamentous cyanobacteria (*Scytonema*) are important in thrombolite formation. Moore and Burne (1994, p. 21) noted that fine laminae, initially present in the Lake Clifton structures, are synsedimentarily destroyed during mesoclot formation as carbonate precipitation continues, and also that the interframework fenestrae are intrinsic features “primarily related to the topography of the surface of the developing microbialite rather than to excavation of the structure by metazoan activity.” Great Salt Lake “algal mounds,” that consist of precipitated aragonitic framework and internal sediment (Halley, 1976, p. 439, Fig. 3), have a sub-centimetric thrombolitic fabric. Ferris et al. (1997) suggested that thrombolitic fabrics in Kelly Lake, British Columbia, reflect a greater degree of calcification than in stromatolites.

Post-depositional thrombolites Aitken (1967, p. 1171) noted that thrombolite macroclots are prone to accentuation by recrystallization. This emphasizes a fundamental difference from stromatolites, whose laminae are essentially primary and not likely to be significantly enhanced by diagenesis. In contrast, clots could be significantly enhanced, e.g., by selective dolomitization, and in some cases secondarily produced, e.g., by bioturbation.

Stromatolites and thrombolites through time

Microbialite types are generally long ranging. Nonetheless, they show some distinctive differences in time distribution (Figure 11). Marine sparry crusts and hybrid stromatolites are typically Precambrian, and fine-grained stromatolites and thrombolites mainly range Neoproterozoic to the present day. Wrinkle structures show the longest range, whereas lithified coarse-grained carbonate thrombolites and stromatolites are only known from the past 10 Ma or less.

Archaean and Proterozoic

Stromatolites are relatively scarce in Archaean rocks until nearly the end of the eon. Their history begins with ~3.45 Ga coniform examples in the Pilbara region of Western Australia. These show fine continuous laminae (Lowe, 1980, 1983) and sparry microfabrics (Hofmann et al., 1999, Fig. 3). Their origins, and those of other Pilbara stromatolites, have been debated (e.g., Lowe, 1994, 1995; Buick et al., 1995), with recent studies supporting a biogenic origin (Hofmann et al., 1999, p. 1260–1261; Allwood et al., 2006, p. 717). Wrinkle and associated structures also suggest the presence of microbial mats in ~2,900 Ma siliclastic sediments of South Africa (Noffke et al., 2008), but these too are generally scarce. However, stromatolites are abundant in the ~2.55 Ga Campbellrand-Malmani carbonate platform of South Africa, locally forming elongate domes 10 m across and 40 m or more in length (Beukes, 1987, p. 9). In addition, these late Archaean carbonates contain distinctive “fenestrate microbialites” (Sumner and Grotzinger, 2004) consisting of millimetric to centimetric areas of light colored cement outlined by thin net-like layers (Figure 12). These have been interpreted as wispy convoluted microbial mats that were encrusted by calcite as they formed (Sumner, 1997a, b).

Large, often decametric, stromatolites are also conspicuous components of Proterozoic carbonate platforms (e.g., Grotzinger, 1986, p. 833; Petrov and Semikhatov, 2001, Fig. 6, p. 269). Where they are well preserved, they often show interlamination of sparry and micritic layers (e.g., Sumner and James, 1996, p. 217). These dark-light layers that appear to represent alternations of lithified mat and abiogenic crust, are typical of “hybrid stromatolites” and are well seen in some *Conophyton* and *Baticalia* (Riding,
2008). Such incorporation of thin abiogenic sparry crusts appears to have contributed significantly to the size and relief of stromatolites throughout the Palaeo- and Mesoproterozoic. During the same period, extensive sheets of very small "microdigitate" stromatolites, typically <5 mm wide and <20 mm high (Hoffman, 1975, p. 262) were common in shallow peritidal environments (Figure 13). These "tufas" have been interpreted as essentially abiogenic (Grotzinger and Read, 1983).

An important control on Archaean-Proterozoic stromatolite formation was gradual decline in seawater carbonate saturation state (Grotzinger, 1989b; Grotzinger and Kasting, 1993). This progressively reduced stromatolite abundance (Grotzinger, 1990) and mediated a long-term trend from sparry crust to micritic carbonate sediments (Grotzinger and Kasting, 1993; Kah and Knoll, 1996). Transition to carbonate mud-dominated platforms ~1,400–1,300 Ma ago (Sherman et al., 2000) preceded the appearance of sheath-calcified cyanobacteria ~1,200 Ma ago (Kah and Riding, 2007). This significant transition, which led to Neoproterozoic development of calcimicrobial thrombolites (Aitken and Narbonne, 1989; Turner et al., 1993, 2000a, b), could reflect induction of CO₂-concentrating mechanisms (CCM) in cyanobacteria in response to fall in CO₂ levels below a threshold near ten times present-day levels (Riding, 2006) (Figure 14). CCMs are responses to reduced availability of inorganic carbon for photosynthesis, and in cyanobacteria include active bicarbonate uptake that locally increases sheath pH, promoting calcification (Merz, 1992). Calcified filaments, such as *Girvanella*, altered microbial carbonate fabrics, disrupting stromatolite layering and promoting thrombolitic macro-clotted fabric. In subtidal environments, Neoproterozoic stromatolites are commonly interlayered with thrombolites with filamentous, clotted, and spongy "cellular" fabrics (Aitken and Narbonne, 1989) comparable with those of Cambro-Ordovician thrombolitic bioherms (Turner et al., 1993; 1997, p. 441, 449; 2000a, Figs. 6e, 8h and i).

**Stromatolite decline**

Stromatolites show long-term decline in abundance that may have commenced as early as the Palaeoproterozoic (Grotzinger, 1990) and is still observed in the Phanerozoic. Fischer (1965) suggested that decline since the Ordovician could reflect both reduction in carbonate saturation and competition by eukaryotes. Competition was subsequently emphasized when it appeared that marked late Proterozoic fall in stromatolite morphotypic diversity coincided with metazoan evolution (Awramik, 1971), but inception of decline prior to the appearance of metazoans implicates reduction in saturation state as the major influence (Grotzinger, 1990). It was also suggested that Cambrian thrombolites reflected disruption of stromatolites by burrowing organisms (Walter and Heys, 1985, pp. 150–151). However, Cambrian-Ordovician thrombolite fabrics are dominated by calcimicrobes that resist disruption (Kennard and James, 1986, p. 494) and
it has since been recognized that thrombolites appeared in the Neoproterozoic (Aitken and Narbonne, 1989), and possibly ~1.9 Ga in the Palaeoproterozoic (Kah and Grozinger, 1992).

This leaves the question of the significance of trends in stromatolite diversity. Stromatolite shape reflects original synoptic relief, determined by accretion rate relative to adjacent sediment (Figure 15). Low relative accretion rate results in low relief that makes stromatolites more prone to lateral incursion by sediment, thereby fostering complex shapes such as digitate forms (Figure 16). In contrast, high relative accretion rate results in high relief and simple shapes, such as domes and cones. Consequently, although mid-Proterozoic increase in morphotypic diversity, e.g., in branched stromatolites, has been regarded as a proxy for abundance, it more likely reflects low synoptic relief due to reduced relative accretion rate. Paradoxically, therefore, increased diversity could be sign that stromatolite growth was in decline due to reduced microbial growth and/or reduction in synsedimentary lithification.

**Snowball Earth**

The prolonged warm Mesoproterozoic interval (Jenkins, 2003) was followed by Neoproterozoic (Sturtian, ~0.7 Ga Ma, Walter et al., 2000; Marinoan, ~0.635 Ga, Bodiselitsch et al., 2005) glaciations. Lower temperature and pCO₂ levels would have decreased seawater saturation state, hindering microbial calcification generally. Cooling would also have favored diffusive entry of CO₂ into cells and therefore may have slowed CCM development, further reducing cyanobacterial calcification. Nonetheless, microbialites are locally conspicuous in Cap Carbonates that immediately follow glacial deposits (e.g., Hoffman and Schrag, 2002; Corsetti and Grozinger, 2005). Cap Carbonates have been suggested to reflect precipitation from seawater highly saturated for carbonate minerals as a result of alkaline upwelling and enhanced terrestrial weathering (Grotzinger and Knoll, 1995; Hoffman and Schrag, 2002). In the ~600–700 Ma Noonday Dolomite of California, narrow laterally amalgamated stromatolites define tubes of intervening detrital sediment fill (Figure 17). The overall organization of some Cambro-Ordovician *Favosamaceria* thrombolites (Shapiro and Awramik, 2006) resembles these “tubestones” (Corsetti and Grozinger, 2003, p. 360).

Following Neoproterozoic “snowball” glaciations, global warming and O₂ rise could have reactivated CCM development, and rising temperature, calcium (Brennan et al., 2004), and pCO₂ (Berner and Kothavala, 2001) levels would have increased seawater saturation state, stimulating microbial calcification (Riding, 2006). This favored microbialite resurgence, and dendrolites, thrombolites, and stromatolites all became widespread in the early Cambrian (Rowland and Shapiro, 2002) (Figure 18).

**Phanerozoic**

**Secular distribution**

Both reefal microbial carbonates (Kiessling, 2002, Fig. 16) and calcified cyanobacteria (Arp et al., 2001) decline in abundance during the Phanerozoic, but this trend shows marked fluctuations. They are common in the late Cambrian-early Ordovician and late Devonian-early Mississippian, and scarce during the Cenozoic.
Fischer (1965) suggested that eukaryote competition and reduction in carbonate saturation state contributed to stromatolite decline from the Ordovician onward.

**Carbonate saturation**: Comparison with seawater saturation state for CaCO$_3$ minerals calculated from modeled seawater and CO$_2$ values shows broad positive correspondence with peaks of microbial/cyanobacterial carbonate abundance during much of the Palaeozoic and Mesozoic (Riding and Liang, 2005), supporting Fischer (1965). Lack of correspondence during the interval ~1,200–1,000 Ma ago could reflect development of cyanobacterial sheath calcification, reflecting inception of CO$_2$-concentrating mechanisms (CCMs) as CO$_2$ levels declined to ~10 times present atmospheric level (PAL) (Riding, 2006). Inferred Proterozoic CO$_2$ trend based on Sheldon (2006), Kah and Riding (2007), Hyde et al. (2000) and Ridgwell et al. (2003). Phanerozoic CO$_2$ trend from Berner and Kothavala (2001, Fig. 13). Threshold for CCMs (10 times PAL CO$_2$) based on Badger et al. (2002). Late Neogene inception of Coarse agglutinated stromatolites and thrombolite could in part reflect incorporation of diatoms into microbial mats and also generally low values of seawater carbonate saturation.

**Competition**: The role of metazoan competition in late Proterozoic and early Palaeozoic stromatolite history is uncertain, and it is debatable whether metazoan grazing significantly affected stromatolite development (Pratt, 1982b) so long as carbonate saturation was high enough to ensure extensive early lithification of microbial mats. Nonetheless, it seems likely that from at least the mid-Ordovician onwards, overgrowth by skeletonized algae and invertebrates inhibited the formation of domical stromatolites and thrombolites. Subsumed within complex reef structures, microbial carbonates would instead have formed patchy and irregular crusts on and around skeletal organisms. Nonetheless, they were often important reef components (Kiessling, 2002, Fig. 16).
Disaster biotas: The concept of stromatolite decline resulting from algal-metazoan diversification (Fischer, 1965; Garrett, 1970; Awramik, 1971) has also given rise to that of stromatolite resurgence in the aftermaths of mass extinctions (Schubert and Bottjer, 1992, p. 885). In this view, if metazoans can competitively exclude microbial carbonates then temporary reduction in metazoan abundance and diversity in the immediate aftermaths of mass extinctions should permit temporary increase in microbial carbonates. Schubert and Bottjer (1992, 1995) interpreted early Triassic stromatolites as “post-mass extinction disaster forms.” However, whereas microbial carbonate reefal abundance also increased noticeably in the aftermath of late Devonian extinction, it did not increase following end-Ordovician, end-Triassic, and end-Cretaceous mass extinctions (Riding, 2006). Nonetheless, it is likely that in these situations, unconstrained by algal and invertebrate reef organisms, microbial mats were able to develop distinctive morphologies, and large domes, columns, and digitate structures have been reported, e.g., at the Permian-Triassic boundary in Sichuan, China (Kershaw et al., 1999).

Thrombolites: Flügel (2004, p. 378) suggested that thrombolite abundance also declined after the Cambrian, although they were still locally conspicuous, e.g., in the Silurian (Kahle, 2001), Devonian (Shapiro, 2000, p. 166), Mississippian (Webb, 1987, 2005), and near the Permian-Triassic transition (Kershaw et al., 1999; Ezaki et al., 2008). Thrombolites have been widely reported in the mid-late Jurassic (see Mesozoic-Cenozoic thrombolites, above), broadly coincident with the last major peak of abundance of calcified marine cyanobacteria (Arp et al., 2001, Fig. 3d).

Evaporite stromatolites: In addition to metazoan extinction, localized increase in carbonate saturation in evaporite basins will favor microbialite development. Pope et al. (2000, p. 1139) noted that isopachously laminated stromatolites, which they considered to be dominantly abiogenic, are well developed in association with major evaporite successions and cited examples in the Proterozoic and Phanerozoic, including the Silurian Michigan Basin of North America and the late Permian Zechstein Basin of northern Europe (Pope et al., 2000,
Figs. 7, 9). These conditions therefore marked temporary returns to conditions that promoted formation of the sparry and hybrid crusts typical of the Archaean and early Proterozoic.

Neogene coarse-grained thrombolitic stromatolites

Well-known present-day examples of columns and domes occur in wave- and current-swept environments at Shark Bay (Logan, 1961) and the Bahamas (Dravis, 1983; Dill et al., 1986; Riding et al., 1991b; Reid et al., 2000). They accrete bioclastic and ooid sand, and have crudely layered (Logan, 1961) and thrombolitic (Aitken, 1967, p. 1171) fabrics. Several factors conspire in their formation: water movement, mat community, and seawater chemistry. High-energy grainy conditions facilitate trapping by lifting sand to the accreting mat surface, and simultaneously deter overgrowth by reefal encrusters (Dill et al., 1989, p. 10). At Shark Bay, seasonal hypersalinity also limits competitors. Together with rapid accretion this allows decimetric, and locally metric, columns to develop. Accretion is high because the mats are thick and soft, with abundant EPS (Decho et al., 2005). In addition to cyanobacteria, they contain diatoms and filamentous green algae that enhance trapping ability (Awramik and Riding, 1988; Riding et al., 1991b). The upper mat remains soft and sticky because it is largely uncalcified, and the early lithification necessary to support these large columns mainly occurs in, or below, the lower part of the mat. Microbial lithification by sulfate reduction (Visscher et al., 2000) is limited to very thin micritic crusts (Reid et al., 2000), cyanobacterial sheaths are uncalcified (Reid et al., 2000, p. 992), and calcification of algal filaments mainly occurs in cavities (Dravis, 1983; Whittle et al., 1993, p. 224). Formation of these coarse-grained thrombolitic stromatolites is therefore largely due to their thick, soft, EPS-rich mats. These in turn reflect a combination of Cenozoic circumstances: (i) seawater saturation state that is too low for cyanobacterial sheath impregnation, but permits early lithification and (ii) the presence of fast-growing microalgae, such as diatoms, which – from a geological standpoint – are relative newcomers to mat communities (Figure 14). Coarse-grained thrombolitic stromatolite domes and columns are well developed in the late Miocene of South-east Spain (Riding et al., 1991a; Braga et al., 1995; Feldmann and McKenzie, 1997) (Figure 19) but are not known in older rocks.

Microbialites, Stromatolites, and Thrombolites, Figure 18 Late Cambrian (Trempealeauan) thrombolite overlain by stromatolite, Smoky Member, Nopah Fm, Dry Mountain, California, north-western Death Valley National Park, USA. Pen ∼15 cm long.

Microbialites, Stromatolites, and Thrombolites, Figure 19 Coarse-grained, oolitic, composite leiolite-stromatolite-thrombolite domes, late Miocene (Messinian), Joyazo, Almería, South-east Spain. Red pen, lower left, ∼15 cm long.
Summary

During the Archaean and much of the Proterozoic, both microbial mat growth and abiogenic precipitation were involved in stromatolite development that, locally, was particularly abundant from the latest Archaean to early Neoproterozoic (Grotzinger and Knoll, 1999). The combination of these biogenic and abiogenic factors was responsible for the rapid growth that enabled stromatolites, ranging from extensive small microdigitate sheets (Grotzinger and Read, 1983) to decametric domes (e.g., Hofmann, 1998, pl. 8a), to dominate carbonate platforms for 1,500 million years. Well-preserved stromatolites with dark-light lamination (Sami and James, 1996; Petrov and Semikhatov, 2001) suggest that lithified mat growth alternated with abiogenic sparry crust precipitation on a millimetric scale to form inter-layered hybrid crust stromatolites (Riding, 2008).

Decline in sparry crusts and hybrid stromatolites ~1,000 Ma ago probably largely reflects reduction in seawater saturation (Grotzinger, 1990). This change also broadly coincided with the inception of cyanobacterial sheath-calciﬁcation in the Mesoproterozoic. This important development may reﬂect induction of CO2-concentrating mechanisms (CCM) to assist photosynthetic carbon uptake as CO2 levels declined (Riding, 2006). It transformed microbialite fabrics, and calcified microbe thrombolites and stromatolites with filamentous fabrics were conspicuous in shallow subtidal environments until the early Ordovician. Subsequently, stromatolites and thrombolites were widely subsumed within algal-invertebrate reefs. In these closely packed habitats, they lacked space to develop classic dome and column morphologies, and instead mainly formed reefal crusts and irregular masses. Only where competitors were environmentally excluded, or absent (as in mass extinction aftermaths), did microbial domes briefly develop extensively.

Both competition and declining carbonate saturation limited the abundance of marine microbialites from the Ordovician onwards (Fischer, 1965), and they are much less widespread and abundant in the Cenozoic than in the Palaeozoic. But microbialite communities have the ability to reinvent themselves. Diatoms together with other microalgae have signiﬁcantly enhanced mat trapping ability. In environments where reefal overgrowth is limited, and coarse grains abundant, these soft mats can create large coarse-grained columns, as at Shark Bay and Lee Stocking Island. Internally these have distinctive crudely layered thrombolitic stromatolite fabrics (Aitken, 1967); in external shape, they closely resemble some Palaeoproterozoic stromatolites.

Microbialites have changed signiﬁcantly during their extraordinarily long history in shape, size, fabrics, and abundance. They have responded to microbial evolution, to environmental changes that have affected both carbonate sedimentation and microbial metabolism, and to the evolution of other organisms. To add to this complexity, whereas stromatolites are essentially lithiﬁed microbial mats, they may be intimately associated with abiogenic crusts.

Microbialites archive important geobiological changes in atmospheric composition, seawater chemistry, met evolution, and biotic interaction. Their study continues to offer many challenges and opportunities.

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Cross-references
Biofilms
Calcified Cyanobacteria
Cap Carbonates
Microbial Biomineralization
Microbial Communities, Structure, and Function
Microbial Mats
Microbialites, Modern
Organomineralization
Snowball Earth

MICROBIAL-METAL BINDING

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Definition
The accumulation of metal cations to microbes, largely through adsorptive processes to the outer cellular surfaces.

Overview
All bacteria have low isoelectric points (below pH 2 in most cases), and consequently, they interact with soluble metal cations and have them intimately associated with their surfaces (Harden and Harris, 1953). Considering their ubiquity in the near-surface environment and their characteristically large surface area-to-volume ratios, bacteria can have a significant influence on metal mobility and speciation in these settings.

Some bound metals (e.g., Ca and Mg) serve the purpose of stabilizing the negative charges of the anionic functional groups, and thus are relatively "fixed" into place, while other metals are much more exchangeable and provide a temporary positive charge to counter the negative charge induced by the deprotonation of the cell’s surface functional groups (Carstensen and Marquis, 1968). The strength of the metal–microbial bond is quantified by the surface complexation/binding constant (K'M), where M refers to the specific metal of interest. The greater its surface complex formation constant, the less likely...
a metal cation will be desorbed into solution. Metal cation sorption is also directly affected by pH, which dictates metal partitioning (or speciation) between solid and soluble phases, and hence, controls its mobility, reactivity, and toxicity in aquatic environments. Of particular importance here is the hydrolysis constant, which measures the tendency of proton release from the hydration sphere of the cation. Figure 1 depicts a generalized adsorption edge curve for a metal binding to a bacterial surface. As pH increases, the cell wall deprotonates and adsorption of the positively charged metal cation increases, but in some instances, adsorption may actually decrease at high pH values as metal hydrolysis is enhanced.

Much of our understanding about how bacteria bind metals stems from the pioneering work of Terry Beveridge on metal accumulation by the Gram-positive bacterium, Bacillus subtilis (Beveridge and Murray, 1976). His research demonstrated that it was the carboxyl groups within the peptidoglycan that were the most electronegative sites, and that the bulk of the binding capacity was associated with the cell wall. In similar studies with Gram-negative cells, such as Escherichia coli, it was demonstrated that they did not bind as much metal from solution as did their Gram-positive counterparts, a pattern stemming simply from the smaller amounts of peptidoglycan associated with the E. coli cell walls (Beveridge and Koval, 1981). Many Gram-positive and Gram-negative bacteria also produce extracellular polymers (EPS), and due to their hydrated nature, dissolved metals can freely diffuse throughout the structure, binding to the anionic carboxyl groups of uronic acids and the neutrally charged hydroxy groups of sugars (Geesey and Jang, 1989). By possessing a large and reactive surface area, it is thus not unexpected that a number of studies have also documented that encapsulated bacteria bind more metals than nonencapsulated varieties (Rudd et al., 1983). Indeed, species producing EPS can tolerate higher metal concentrations than those that do not, and it has been shown that the proportion of encapsulated bacteria increases in metal-polluted sediment, whereas mutants that cannot produce capsules die off (Aislabie and Loutit, 1986).

Irrespective of the bacteria studied, what was repeatedly observed was that considerable variations in metal immobilization could be displayed by a single species. In some instances, cell walls were diffusely stained (Figure 2), while at other times so much metal was fixed to the cell surface that it formed a distinct mineral phase (Figure 3). This led Beveridge and Murray (1976) to originally propose a two-step mechanism for the metal adsorption process; the first step in time is an electrostatic interaction between the metal cations and the anionic sites in the cell wall/EPS. This interaction then acts as a nucleation site for the deposition of more metal cations (and anions) from solution, potentially leading to biomineralization. The size of the deposit depends on a number of variables, including the concentration of ions and the amount of time through which the reactions proceed. If sufficient time exists, then the mineral product grows in size within the intermolecular spaces until it is physically constrained by the organic polymers. The end result is a bacterial wall/EPS that contains copious amounts of metal, often approaching the mass of the bacterial cell itself (Beveridge, 1984).
Although binding of cations to a microbial surface is largely an electrostatic phenomenon, the structural and compositional complexity of the wall or EPS, as well as the unique physicochemical properties of each element, adds a level of complexity to the overall process. In other words, protons and each different cation should be capable of interacting in a distinctive way with surface ligands, such that a cell’s surface will display varying affinities for binding different cations. Moreover, in natural systems, a multitude of cations (and anions) exist that should compete with one another for complex formation. This realization has led to a number of metal binding studies that have compared the relative affinities of protons and various cations for the anionic ligands on the cell surfaces of different microorganisms, by techniques that involve displacing one by another. Those studies have highlighted two very important points, the first being that metals and protons compete for the same surface sites, and as solution pH decreases, the functional groups become protonated, displacing loosely bound metal cations (Fowle and Fein, 2000). The second finding is that microbial–metal interactions are largely abiotic, nonspecific, and reliant upon thermodynamics (Warren and Haack, 2001). Some of the most important factors that influence metal binding to cells are (1) the ionic potential of the solution, (2) cell wall/EPS ligand spacing and their stereochemistry, (3) the ligand composition, and (4) the balance between the initial electrostatic attractions between a soluble metal cation and the organic ligands, and the subsequent covalent forces that arise from electron sharing across a metal cation–ligand molecular orbital (Williams, 1981). These properties are largely understood, and given sufficient information about the environment in which a microorganism is growing, it is possible to extrapolate and predict metal binding patterns on a cell surface.

In the past decade, metal binding experiments have begun to emphasize the stability constants for metal–organic ligand interactions and elucidate how metal binding correlates with cell surface reactivity during changes in solution chemistry. Moreover, unlike many of the early biosorption studies that were carried out in supersaturated conditions with respect to the metal of interest, more recent studies have focused on describing metal–microbe interactions at more realistic undersaturated conditions. The goal for much of this research is to develop geochemical speciation models that describe how microorganisms interact with metals and mineral surfaces under varying geochemical conditions, thus enabling their use in reactive transport models (Fein, 2000 for review). The reactions can be quantified using two different approaches: (1) bulk partitioning relationships or (2) surface complexation models (SCM). In the first instance, partitioning models, such as $K_d$, Freundlich and Langmuir isotherms, can easily be applied to complex systems because they do not require a detailed understanding of the nature of the surfaces or the adsorption/desorption mechanisms involved. However, they are system-specific, meaning that the results from a set of experiments can be inapplicable to different systems. In contrast, the SCM takes into account the effects of changing pH, solution composition and ionic strength, the acid–base properties of surface functional groups, competitive sorption with other solutes, and solid-phase reactivity and mineralogy. It then draws upon that information to extrapolate to conditions beyond those tested in the laboratory. However, in order to utilize these models in more complex settings, more detailed understanding is required of not only surface and aqueous speciation but also the adsorption and desorption mechanisms.

New experimental techniques are helping to elucidate both the mechanisms and stoichiometry of metal–microbe interactions. Spectroscopic data provide more direct evidence of the metal coordination environment than traditional SCM and partitioning models. For example, metal binding onto B. subtilis has been examined by X-ray absorption spectroscopy (XAS) and provided estimates of metal:functional group stoichiometry (Boyanov et al., 2003; Kelly et al., 2002). Mechanisms of metal binding have been directly probed via time-resolved laser-induced fluorescence spectroscopy (TRLFS) and have shown that some metals preferentially bond to one functionality in the cell wall (Panak et al., 2000), while others are less specific (Texier et al., 2000; Markai et al., 2003). A notable recent advance in constraining the thermodynamics of metal–microbe adsorption reactions was the combination of calorimetric data with SCM to generate site-specific enthalpies and entropies of metal adsorption onto the cell wall of B. subtilis. This work indicated that heavy metals bind to the cell wall via inner sphere complexation (e.g., no interlayer water molecules) with multiple anionic
oxygen ligands. Stoichiometry and temperature dependence of the metal–bacteria adsorption reactions can also be extracted using this approach (Gorman-Lewis et al., 2006).

Summary
Microbial–metal adsorption includes the electrostatic and chemical association of metals with the organic functional groups of microbial cell surfaces. This can be considered a passive process driven by the electronegativity of the microbial cell wall/EPS and the Gibbs free energy of the adsorption reactions. These adsorption reactions seem to be dominated by binding of cations to the oxygen containing moieties on the cell surface. Future advances in elucidating the mechanisms of metal adsorption to microbes will come from combining novel analytical techniques, such as colorimetry and spectroscopy, with bulk partitioning studies and site-specific adsorption models.

Bibliography

Cross-references
Carbonates
Clay Authigenesis, Bacterial Dolomite, Microbial Metalloenzymes Microbial Biomineralization Microbial Surface Reactivity Nanocrystals, Microbially Induced Organomineralization

MICROBIOCORROSION

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Definition
Destruction of rocks and minerals by biological activities has been termed bioerosion (Neumann, 1966). It includes mechanical as well as chemical effects, that is, bioabrasion and biocorrosion (Schneider, 1976; Golubic and Schneider, 1979). However, both the processes often co-occur; they are functionally interconnected and mutually supportive. Bioerosion can result from the activity of macro- or microorganisms and, thus, is called macrobiocorrosion and microbiocorrosion. microbiocorrosion can also be closely associated with microbial rock formation and consolidation in stromatolitic structures (Reid et al., 2000; MacIntyre et al., 2000; Garcia-Pichel et al., 2004; Dupraz and Visscher, 2005). In fact, the oldest known fossils of microboring organisms were located in lithified horizons of silicified stromatolites (Zhang and Golubic, 1987).
Bibliography


MICROSENSORS FOR SEDIMENTS, MICROBIAL MATS, AND BIOFILMS

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Synonyms
Amperometry; Diagenesis; Diffusion-reaction; Micro-electrode; Microenvironment; Potentiometry

Definition
Microsensors are needle-shaped sensors (Figure 1) that can be inserted in biologically active matrices, such as sediments, to measure directly concentrations of certain compounds.

Introduction
In sediments, microbial mats, and biofilms steep gradients of substrates and products develop, due to high metabolic activities and mass transfer limitations (see Chapters Biofilms; Microbial Mats; Sediment Diagenesis – Biologically Controlled). Stratifications of microenvironments develop that determine the existence and activity of microbial consortia. For example, in active biofilms oxygen can penetrate less than 50 μm (de Beer et al., 1994c; de Beer et al., 1993), in sediments oxygen penetrates typically less than 2 mm (de Beer, 2001; Jørgensen and Revsbech, 1985; Meyers et al., 1987; Revsbech, 1983; Sorensen et al., 1981; Sweerts and de Beer, 1989). Below the oxic zone anaerobic microbial processes can occur, such as denitrification and sulfate reduction (see Chapter Sulfur Cycle), that determine the element cycling (the coupled degradative and chemolithotrophic processes) in the system as a whole. Stratifications on such a small scale cannot be studied by more classical techniques such as pore-water extraction and chemical analyses. Extraction is impossible on such small scales, as it is highly destructive for the system and concentrations will have changed during the extraction procedure. To study such stratified systems we need microsensors that are minimally invasive and directly measure the concentrations without disturbing the system in which we measure. Moreover, as the system is not disturbed by microsensor analyses, we can perform sequences of measurements and, for example, study how the system responds to environmental changes. Microsensor measurements have enormously enhanced the insight in the vertical distribution of microbial processes in sediments. Microsensors were originally developed for physiological studies in animal and plant tissues, and even for intracellular measurements (Ammann, 1986; Hinke, 1969; Thomas, 1978), but soon applied in environmental studies (Bungay et al., 1969). They became an established technique for ecological studies by the developing work of Revsbech, who made them more robust and invented a large diversity of sensors (Kühl and Revsbech, 2000; Revsbech and Jørgensen, 1986).
Principle
The microsensors most commonly used for environmental studies are of the electrochemical principle, i.e., the compound to be measured induces a current (amperometric) or a potential (potentiometric) proportional to the concentration of the solute. The sensors are commonly made of glass, as this is easy to pull to fine tips, is inert and has excellent isolation properties. It involves the pulling of micropipettes in flames and electrical coils to exactly the desired geometry, thickness, and tip opening, followed by inserting the actual electrodes into these capillaries (Figure 1). The electrodes are mostly platinum wires, etched to micron thickness, and coated with a thin insulating glass layer. The making of microsensors is a fine art. For detailed descriptions of sensor preparation and electrochemical principles the reader is referred to various manuals and reviews (Gieseke and de Beer, 2004; Kühl and Revsbech, 2000; Revsbech and Jørgensen, 1986; Thomas, 1978).

The amperometric sensors consume the compound they measure, but since microsensors are so small this effect can be ignored. The tip diameter of some types can be less than 1 µm, but for most studies ranges between 3 and 15 µm are common, so the sensors are of similar size as the microbes studied. The size of the sensor must be carefully chosen. Small sensors respond faster and disturb the local microenvironment less, but are more fragile than larger sensors. The size of the sensor is a compromise between robustness and spatial resolution less. For soft and extremely active biofilms microsensors of 1–3 µm are ideal; for measurements in coarse sand, tip diameters of 300 µm are used. Also for such big sensors, the actual sensing surface is ca 1 µm, the size is determined by the thick glass wall (Table 1).

Essential equipment
Microsensors can be used in the laboratory and in the field. They can function also under high pressure, and can provide direct data from deep sea environments. Typical applications are measurements of concentration profiles and concentration changes upon a perturbation at a defined location. Microsensors are always positioned by micromanipulators mounted on a stable, heavy stand. The micromanipulator is preferably motorized, and controlled by a computer that is also used for data-acquisition. One can determine the exact position of the sensor to the surface of the studied object, with help of a dissection microscope. For in situ use all electronic components must be packed in water-sealed pressure housings. The most reliable in situ profiler consists of a single cylinder with amplifiers and computer for data storage and motor control. The sensors are mounted on special plugs directly on the cylinder, which can be moved vertically along a rail in steps of 12.5 µm (or multiples of this) by an external motor, according to a preprogrammed protocol. It is powered by a battery and can operate up to 72 h, at a depth of up to 6,300 m.

Applications
Microsensors have been intensively used in studies on sediments, biofilms, and microbial mats (Kühl and Revsbech, 2000). The microscale distribution of substrates is a function of both mass transfer and local conversion rates, so if we have two of the phenomena quantified

<table>
<thead>
<tr>
<th>Compound measured</th>
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<td>8</td>
<td>Jeroschewski et al. (1996)</td>
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<td>S²⁻</td>
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<td>6</td>
<td>Revsbech et al. (1983)</td>
</tr>
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</table>

The sensors marked with # are only suitable for freshwater studies, the others can be used in all salinities.

Microsensors for Sediments, Microbial Mats, and Biofilms, Table 1 Overview of microsensors suitable for environmental studies

MICROSENSORS FOR SEDIMENTS, MICROBIAL MATS, AND BIOFILMS 659
we can calculate the third: from the microprofiles and mass transfer we can determine the distribution of microbial activity (Berg et al., 1998; Gieseke and de Beer, 2004; Revsbech et al., 1986), and from the microprofiles and the local conversion rates we can determine the mass transfer rates (de Beer et al., 2006; de Beer et al., 2004). The latter possibility is rather rarely considered, but the most direct way to determine advection and areal conversion rates in advection-dominated sediments.

Calcification is for geology a highly relevant (micro) biological process (see Chapters Microbial Biomineralization; Stromatolites). The large background calcium concentrations of ca 8–10 mM make it difficult to determine calcification rates from a mass balance. With microsensors it is possible to measure micromolar concentration changes in seawater, in time or over distance, and thus to study the relation between (de)calcification and metabolic activities (photosynthesis and respiration). In Figure 2 the effect of light on coral reef sediments (Heron Island, Great Barrier Reef) is demonstrated: by photosynthesis the oxygen concentration peaks in the photic zone, due to CO₂ fixation the pH shifts up and calcium carbonate precipitates (concentration minimum). In the dark (right panel) calcium diffuses out of the sediment. The photic layer in which these processes occur is less than 5 mm thick.

Microsensors are extensively used to study photosynthesis in microbial mats (Hoehler et al., 2002; Polerecky et al., 2007; Revsbech et al., 1983; Wieland and Kuehl, 2000; Wieland et al., 2005) (see Chapter Hypersaline Environments), (de Beer and Schramm, 1999; de Beer et al., 1994b; Santegoeds et al., 1998; Schramm et al., 1996, 1999), in sediments (Revsbech, 1983; Sweerts, 1990; Sweerts and de Beer, 1989; Sweerts et al., 1990). Using diver-operated microprofilers well-controlled in situ measurements are possible (Weber et al., 2007; Ziebis et al., 2002). However, the most exciting applications are autonomous deployments on sites too remote or too harsh for human guidance: (1) direct in situ measurements during tidal cycles give insight in transport processes in permeable intertidal flats (de Beer et al., 2004; Roy et al., 2007; Werner et al., 2006a,b), (2) in situ measurements in the deep sea supply physiological data communities associated with gas hydrates and high pressure volcanism (de Beer et al., 2006; Glud et al., 1993, 1994; Wenzhöfer et al., 2001a,b; Wenzhöfer and Glud, 2002). It is thinkable that some sort of microsensors will be used in future exploration of other planets.

**Summary**

Microsensors can measure the microenvironment of dense and highly active microbial communities on the scale of microbes. Microsensors are used in field studies and in controlled laboratory experiments. As the sensors are very small they do not disturb the structure or the
pore-water chemistry, allowing precise, rapid, and online measurements of steady-state microprofiles, or concentration dynamics upon a perturbation. Both types of measurements are useful for high spatial resolution studies on the distribution of microbial activities, and on studies on mass transfer phenomena.

Bibliography


Cross-references

Biofilms
Biofilms and Fossilization
Cyanobacteria
Deep Biosphere of the Oceanic Deep Sea
Hypersaline Environments
Microbial Biomineralization
Microbial Mats
Microbiomes, Modern
Photosynthesis
Sediment Diagenesis – Biologically Controlled
Sulfur Cycle

**MOLAR-TOOTH STRUCTURE**

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**Definition**

Molar-tooth structure is a synsedimentary, combined deformation and early diagenetic feature occurring in calcareous strata of mainly Precambrian age. It consists of arrays of closely spaced, sharply defined, upright veins, and subordinate horizontal sheets and spheroids composed of calcite microspar. Veins are vertically obliquely oriented, discontinuous, typically strongly squashed or crumpled, and often brecciated.
Geological age
Molar-tooth structure (MTS) was first named in 1885 from its appearance on limestone bedding planes in the Mesoproterozoic Purcell Supergroup (= Belt Supergroup in USA.) along the border between southwestern Canada and adjacent northwestern Montana. Almost all occurrences are in Mesoproterozoic to late Neoproterozoic (Ediacaran) strata, representing a 1 billion-year time span from about 1,600 to 600 Ma, but there are several examples noted from the Paleoproterozoic and one from the Neoarchean (James et al., 1998; Pratt, 1998b; Shields, 2002; Meng and Ge, 2002). Importantly, a few Phanerozoic cases have also been reported (Figure 1c; Pratt, 1982; Rossetti, 2000).

Geobiological implications
Organisms and organic matter have been alleged to be implicated in MTS formation in various ways. Its rarity after the Neoproterozoic has been ascribed generally to substrate modification by animals. However, the drastic reduction of MTS occurrences took place before the known appearance of infaunal invertebrates and, instead, Shields (2002) saw the decline as due to changes in the chemistry of seawater, especially a decrease in CaCO₃ saturation caused by a drop in atmospheric pCO₂. There may have been an evolutionary control on the shape and mineralogy of lime mud, leading to the precipitation of granular lime mud which was thus prone to fluidization (Pratt, 1998b, 2001). Pratt (1998b) postulated that the rheology of carbonate sediment altered with the changing nature of organic matter and the microbiota brought about by the “Cambrian explosion.” Biomarkers in late Neoproterozoic examples represent cyanobacterial, anaerobic bacterial, and eukaryotic algal precursors in the sediment (Kuang et al., 2004). According to some interpretations, MTS was originally void space created by CO₂ generated by the biodegradation of organic matter.
(Furniss et al., 1998; Pollock et al., 2006) or by CO$_2$-clathrate destabilization (Marshall and Anglin, 2004). James et al. (1998) suggested that a surface microbial mat sealed the sediment. If not physicochemical, precipitation of CaCO$_3$ crystals in the veins (Pollock et al., 2006) or as sediment grains before being fluidized (Pratt, 1998b, 2001) may have been triggered by dissolved organic molecules, nucleated on organic particles, or induced by photosynthesis.

**Shape**

MTS occurs mostly in both clean and argillaceous subtidal lime mudstones (Figures 1a–c, 2a and b), but a few are in shales (Figure 2c). Individual vein arrays crosscut up to ~0.5 m of sedimentary bedding; locally, they can comprise more than 50% of the host rock. Veins are discontinuous, range from fairly uniform to variable in width up to ~5 mm along their length, and typically exhibit tapered terminations. Although more or less straight to gently curved in some beds, mostly veins are strongly squashed or crumpled. The whole array may be tilted in one direction. On bedding planes, veins describe variably continuous subparallel, crisscrossing, or irregularly reticulate lines. The upper and lower surfaces of horizontal veins match, indicating that they are dilatational sheet cracks. Crudely spheroidal blobs are up to ~1 cm across. Two crosscutting episodes of vein formation are common, and they typically show differing degrees of deformation.

In some argillaceous and silty units, as many as four consecutive phases of vein generation can be delineated, with each containing progressively less calcite and more terrigenous particles, and consequently appearing less and less distinct from the matrix.

The matrix usually shows soft-sediment deformation especially in the form of small-scale folding. Typically, the matrix along with the enclosed MTS was deformed plastically together. Veins may be dislocated and the segments shingled in both vertical and horizontal directions, but they are also often stretched, boudined, or smeared. In many cases, a subsequent event caused the still plastic matrix to be deformed around veins that had become stiff due to calcite cementation, which also resulted in brecciation of the veins. After consolidation of the matrix, a further phase of deformation caused small mode I cracks in the veins but little displacement. These features are evidence for variably directed compressional, tensile and shear stresses that were imposed often repeatedly during the separate rheological evolution of both components.

Seafloor erosion affected many beds with MTS. The more resistant veins commonly protruded above the substrate, and vein segments and fragments were winnowed out and concentrated into interbedded intraclastic grainstones (Figures 1b and 2b). In a so far unique case, cross-lamination in vein fills indicates that they were exhumed and reworked by waves before cementation (Bishop and Sumner, 2006).

**Composition**

Locally MTS consists of micrite. However, in most cases it is composed of interlocking, equant, inclusion-free microspar crystals 7–15 µm across (Figure 3a). Backscattered electron microscopy and cathode-luminescence reveal that in some examples these crystals consist of
well-sorted rhombs and multifaceted euhedra with calcite cement overgrowths (Figure 3b; Pollock et al., 2006; Bishop and Sumner, 2006). These cores may be the original mud particles or a product of synsedimentary recrystallization. Similar grains occur in the matrix mudstone or shale. Micropar in MTS is typically uniform in size but locally there is variation that describes crude wall-parallel lamination; indistinct shear veins or cracks can be detected in some examples. In some units the MTS is recrystallized to pseudospar.

Where veins penetrate interbedded siltstone or sandstone layers the microspar may contain silt or fine sand plus minor amounts of clay (Figure 3c; Pratt, 1998a, 1999; Bishop and Sumner, 2006). MTS may grade into dikelets and thin sills of injected silt and sand of the kind that have been termed "dikelets and thin sills of injected silt and sand of the kind that have been termed "syneresis" cracks (Pratt, 1998a; cf. Tanner, 1998; Shi et al., 2009).

The calcite in MTS exhibits $^{13}$C values in the range of about $-1.5\%$ to $3.5\%$, which are similar to those in co-occurring sedimentary carbonate particles (Frank and Lyons, 1998; Pratt, 1998b; Marshall and Anglin, 2004; Bishop et al., 2006; Kuang et al., 2007).

**Formation**

For more than a century, the enigmatic nature of MTS was tolerated but largely ignored. Because fragments of MTS comprise sedimentary particles, it was eventually recognized as having formed intrasтратally just below the sediment–water interface. Some geologists presumed MTS to be a fossil algal or microbial object. Later it was accepted as a deformation feature involving substantial shrinkage of the host sediment – although not due to desiccation because there is no evidence for associated subaerial exposure.

Pore-lining cement fabrics are absent in MTS even though they are present in co-occurring grainstones. Nevertheless, it is still debated whether or not MTS was initially a system of cavities, in which case CaCO$_3$ precipitated later as tiny crystals inside them (Fairchild et al., 1997; Furniss et al., 1998; Pollock et al., 2006; Bishop and Sumner, 2006; Bishop et al., 2006). The presence in MTS of sand, silt, and clay impurities as suspended particles, clusters of grains, or seams is difficult to reconcile with this interpretation.

The possibility that MTS began as voids forced open by bubbles of CO$_2$ passively generated by the oxidation of organic matter in the sediments is unlikely because of the extreme amount of organic matter required, the shallow-water, oxidizing depositional setting, the linear to reticulate shape of vein arrays, the evidence for concomitant sand and silt injection, and the complex nature of the stresses demonstrated by accompanying physical deformation (Pratt, 1999). Because of the tropical sedimentary environment of host limestones, the involvement of CO$_2$-clathrates is implausible. In most units, the absence of closely associated high-energy features is further evidence against the possibility of void opening by wave-induced loading (Bishop et al., 2006).

Pratt (1998b) explained MTS as a seismically induced deformation feature of calcareous sediment possessing unique geotechnical properties for which modern analogues are lacking. By this model, cyclic loading during earthquakes caused elevated fluid pressure and loss of shear strength in the muddy sediment. During abrupt dewatering, veins, sheet cracks, and spheroidal pockets were jacked open by the injection of granular lime mud that was segregated from the sediment. Examples where more than 50% of limestones consist of MTS indicate that a substantial proportion of the lime mud was mobilized. However, shaly host rocks still contain poorly defined seams and residual disseminated calcite grains that did not find their way into veins. Incorporated sand and silt show that dewatering and injection were both downwardly and upwardly directed. Spheroids formed where dewatering was localized and fluids did not escape to the surface. All this caused a striking shrinkage of the matrix and compaction of upright veins. As shaking proceeded,
they were squeezed, sheared, and stretched in different directions. After cementation of the mud infill, subsequent events deformed the matrix again, generated more veins, and brecciated the first set of veins, until the sediment was too stiff to deform further. Indeed, in almost every case, distortion of MTS and the matrix testifies to differential rheologies and progressive evolution from liquefaction through plastic deformation to brittle failure, as would be expected if affected by repeated earthquakes during dewatering and early diagenesis. A similar seismic model has been invoked for sand and silt dykelets (syneresis cracks), except that synsedimentary cementation was not involved (Pratt, 1998a).

**Bibliography**


**Cross-references**

Calcite Precipitation, Microbially Induced Carbonate Environments Carbonates Deep Fluids Microbialites, Modern

**MOONMILK**

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**Synonyms**

Bergmilch; Lac luna; Lac montanum; Mannmilch; Mondmilch; Montmilch; Mundmilch

**Definition**

Moonmilk is a carbonate deposit that occurs within various subterranean systems. Moonmilk has a white to gray color and, in contrast to rigid cave deposits (speleothems) such as stalactites and stalagmites, exhibits a soft, muddy texture of microcrystalline aggregates. These aggregates are mainly composed of calcite, and to a lesser extent of aragonite, monohydrocalcite, hydromagnesite, sulfates, and nitrates (Martínez-Arkazo et al., 2007; Richter et al., 2008; Cañaveras et al., 2006; Borsato et al., 2000). The calcite shows an aragonite-like needle form (“lublinite”), which is normally associated...
with soil bacteria. The crystal needles have a diameter of about 0.1 µm and a length of ca. 8–10 µm. The name “moonmilk – Mondmilch” is derived from the name of a cave, “Mondmilchloch,” located at the Pilatus mountain (Emmental Alps, Switzerland) (Fischer, 1988).

**Geobiological implications**

Moonmilk differs in many aspects from the traditional cave carbonates such as stalagmites and stalactites (Moore and Sullivan, 1997; Bögli, 1978). Its formation is strongly influenced by microbial activity, as suggested by the presence of microbial aggregates, biofilms, and/or fungal mycelia (Cañaveras et al., 1999, 2001, 2006; Borsato et al., 2000; Gadd, 2004; Burford et al., 2003). Moonmilk is a result of (1) microbially mediated weathering and **microbiocrystallization** of carbonate host rock under highly humid conditions and (2) microbially mediated re-precipitation of various carbonate minerals. After dissolution of the mineral components, the residue exhibits high amounts of organic remains. Besides many fungal and actinomycetes, the bacterium *Macromonas bipunctata* plays a major role in the formation of moonmilk. *M. bipunctata* is a lophotrichous, heterotrophic and strictly aerobic betaproteobacterium that has been classified as a colorless sulfur bacterium capable of oxidizing sulfide (Dubinia and Grabovich, 1984). Within **extracellular polymeric substances (EPS)** of *M. bipunctata* and further microbes, calcite seed nuclei are formed to precipitate “ublinite” moonmilk crystals. *M. bipunctata* was originally described as *Pseudomonas bipunctata* by Gicklhorn (1920) and renamed by Utermöhl and Koppe (1924).

**Bibliography**


**Cross-references**

- Biofilms
- Carbonate Environments
- Carbonates
- Karst Ecosystems
- Microbial Mineralisation
- Microbial Mats

**MUD MOUNDS**

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**Synonyms**

Biodetrital mound; Carbonate mounds; Carbonate mud mound; Lime mud mound; Microbial mound; Mudbank; Reef mound; Stromatactis mounds

**Definition**

Mud mounds are biosedimentary buildups, part of the reef system and are dominated by fine-grained carbonates (up and more than 50% of rock volume), which form heterogeneous polygenetic matrix-supported fabrics (stromatolitic, thrombolitic, leiolitic, fenestral, laminar, reticulate, Stromatoloid, etc.). These fabrics are composed of both allomicrites and automicrites (abiotic, biogenetically induced, and biogenetically controlled). Production and accretion mechanisms in mud mounds are varied and not always clear, but are associated with the activity of microbial benthic communities (in different degrees of participation). Metazoans, being important colonizers, can or cannot occur but never produce a primary skeletal framework. Mud mounds colonized the oceans from the Proterozoic times, and they grow up from deep aphotic basinal settings to shallow water platforms.
Evolution of the term mud mound

Probably, the core of the Silurian Wabash buildup (Indiana, EEUU) was the first carbonate deposit referred to in the literature as a “carbonate mud mound” and was described as a mound of calcisiltite containing crinoid, bryozoan, ostracode bioclastics, and stromatactis, but essentially no stromatoporoids or corals (Textoris, 1966). The Silurian mud mound facies were compared by Textoris with similar buildups reported from other parts of the world (Devonian of Canada, Carboniferous of Europe and EEUU).

When reefs turned mud mounds

In the 1960s, the lack of a recognizable skeletal framework in these buildups pointed out their differences with the “classical” reef idea. During some years, most works on mud mounds were focused on the study of their scarce fauna and few were mentions to the origin of the carbonate mud, paradoxically the main component. Wilson (1975) extended in his book the definition and denomination of “limestone mound” to many other Phanerozoic buildups, which were seen as dominated by detrital, bioclastic micrites with minor organic boundstone and perceived to accumulate both through hydrodynamic processes and in situ organic production. Thus, during the 1970s, the origin of the matrix was explained by the baffling theory: the carbonate mud, externally produced, was baffled by metazoans (crinoids, bryozoans, sponges, etc.). The role of early submarine cementation was also taken into consideration to explain the stabilization of mud mound depositional slopes. Baffled micrites and subsequent cementation was the proposed origin; thus, mud mounds were compared with very different types of Holocene buildups: shallow-water seagrass mudbanks and deep-water coral lithoherms.

When some mud mounds turned into microbial reefs

From the 1970s to the 1980s, some fabrics and textures present in the mud mounds were related with “cryptalgal” and other biosedimentary structures as stromatolites and thrombolites (Monty, 1976; Pratt, 1982). The mud mound matrix could be now bound, trapped, and precipitated by the activity of microorganisms (“blue-green algae” – cyanophytes and cyanobacteria).

From then, the source or origin of the carbonate mud, the matrix problem, will be the leitmotiv in the study of mud mounds. Bosence et al. (1985), from the recent carbonate mounds from Florida Keys, proposed three mud mound models based on the relation between the external and the internal sediment (carbonate mud) supplies in the mound: export, import, and self-sufficient models, respectively. From the 1970s and clearly in the 1990s, mud mounds began to be seen as “self-sufficient” mounds, and the role of microbial communities in their construction gained more importance (Tsien, 1985; Lees and Miller, 1985; Camoin and Maurin, 1988).

James and Bourque (1992) proposed a conceptual classification of reefs and “mounds,” where mounds are those structures which were built by smaller, commonly delicate and/or solitary elements in tranquil settings. They differentiated three types of mounds: “microbial” mounds, “skeletal” mounds, and “mud” mounds. Microbial and skeletal mounds are grouped into “biogenic mounds” because they are organically controlled, whereas the term “mud mound” was restricted by the authors to those formed by inorganic accumulation of mud with variable amounts of fossils. The authors pointed out that the division between biogenic mounds and mud mounds depends on the nature of the accumulation/construction controls, not on the percentage of fossils.

One of the main outstanding questions concerning the mud mounds is related to the modes of formation of the carbonate mud matrix and their recognition. The increase in the number of studies on microbial carbonates and their formation and fabric classifications (Kennard and James, 1986; Burne and Moore, 1987; Riding, 1991) led to new horizons in the matrix problem. Fabrics and geochemical signatures from automicrites of recent cryptic microbialites and controls in their formation (Reitner, 1993) were found to be similar to those recorded in Cretaceous and Jurassic mud mounds (Neuweiler, 1993; Keupp et al., 1993; Reitner et al., 1995; Leinfelder and Keupp, 1995). Thus, modern automicrites began to be used as modern counterparts of some mud mound end products.

Some authors began to use the term mud mound in a descriptive nongenetic way as carbonate buildup consisting of more than 50% of mud and/or peloidal mud (Reitner and Neuweiler, 1995), having depositional relief, and forming part of a polygenetic and continuum spectrum ranging from “biodetrital” to “microbial” mud mounds (Bosence and Bridges, 1995). Pratt (1995) considered mud mounds as ecologic reefs because they possess “rigid frameworks” produced by both microbial activity and synsedimentary cementation.

Last formal definition of mud mound has been given by Riding (2002), as carbonate mud-dominated (micrite and fine-silt) deposits with topographic relief and with few or no stromatolites, thrombolites, or in-place skeletons. Riding added that the deposits can be organic and/or inorganic in origin and it can be difficult to distinguish their origins.

General features

Mud mounds have varied external forms; the term mud mound is used here not in a morphological way due to the fact that not all of them are mounds or dome-shaped; they can be tabular-shaped and can occur as isolated massive bodies or as stacked or amalgamated bodies, spreading over kilometers. The biggest mud mound complex is the Waulsortian mudbank from Ireland.

They could develop as a high synsedimentary relief and show depositional dips from 10° up to 50°, can be or cannot be flanked by bioclastic limestones, and grow up from
basinal to platform settings. Mud mounds are composed mainly of fine-grained carbonate-matrix-supported fabrics, which are called, depending on the classification schemes followed, mudstones, wackestones, biomicrites, etc. These carbonate muds are texturally and genetically varied. The presence of cavities can be a characteristic feature in some mud mounds. Their macrofauna are diverse and can be important volumetrically in some mounds, although they never constitute a skeletal framework.

Types of carbonate muds
Carbonate muds are formed by pure aphanitic micrites and/or mixture of silt-sized calcite particles (up to 62 µm) as peloids. Then, they can be texturally grouped into aphanitic and peloidal micrites. Both types occur separately, or there is grading between them; they also occur as neomorphosed microspars. Microstructurally, they appear as dense and/or clotted/grumelar/spongiosan microfabrics, which constitute at mesoscale, stromatolitic, thrombolitic, fenestral, and/or cryptagal/cryptomicrobial/leiolitic fabrics (summarized in Riding, 2000).

The carbonate muds can be classified in relation to the locus of their production. The muds externally produced and later imported into the mound are named allomicrites (Figure 1). They are stabilized at mound surface by mound biota (eukaryotes and prokaryotes) through baffling, trapping, and/or binding mechanisms and early cementation.

The in situ produced carbonate muds are named automicrites. They are formed through abiogenic, biologically induced, and biologically controlled mechanisms (summarized in Flügel, 2004). The biologically related mechanisms are known as nonenzymatically and enzymatically controlled carbonates. Mud mounds seem to be specially dominated by the nonenzymatically carbonate production of automicrites via nonliving substrates (organomineralization of the organic matter derived from decaying organisms) and/or via metabolic processes (by heterotrophic, chemolithotropic, and phototrophic bacteria and cyanobacteria). In the first case, the
resulting automicrites are known as organomicrites. The automicrite production occurs at mound surface as well as at subsurface.

The mud mound system comprises both soft (allomicrites and automicrites) as well as firm/early indurated carbonate muds (automicrites), which can suffer from reworking processes (chemical, physical, and/or biological ones) resulting in paraautochthonous muds. These secondary muds or the next successive generations of in situ muds, therefore, resemble a mixture of previous ones. This system should be analyzed as a continuous series of processes of automicrite production, allomicrite input, and internal reworking.

The filling of mound cavity system records many of the last mud mound synsedimentary episodes: (1) the entry of allomicrite by gravity or currents, (2) the colonization by cryptic biota, (3) production of automicrites, (4) reworking processes, and (5) the early marine cementation phases as well as burial-related phases.

So mud mounds have polygenetic carbonate mud-dominated fabrics resulting from automicrites production, allomicrites input, and recycling processes (Figure 1). But some microfabrics have been related at the same time with different types of biogenic as well as abiogenic processes, and discriminating between some processes and their products is not always possible.

Mud Mounds, Figure 2  Microbial mat-based model for accretion of mud mounds and development of cavities. (Modified from Pratt, 1982.)
Biota

Dominant invertebrate macrofossils on mud mounds were suspension feeders as pelmatozoans, bryozans, and sponges—siliceous and calcareous types, although others such as brachiopods, molluscs, and arthropods could be sporadically important. Solitary and colonial corals occasionally occurred on mud mound records as well as calcareous algae, but they were much less common (not as colonizers of the shallow-water mud mound phases). Others such as benthic foraminifera and polychaete worms also occurred as part of the mud mound biota from the Carboniferous times. In general, the biota of mud mounds was mainly dominated by heterozoan assemblages.

In mud mounds, excluding the evident microbial fabrics (stromatolitic, thrombolitic and others), the main signal or product of microbial activity is hidden in the carbonate mud (biogenic automicrites) and in some authigenic mineralizations (iron pigments, Fe/Mn surfaces, framboidal pyrites, etc.). There is also direct evidence of calcified microbes (Girvanella, Renalcis, Epiphyton, Bacinella, Rivularia-like forms, etc.), and most of them have been interpreted as cyanobacterial fossils. Other evidence of microbial activity is the presence of unidentified filament-rich microfabrics, filaments and spherulites as moulds or casts, and peloids.

Sponge–bacterial assemblages have also played an important role in the initiation and first stages of many mud mound developments (early cementation of sponge tissues is related with the activity of symbiotic bacteria as well as the organomicrite production through the decaying of their tissues).

Cavities

There is a wider spectrum of cavity types in mud mounds (stromatactis, zebra, irregular, and sheet-like shapes). Probably, the most famous are the stromatactis, with smooth, flat base and irregularly digitate roofs, matrix-supported in general, although some can be partially or totally sheltered by remains of fossils. Filled with centripetal marine fibrous cement crusts and varied internal sediments (allomicrites and automicrites), stromatactis were abundant in Paleozoic mud mounds, especially from the Ordovician–Mississippian interval.

The origin of stromatactis has been largely debated, and today it remains controversial. Several hypothesis has been used (summarized in Flügel, 2004, 194 p.), which can be grouped in “organic” (recrystallized fossils, related with decaying organism, burrowing, etc.) and “inorganic” theories (dissolution, collapse, internal erosion between submarine crusts or bioclasts, dewatering and compaction of the muds, recrystallization, enlargement of preexisting cavities, presence of gas hydrates, etc.). In the organic cases, there are two models: the cryptalgal/microbial mat model and the sponge model.

The microbial mat model was initially proposed by Pratt (1982) to explain the mud mound accretion and its final observed fabrics (Figure 2). The fabrics and the resulting cavities were the result of the combination of three variables: (1) the distribution of the microbial mat, (2) the rate of sediment loading onto the mat, and (3) the degree of winnowing. The distribution of the microbial mat (laterally continuous or in patches) produces the resulting fabrics (laminar or reticulate, respectively). The winnowing of the unconsolidated sediment between the microbial bounds and cemented sediment produces the cavities.

In the sponge model (Bourque and Boulvain, 1993), the cavities are formed by the partial collapse of the sponge body as result of early diagenetic processes through four stages (Figure 3): (1) zone of the living community, (2) zone of sulphate reduction, (3) zone of oxidation of ferrous iron, and (4) zone of marine cementation. In the living surface, the mud is trapped by sponges and/or produced by microbial activity. Below the surface, the microbial decay of sponges occur; part of the sponge body and the mud are cemented, and other sponge parts are in collapsed state; the later internal erosion of the unconsolidated material produces the open cavities. A few meters
below, in the ferrous iron oxidation zone began the incipient marine cementation of these cavities forming the stromatactis.

Types of mud mounds through the Phanerozoic record

The classification and distribution of mud mounds through the record depend on the “mud mound versus reef concept” of almost each researcher and thus remain controversial in several aspects. Mud mounds have existed from Proterozoic times and through the Phanerozoic record (see reviews by Monty, 1995; Pratt, 1995), and important growth episodes occurred in early Cambrian, late Devonian, and Mississippian times. Cambrian to Ordovician mud mounds (Figure 4) were characterized by different fabrics and assemblages (stromatolitic, thrombolitic, and massive fabrics with calcimicrobes, and cambrian archaeocyathid-sponge-calcimicrobial assemblages). From the Ordovician to the Mississippian, some of the carbonate mud-rich buildups, which typify this interval, have been regarded as the classical massive mud mounds with abundant stromatactis (Figure 4) as the ‘red stromatactis mounds’ (Recif Rouges-type mound) and the Waulsortian mudbanks (Figure 5a and b). Clear microbilite-related fabrics (stromatolites and thrombolites) are extremely rare in classical mud mounds, although they show other nonskeletal reef frameworks (calcimicrobial and biocementstones sensu, Webb, 1996), as well as filaments and some characteristic features (clotted, peloidal micrites, and fenestral microfabrics). The parallel diversification of skeletal mound macro- and micro-biota produced more varied metazoan–microbialitic assemblages. One of the most persistent through the mud mound record is the sponge–microbialitic association, very common in Jurassic mud mounds as well as in the deeper initial stages of many Paleozoic mounds. Early Cretaceous is considered the last significant moment of organomineralic mud mound development (Neuweiler et al., 1999), although

**Mud Mounds, Figure 4** Distribution of mud mounds through the geologic record. (A) Precambrian to Quaternary time divisions. (B) Distribution and relative importance of nonenzymatic reef frameworks (from Webb, 1996), characteristic also in mud mounds. (C) Distribution of (1) microbial and (2) biocemental mud mounds. (Modified from Bosence and Bridges, 1995.) (D) Distribution of stromatactis and stromatactoid cavities, the size of the bar represents their relative importance in mud mounds. (E) Interval distribution of the main typologies of mud mounds.

**Mud Mounds, Figure 5** Classical mud mounds. (a) Close view of a Devonian Recifs Rouges-type mound, Beaufachateau Quarry, Belgium. (b) Carboniferous Waulsortian mudbank, Waulsort, Belgium.
other authors mentioned that the latest mud mounds are Miocene (Pratt, 2000).

Ecological zonation models

The Récifs Rouges-type mounds and the Waulsortian mudbanks are probably the most famous and are well known as “classical mud mounds” (Figures 4 and 5) Devonian (Frasnian) mounds from Belgium show a shallowing upward succession from deep basal greenish-grey shales through their mud mound facies: red lime mudstones to wackestones with stromatactis, pink wackestones with millimeter stromatactis to crudely bedded grey wackestones to packstones, and bindstones, and their associated biota. Similar facies (red stromatactis mounds) and assemblages have been found in other mud mounds through the Paleozoic record (Bourque, 1997). The carboniferous Waulsortian mudbanks of Belgium show a four grain-type assemblages related with the growth of these mounds from deep subtidal, aphytic to photic conditions. A depth-related phase model was recognized by Lees and coworkers (summarized in Lees and Miller, 1995) and applied to Waulsortian mudbanks of Europe and North America. Bourque (1997) compared Récifs Rouges-type mounds from Silurian of Canada and Devonian of France and Belgium with carboniferous mounds from Algeria and the Waulsortian mudbanks model and established an ecological zonation model formed by four benthic communities zones (Figure 6): (1) the sponge zone, (2) the fenestellid–sponge zone, (3) the delicate skeletons–sponge zone, and (4) the delicate skeletons–microbial encrusters zone.

Controlling factors

Today, many parameters about the initiation, accretion, and spread of many mud mounds seem to be enigmatic. However, some others begin to be more clear.

Locations of some mud mounds have been related with submarine cold seeps, vents, faults, halokinetic and argilokinetic settings, and local antecedent topographic irregularities, have been associated to nutrient-enriched areas (by endo-, down- and/or up-welling currents), and could be also related with stratified basin waters and fluctuating oxygen minimum zone. Many of them grew up into aphyotic to diphotic depths (more than 200 m); however, they could reach and colonize the euphotic zone. Some grew at lower sedimentation rates and/or during sedimentary starvation during TST episodes, although this is not a general trend for all of them. Most of these factors are oceanographic and/or tectonically basin-related parameters and could co-occur together in many cases.

Those factors (biological and chemical parameters) associated with the carbonate mud precipitation related with eukaryotes and prokaryotes should be taken into consideration:

(a) Production of acidic organic macromolecules (AOM) and an increase in carbonate alkalinity via (1) bacterial heterotrophic pathways (ammonification and sulphate reduction), (2) carbonate, and (3) silicate weathering. AOM and high alkalinity favor the organomicrite production in microbialites and mud mounds (Reitner and Neuweiler, 1995).

(b) Extracellular polymeric substances (EPS)-biomineralization of microbial mats and biofilms.

(c) Calcification of cyanobacteria.

<table>
<thead>
<tr>
<th>Cambrian-devonian Recifs rouges-type mounds</th>
<th>Carboniferous mud mounds</th>
<th>Depth-related communities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silurian (Canada)</td>
<td>Devonian (France)</td>
<td>Devonian (Belgium)</td>
</tr>
</tbody>
</table>

- **Fenestellid**
  - Stromatoporoids
  - Calcareous algae
  - Microbial encrusters

- **Cinoids**
  - Bryozoans + mud

- **Sponge** (spicules)
  - Sponges
  - (Red stromatactis limestone)

- **Sponges** (Red stromatactis limestone)
  - Sponges
  - (Red stromatactis limestone)

- **Sponges**
  - Sponges

- **Phase D:** C + grain coating calcareous algae

- **Phase C:** B + Plurilocular foraminifera

- **Phase B:** A + Sponger spicules
  - Phase A: fenestellids, cinoids, ostracods

- **Phase B:** Delicate skeletons and sponges + mud

- **Phase D:** Delicate skeletons and microbial encrusters

- **Photic**

Mud Mounds, Figure 6 Ecological zonation assemblages in some Paleozoic mud mounds. (Modified from Bourque, 1997.)
Conclusions

Mud mounds are considered mainly as nonenzymatic carbonate deposits that formed a polygenetic and very continuous record of buildups through the Phanerozoic and reached a maximum development during the Paleozoic times. They are of considerable importance not from an economic point of view (hydrocarbon reservoirs, major mineral host-rock and base deposits, ornamental rocks) alone. In fact, they represent an excellent record for the study and establishment of (1) the interactions between the marine microbial and nonmicrobial benthic communities (paleoecologic and paleoenvironmental parameters), (2) marine carbonate production models (mud mound factory), and (3) proxies in palaeoecographic reconstructions (nonenzymatic carbonates are formed in equilibrium with ambient sea water).

Mud mounds are characterized by different modes of carbonate mud production (abiogenic, biologically induced, and biologically controlled autochmricite) and sedimentation input (allochmricite). The key of mud mounds understanding lies behind here: to recognize and differentiate between the autochmricite and allochmricite relationship and their contribution to the mud mound accretion.

Bibliography


Cross-references
- Bacteria
- Biofilms
- Calcite Precipitation, Microbially Induced
- Cyanobacteria
- Extracellular Polymeric Substances (EPS)
- Microbial Communities, Structure, and Function
- Microbial Mats
- Microbialites, Modern
- Reefs
- Waulsortian Mud Mounds

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**MUTUALISM**

Mutualism is a form of a symbiosis, in which both organisms involved benefit. See entry “Symbiosis” for further reading.

**MYCORRHIZAE**

A mycorhiza (pl mycorrhizae, from the Greek “mycos” = fungus and “rhiza” = root) is a plant-fungal association that comprises the most widespread type of terrestrial symbiosis. See entry “Symbiosis” for further reading.
NAN(N)OBACTERIA

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Synonyms
Living nanovesicles; Nanobes; Nanocalcifying particles (NCP); Nanoforms; Nanoglobules; Nanons; Nanorganicisms; Nanoparticles

Biologists used the word nanobacteria to describe organisms that possibly enhance biomineralization such as that of kidney stones in humans, whereas nannobacteria is used by geologists for organisms responsible for mineral precipitation in terrestrial and extraterrestrial rocks.

Definition
Nan(n)obacteria are tiny globular objects, ranging in diameter from 20 to 200 nm, with the ability to nucleate minerals. They are the smallest known, self-replicating particles with slow growth rate. Because they are by definition down to 1/10 the size of bacteria, it raises the problem of whether or not an organism of this size is large enough to house necessary cell components such as DNA, RNA, and plasmids.

Introduction
The term “nan(n)obacteria” has been originally proposed by Folk (1993) to describe 20–200 nm wide nanovesicles observed in carbonates using scanning electron microscopy (SEM). Kajander and Çiftcioglu (1998) defined the role of similar tiny particles in kidney stone formation. Based on their mineralogical properties, nan(n)obacteria have also been called calcifying nanoparticles (CNP) by these authors. Nan(n)obacteria-like particles have been observed in Martian meteorites, thereby raising questions about a primitive form of life (Bradley et al., 1997). CNP are studied extensively in pathogenic diseases as infectious agents by nucleating crystals (hydroxyapatite). Therefore, some comparisons have tentatively been made with the geological record. Although they display some evidences of bacterial nature, their living status is subject to controversy because of their small size and the fact that DNA extraction remains unsuccessful. On one hand, biologists discuss their metabolic existence and on the other hand geologists argue for widespread organic fossilization in palaeoenvironments. When interrelations between geology and microbiology are possible, they are looked at only from the point of view of experimental procedures (e.g., one emphasizes the use of DNA probes in geological samples). This prohibits an objective comparison of these very small particles.

Reported nan(n)obacteria in the literature
In the geological record, nan(n)obacteria-like particles are encountered mainly in carbonates: in calcite (Dix et al., 1999; Chen et al., 2005; Mamet and Prétat, 2006), microbialites (Camoin et al., 1999), dolomite (Figure 1; Vasconcelos et al., 1995; Gournay et al., 1999; Wright, 1999; Akççek, 2009), hydrothermal springs (Casanova et al., 1999), and oncrite laminations (Cavalazzi et al., 2007). They have also been observed in sandstones without mineral precipitate (Uwins et al., 1998) and on quartz overgrowth in crude oil (Spark et al., 2000).

In medical biology, CNP have been the focus of numerous studies on various pathogenic diseases (see Urbano and Urbano (2007) for a review), for example, gallbladder (Wang et al., 2006), nasopharyngeal cancer (Zhou et al., 2006), and renal stone formations (e.g., Kajander and Çiftçioglu (1998) and Jones et al. (2009)).
Characterization of purported nan(n)obacteria

Nan(n)obacteria exhibit some characteristics, which seem to correspond to the classical criteria indicating a biological origin: (1) a typical bacterial appearance (Kajander and Çiftçioglu, 1998; Uwins et al., 1998); (2) a slow growth in liquid cell culture media; (3) an expression of specific antigens (Hjelle et al., 2000; Miller et al., 2004) by using monoclonal antibodies commercially available through Nanobac Oy (Finland) as 8D10 and through Santa Cruz Biotechnology, USA, named GM-CSFRα; (4) a staining with nucleic fluorochroms (Uwins et al., 1998; Miller et al., 2004) using 4',6-diamidino-2-phenylindole (DAPI), acridine orange, Hoechst 33258, Feulgen, and/or Picogreen; (5) a resistance to antibiotics such as tetracycline and citrate (Maniscalco and Taylor, 2004); (6) a sensitivity to calcium-chelating agents (Rawal and Pretorius, 2005); and (7) a DNA sequencing tentatively assigned to the α-2 subgroup of proteobacteria, which also includes Brucella and Bartonella species occurring within and outside of host cells (Kajander and Çiftçioglu, 1998; Çiftçioglu and Kajander, 1998). According to Čisar et al. (2000), they could come from PCR contaminant.

Possible origins for nan(n)obacteria

Due to the very small size of purported nan(n)obacteria, the hypothesis that they might represent living entities is very controversial. Several, alternative origins have been proposed:

- **Inorganic origin:** Inorganic precipitates (Vecht and Ireland, 2000; Čisar et al., 2000) and artifacts due to sample preparation (e.g., coating for SEM, acid treatment, polishing artifacts) have been considered as an alternative origin for these nanostructures. However, nanostructures can also be observed in samples that have not been exposed to these techniques (Folk and Lynch, 1997).

- **Extracellular polymeric substances (EPS):** Reitner et al. (1995) have proposed that nanospheres were an initial calcification of EPS. They have been interpreted as early stages of dolomite precipitation in anaerobic cultures (Bontognali et al., 2008) and aerobic cultures (Figure 2; Sanchez-Roman et al., 2008). Similar observations have been made on aragonite nanocrystals that contained organic molecules (Benzerara et al., 2006).

- **Proteins:** Tissue degradation caused by microbial activity can create nanobacteria-like particles of proteinous origin (Schieber and Arnott, 2003). In the case of kidney stones, it has been confirmed that nan(n)obacteria are in fact related to blood proteins (Martel and Young, 2008), that is, fetuin (Raoult et al., 2008).

- **Small bacteria:** Dwarf bacteria generally show a decrease in cell size. This is assumed to be the case of bacteria exposed to low nutrient or starvation conditions (Velimirov, 2001). Ultramicrobacteria exhibit a very low growth rate (Torella and Morita, 1981). The distinction between ultramicrobacteria and starvation forms is based on their physiological properties (Velimirov, 2001).

- **Viruses:** Stressed bacteria overproduce vesicles that are apparently used to selectively eliminate unwanted material, for example, unfolded proteins (Soler et al., 2008). All fluorescent small-size particles are assumed to correspond to a DNA virus (Soler et al., 2008). Membrane vesicles seem to be produced by most bacteria and have been...
detected in natural environments, including biofilms (Schooling and Beveridge, 2006).

Minimal conditions for a living cell
Theoretical calculations have been made in order to define the smallest size needed for a cell to have metabolic processes and accommodate the smallest genome. According to these assumptions, the minimal cell size should fall within the range of 100 nm (Boal, 1999), 140 nm (Maniloff et al., 1997), and 170 nm (Adams, 1999). Assuming a cell genome housing 250 genes (which is considered the minimal number needed to support heterotrophic cell growth in a nutrient-rich environment with a minimal metabolic pathway), a minimum diameter of 50 nm has been established, which could contain eight genes (Adams, 1999). Nevertheless, a cell with a size of only 50 nm, a 5 nm cell wall, 2 ribosomes, 520 protein molecules, and a DNA strand containing 8 genes is considered too small to function like recent bacteria, based on the smallest known living organism, that is, *Nanoarchaeum equitans* (Huber et al., 2002).

Universal origin for nan(n)obacteria?
The analogy between nan(n)obacteria-like particles in pathogenic diseases and geological records is often made even though the methodology differs. Nan(n)obacteria have not been found in any clinical condition that does not involve calcification, which is not the case of some geological samples (Uwins et al., 1998; Schieber and Arnoff, 2003). Recently, nan(n)obacteria-like particles associated with kidney stone formation have been assigned directly to blood proteins reacting with antibodies that were so far claimed to be specifically associated to nan(n)obacteria (Martel and Young, 2008; Raoult et al., 2008). The latter authors suggested that they are nucleic-acid free. An alternative origin has been proposed where nan(n)obacteria could be the by-product of the living activities of bona fide bacteria inhabiting kidney stones (Aloisi, 2008). If we consider that nan(n)obacteria could be EPS accumulative and knowing that EPS contain DNA (Whitchurch et al., 2002; Steinberg and Holden, 2005), it is unlikely that this hypothesis fits with nanobacteria observed by Uwins et al. (1998), where no other microorganisms were detected.

Summary
Nan(n)obacteria emerged as a new form of life during the last two decades, based mainly on their capability to trigger infectious diseases. Their interpretation as simple proteins in kidney stones has been recently ruled out, although similar nano-objects have been also observed in geological records. The controversy about their existence (too small to be biologically viable) is still ongoing today because no universal origin can be attributed to these nanoparticles. The EM imaging does not present enough evidence alone, and molecular and physiological data are required in order to try to define their properties.

Bibliography


**Cross-references**

Bacteria
Biosignatures in Rocks
Dolomite, Microbial
Extracellular Polymeric Substances (EPS)
Microbial Biominalerization
Nanocrystals, Microbiially Induced
Origin of Life
**NANOCRYSTALS, MICROBIALLY INDUCED**

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**Definition**

Mineral particles less than 0.1 µm are a vital component of geochemical cycling in the Earth surface systems. Particles in this size range are generally termed “nanoparticles”; nanocrystals are nanoparticles that exhibit long-range structural order. Their small size implies that they may be relatively mobile in natural systems, as well as more chemically labile than large crystals. They have recently come to the forefront of material science, with new evidence that the properties of some materials are a strong function of particle size, particularly at the nanometer scale.

Nanocrystals are characterized by higher surface strain and disorder, higher surface reactivity, and differences in reaction kinetics compared to larger crystals of the same composition. These properties make them important reactive agents in natural systems, especially given that minerals in surface and near-surface environments are typically smaller and more highly disordered than minerals crystallized under ideal conditions. In general, the interest in biological nanomaterials is focused on specific properties that a given material can offer. For example, the electronic structure of nanocrystals is of particular interest from the perspective of material science. For a review of chemical and physical properties related to nanomineral structure and composition, see Gilbert and Banfield (2005).

The crystallization of biominerals spans eukaryotic (“true nucleus”) and prokaryotic organisms (Lowenstam and Weiner, 1989). Eukaryotes form geologically important structures that may be based on nanoparticles, such as the silica-reinforced shells of diatoms or the calcite shells of coccolithophores and foraminifera (Weiner and Dove, 2003). Prokaryotes dominate, however, among microorganisms that mediate the development of nanoparticles, in terms of their ubiquity in surface and subsurface environments and the compositional and structural range of materials they can form (Gilbert and Banfield, 2005). This article focuses mainly on bacterial nanoparticles that contain metals because of their importance in Earth surface and subsurface environments as well as their relevance to other disciplines related to nanoscience.

**Bacterial nanocrystals**

**Induced versus controlled crystallization**

The development of biominerals or biocrystals may be either biologically induced or biologically controlled by the bacteria (Lowenstam and Weiner, 1989). In biologically controlled mineralization, microbes exert genetic and chemical control over the formation of precipitates that are designed to serve a particular function. In contrast, biologically induced mineralization yields particles that are secondary to a chemical process controlled by the microorganism. In this case, the precipitates serve no physiological purpose.

Biologically induced nanominerals can result from a range of bacterial processes, including respiration, detoxification, and ion uptake or expulsion during metabolism. Although minerals with a range of sizes and crystal structures can be produced as a result of biologically induced mineralization (e.g., Fortin et al., 2008), the nanometer-sized minerals are of particular interest because of their chemical reactivity as well as the potential for transport in natural systems. Reactivity is a consequence of the high proportion of surface atoms, which are chemically uncoordinated at the solution interface and less ordered than fully coordinated atoms in the structure.

Nanomineral formation can be induced in the extracellular environment, as a result of changes in solution chemistry caused by uptake or expulsion of ions (e.g., H\(^+\)) from the cell (Fortin et al., 2008). In addition, bacterial surfaces carry negative and positive charges due to the dissociation of functional groups integral to the cell wall (Beveridge, 1989). Sorption of ions to charged bacterial surfaces or sheaths can lower the nucleation energy for crystal formation and induce thick mantles to develop based on nanoparticles. This process can be observed during the growth of silica sheaths around bacteria and cyanobacteria, which may also incorporate metal ions (Ferris et al., 1986; Konhauser et al., 2004). More examples of induced mineralization are discussed below.

**Extracellular nanominerals**

Bacterial metal respiration is a process in which electrons are removed or accepted by a metal ion to generate energy for the cell. Specific bacteria are capable of either oxidation or reduction; for example, *Gallionella* spp. and *Leptothrix* spp. are known iron oxidizers, whereas *Shewanella* and *Geobacter* are genera associated with metal reduction. Metal oxidation and reduction (“redox”) by bacteria may take place in chemically stratified zones within a sediment or water column, or may occur in microscale zones (Nealson and Stahl, 1997; Sobolov and Roden, 2002). Minerals form when a change in metal valence induced by respiration affects the solubility of a metal, in the presence of a suitable counterion to react with the reduced or oxidized metal species; the minerals form by an induced process rather than a controlled one.

Iron- and manganese-oxidizing bacteria can produce copious quantities of nanoparticulate minerals via a respiratory process that strips electrons from lower valence states of the metal and transfers them to the electron transport chain (Tebo et al., 1997; Kappler and Straub, 2005). In the case of iron, particles of poorly crystalline ferric hydroxide result that are typically 3–5 nm in diameter;
these aggregate strongly and eventually form larger particles of more crystalline iron oxides (Kappler and Straub, 2005).

For metal-reducing bacteria, metals serve as the terminal electron acceptor during respiration rather than being assimilated by the bacteria. Metals transformed by bacteria during dissimilatory respiration that form nanoparticles include Fe, Mn, U, Tc, Cr, V, and Pu (Lloyd et al., 2002; Boukhalfa et al., 2007; Fortin et al., 2008). Of respirable metals, iron is ubiquitous and relatively abundant in most environments, and copious amounts of secondary ferrous or mixed valence precipitates may form. Reduction of FeIII by dissimilatory metal reducing bacteria such as *Shewanella putrefaciens* and *Geobacter metallireducens* can result in extracellular crystals of magnetite (Fe₃O₄) around 10 nm in diameter, which serve no known purpose for the organisms that produce them (Frankel and Bazylinski, 2003), in addition to larger crystals of minerals with different structures and compositions (Fortin et al., 2008).

Nanominerals associated with the bacterial cell wall

Soluble metals, such as species formed by V⁵⁺ and U⁶⁺, can penetrate the outer membrane of Gram-negative bacteria and form nanometer-size precipitates via an induced process in the periplasm upon reduction during respiration, in addition to forming abundant extracellular precipitates (Figure 1) (Suzuki et al., 2002; Lloyd et al., 2002). Soluble cytochromes in the periplasm catalyze the reduction of the metals to lower valence, insoluble species, e.g., U(IV) and V(IV). These precipitates are several nanometers in diameter and may have considerable consequences for metal mobility in the environment (Suzuki et al., 2002). Recent evidence suggests, however, that extracellular proteins in biofilms inhibit the mobilization of nanoprecipitates by inducing aggregation (Moreau et al., 2007). Nanoparticles generally have a strong tendency to aggregate.

**Intracellular nanominerals**

Nanocrystalline precipitates of respired metals generally remain external to the cytoplasm or within the periplasm. It has been observed, however, that reduced iron is reoxidized and compartmentalized within the cytoplasm as clusters of 40–50 nm precipitates at the poles of *S. putrefaciens* CN32 during the dissimilatory reduction of iron (Figure 2) (Glasauer et al., 2002; Glasauer et al., 2007). The particles contain both ferric and ferrous iron, display a low degree of crystal order, and appear to be surrounded by a membrane. The function of the particles, if any, is still unknown.

Some soluble, oxidized forms of metals more scarce than Fe can penetrate the plasma membrane and precipitate as induced nanocrystals in the cytoplasm of bacteria, as well as form extracellular precipitates. Examples of these include gold, silver, tellurium, and cadmium; all are highly toxic, and chemical reduction may be part of a detoxification strategy (Southam and Beveridge, 1994; Klaus-Joerger et al., 2001; Baesman et al., 2007). In the case of gold and silver, the intracellular precipitates are highly crystalline and have distinctive morphologies (Southam and Beveridge, 1994; Klaus-Joerger et al., 2001). During detoxification of cadmium present in the extracellular environment, *Schizosaccharomyces pombe* produces intracellular nanocrystalline particles of cadmium sulfide, around 1.8 nm in size, which can act as quantum semiconductor crystallites (Klaus-Joerger et al., 2001).

The challenge in harnessing bacterial metabolism to produce nanoscale minerals lies in controlling the size...
and crystal structure of the products. Extracellular precipitates are subject to the diverse range of chemical conditions that the cell encounters, resulting in amorphous or poorly crystalline materials with a high proportion of impurities (Gilbert and Banfield, 2005). In contrast, biologically controlled mineralization takes place in the tightly regulated intracellular environment and results in nanoscale minerals with architectures that are exquisitely defined (Bazylinski and Frankel, 2003). The only known example of bacteria that direct the formation of iron nanocrystals are the magnetotactic bacteria. The intracellular minerals they form consist of either magnetite or greigite (Fe₃S₄). Each crystal functions as a single domain magnet, termed a magnetosome. The magnetosomes are aligned in single or multiple chains within the cytoplasm (Figure 3), which increases the magnetic susceptibility. The function of the magnetosomes is unknown, although it has been widely speculated that the crystals help maintain the bacteria in their optimal environment within the freshwater or brackish aquatic systems where they are found (Bazylinski and Frankel, 2003). Magnetosome magnetite is of great interest because of its magnetic properties and crystal structures, and it is anticipated that understanding the molecular genetics of magnetosome formation will lead to the abiotic synthesis of nanocrystalline magnetite particles with the same morphologies and properties, as well as help explaining their occurrence (Bazylinski and Frankel, 2003).

Future directions in microbial nanocrystal research

The use of living or intact bacteria to fabricate nanocrystals requires the cultivation of biomass on a large scale and selective extraction of the mineral particles that form, both of which present challenges. The greatest potential in harnessing bacterial activities for nanocrystal fabrication lies in identifying the genetic systems that control precipitation. The identified systems may then be applied using organisms that are more readily cultivated or to produce larger quantities of nanocrystals. Genetic engineering techniques may be used to exert greater control over the composition and crystal structures of the mineral products. An interesting new research direction in biomaterials considers the impact of biopolymers such as peptides and RNA on nanocrystal formation (Feldheim and Eaton, 2007). Future research may combine bacterial processes that produce abundant nanoparticles with exposure to biopolymers that mediate their formation to generate crystals that have been engineered to impart specific properties.

Summary

Bacterial nanocrystals are of great interest because of their importance in natural environments as well as their practical applications. Their broad relevance has joined scientists across disciplines from geology to biology and material science, which has rapidly advanced the understanding of nanoparticulate matter and its reactivity in natural settings. Controlled nanocrystal synthesis among bacteria is rare and yields relatively low amounts of solid relative to biomass. Induced crystallization processes can result in large volumes of poorly crystalline or morphologically heterogeneous minerals and span a wide range of elemental compositions. Harnessing biological reactions to control microbial nanocrystallization is an outstanding challenge and presents intriguing opportunities for new research paths. Finally, understanding the properties of nanocrystals as well as why some bacteria produce them is crucial to predicting how metals react and transform in natural environments.

Bibliography


Nickel is one of the most important trace metals in biology. While its concentration in most rocks is only between 10 and 150 μg g⁻¹, it is highly enriched in ultramafic rocks, like peridotite and serpentine (Sigel et al., 2006). Nickel is a major trace element and part of the catalytic centers of many important metabolic enzymes.

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(Dobbek et al., 2001; Voordouw, 2002). The Ni-containing CODH is involved in many reactions in anaerobic microorganisms: it catalyzes the oxidation of CO with H₂O to CO₂ and H₂ in carboxydrotrophic bacteria, and assists the oxidation of acetate to CO₂ in sulfate-reducing bacteria and archaea and the disproportionation of acetate to CH₄ and CO₂ in methanogenic archaea. In many groups of anaerobic microorganisms utilizing the reductive acetyl-CoA pathway, such as acetogenic bacteria or methanogenic archaea, CODH is often found in a complex with ACS (Strauss and Fuchs, 1993; Holo and Sirevag, 1986; Drennan et al., 2004). ACS catalyzes the formation of acetyl-CoA from CO₂ leading in combination with the CODH to the synthesis of acetate from two CO₂. These two enzymes play an important role in the global carbon cycle, since up to 10% of the organic carbon produced by photosynthesis is remineralized by anaerobic microorganisms involving CODH/ACS (Drake, 1994). MCR is the key enzyme of methanogenic archaea catalyzing the final step during the reduction of carbon dioxide to methane (Thauer, 1998). All biologically formed methane is produced by methanogenic organisms in the terminal step of anaerobic degradation. The nickel is located in a tetrapyrrole coenzyme named F₄₃₀, which is unique to methanogenic archaea. MCR catalyzes the reduction of methyl-coenzyme M with a reduced coenzyme B, leading to the release of methane and a heterodisulfide of the two coenzymes. The latter can be cleaved in an energy yielding reaction. Furthermore, in a process called “reverse methanogenesis,” methane is anaerobically oxidized with sulfate as electron acceptor. This process – known as AOM – is carried out by an uncultured group of archaea (ANME), which has been shown to contain high amounts of a modified MCR (Krüger et al., 2003; Hallam et al., 2003). Hydrogenases are a family of oxidoreductase enzymes that catalyze the reversible oxidation of molecular hydrogen. Nickel-containing hydrogenases have been isolated from bacteria and archaea (Voordouw, 1992). Finally, nickel is also present in a number of other enzymes, such as urease, a superoxide dismutase, and glyoxylases. The functions and biological roles of these metalloproteins have been described in detail (Sigel et al., 2006).

Recently, due to its strong involvement in geologically important microbial reactions, especially methanogenesis, AOM, and carbon fixation, nickel has raised interest as a potential biosignature for these processes and the involved microorganisms in recent or ancient environments (Reitner et al., 2005; Hausrath et al., 2007).

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Cross-references
Acetogens
Anaerobic Oxidation of Methane with Sulfate
Biosignatures in Rocks
Carbon Cycle
Hydrogen
Metalloenzymes
Methanogens
Sulfate-Reducing Bacteria

NITRIFICATION
Nitrification is the two-step microbial process by which ammonia (NH₃) is first oxidized to nitrite (NO₂⁻) which is, in a second reaction, converted to nitrate (NO₃⁻). See entry “Nitrogen” for detailed reading.
NITROGEN

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Definition
Nitrogen is a nonmetal element with atomic number 7 and atomic mass of 14.00674 u. It was discovered in 1772 by Daniel Rutherford (1749–1819), a Scottish physician, botanist and chemist (Weeks, 1934).

Basic information
Nitrogen mainly occurs as dinitrogen (N\textsubscript{2}), a diatomic, colorless, odorless, and inert gas at standard conditions. The atoms of N\textsubscript{2} are connected via an extremely stable triple bond, the strongest in nature (bond energy \(= 945 \text{kJ mol}^{-1}\)). N\textsubscript{2} constitutes 78.1 vol% of the atmosphere, making up the largest nitrogen reservoir on earth, followed by sediments and the oceans. Other, geobiologically relevant forms of inorganic nitrogen in fresh and marine waters, soils, and sediments include ammonia (NH\textsubscript{3}), its ionic form ammonium (NH\textsubscript{4}\textsuperscript{+}), nitrite (NO\textsubscript{2}\textsuperscript{−}), nitrate (NO\textsubscript{3}−), and gaseous nitrogen oxides (N\textsubscript{2}O, NO\textsubscript{x}) (Bashkin, 2002). An overview about major global nitrogen reservoirs is given in Table 1.

Nitrogen is one of the "biological elements" (beside C, H, O, P, and S), and is a major component of amino acids/proteins, nucleic acids (DNA, RNA), phospholipids, porphyrins, and secondary metabolites. According to the "Redfield–Richards Ratio," the C:N:P ratio of phytoplanktonic biomass is, with some variation, 106:16:1 (C/N = 6.6). The C/N ratio of terrestrial plants is substantially more variable and also tends to be larger than that for marine phytoplankton, mainly due to a higher portion of nitrogen-free cellulose- and lignin-based wooden tissues. Although biomass plays only a minor role as a global nitrogen reservoir (Table 1), nitrogen is, after carbon and oxygen, quantitatively the most abundant element in living organisms and its biogeochemical cycle is of great importance in the biosphere. The key processes involved in the nitrogen cycle are the following. For a schematic overview, see Figure 1).

Biological nitrogen fixation
Biological nitrogen fixation refers to the biological conversion of dinitrogen (N\textsubscript{2}) into ammonia (NH\textsubscript{3}), ammonium (NH\textsubscript{4}\textsuperscript{+}), and various organic compounds, and is the ultimate source of nitrogen for all organisms. There are two major limitations for biological nitrogen fixation (Bashkin, 2002). First, because of the stability of the triple bond in N\textsubscript{2}, nitrogen fixation requires a high energy input. Only some symbiotic and non-symbiotic bacteria, archaea, and algae, the so-called nitrogen fixers or diazotrophs, are able to perform the reduction of N\textsubscript{2}, employing a specific Fe and Mo-bearing enzyme, nitrogenase, which catalyzes the following reaction:

\[
\text{N}_2 + 8\text{e}^- + 8\text{H}^+ + 16\text{ATP} \rightarrow 2\text{NH}_3 + \text{H}_2 + 16\text{ADP} + 16\text{Pi} \quad (1)
\]

Second, nitrogenase is rapidly, and irreversibly, deactivated by oxygen. Therefore, only those organisms that live in anoxic environments or that can locally create such an environment will fix nitrogen. In terrestrial habitats, symbiotic bacteria, of which the genus Rhizobium is the most well studied, are important nitrogen fixers. These bacteria are found on the roots of leguminous plants (peas, soybeans) and some woody shrubs and trees. In aquatic systems, including the oceans, cyanobacteria are considered as the most important players in nitrogen fixation (Stal and Zehr, 2008). In cyanobacteria, nitrogen fixation takes place in particular cell compartments, the heterocysts, which are well sealed from the sites of oxygen production (see entry Cyanobacteria). N\textsubscript{2} fixation in

| Nitrogen, Table 1 Major global nitrogen reservoirs—(After Reeburgh, 1997; Purvaja et al., 2008.) |
|-----------------|-----------------|
| Reservoir       | Tg N (1 Tg = 10\textsuperscript{12} g) |
| Atmosphere      | 3.9 \times 10\textsuperscript{9} |
| N\textsubscript{2} | 1.3 \times 10\textsuperscript{9} |
| Ocean           | 6.0 \times 10\textsuperscript{5} |
| N\textsubscript{2}O | 1.0 \times 10\textsuperscript{5} |
| Marine (plants) | 3.0 \times 10\textsuperscript{2} |
| Marine (animals)| 1.7 \times 10\textsuperscript{2} |
| Terrestrial     | 3.5 \times 10\textsuperscript{4} |
| Soil            | 9.5 \times 10\textsuperscript{4} |
| Sediments       | 4.0 \times 10\textsuperscript{7} |

Nitrogen, Figure 1 Major pathways in the biogeochemical nitrogen cycle. 1 = Biological nitrogen fixation, 2 = Ammonia assimilation, 3 = Nitrification, 4 = Assimilatory nitrate reduction, 5 = Ammonification, 6 = Denitrification (dissimilatory nitrate reduction), 7 = Anammox.
Ammonia assimilation

Ammonia assimilation (uptake) is the process by which NH₃/NH₄⁺ is transferred to organic nitrogen-containing compounds (e.g., amino acids or nucleotides; Madigan and Martinko, 2006). Such direct assimilation of NH₃/NH₄⁺ into biomass has significant energetic advantages for those organisms which are able to use this form of nitrogen. In oxic environments, however, ammonium ions have a very limited lifetime because they are easily consumed in the nitrification process (see below).

Nitrification

Nitrification is the process by which ammonium is oxidized to nitrite and further to nitrate. The process includes two conjugated stages both of which are performed by aerobic bacteria, mainly chemoautotrophs (Meiklejohn, 2006). At present, no organism able to perform both steps simultaneously is known. The first stage of nitrification is the oxidation of ammonium to nitrite, according to the following reaction:

\[
\text{NH}_4^+ + \frac{1}{2} \text{O}_2 \rightarrow \text{NO}_2^- + \text{H}_2\text{O} + 2\text{H}^+,
\]

\[
\Delta G^0 = -290 \text{ kJ/mol}
\]

Representative bacteria that oxidize ammonium to nitrite include Nitrosomonas (very common in soils), Nitrospira, and Nitrocytis species. Notably, it has recently been reported that some bacteria (Thiocapsa sp., Rhodopseudomonas sp.) oxidize nitrite to nitrate in the light, thus using nitrite as an electron donor for photosynthesis. So far, this is the only known photosynthetic oxidation in the nitrogen cycle (Schott et al., 2010).

The second step in nitrification is the oxidation of nitrite to nitrate.

\[
\text{NO}_2^- + \frac{1}{2} \text{O}_2 \rightarrow \text{NO}_3^-, \Delta G^0 = -82 \text{ kJ/mol}
\]

Representative bacteria that oxidize nitrite to nitrate include Nitrobacter (very common in soils), Nitrospina, and Nitrococcus species.

Artificial fertilizers containing reduced forms of nitrogen, namely urea and ammonium sulfate \((\text{NH}_4)_2\text{SO}_4\), may fuel the nitrification process in well-drained agricultural soils. This has considerable environmental consequences, because in the nitrification process, some intermediates like hydroxylamine \((\text{NH}_2\text{OH})\), NO, and N₂O are produced in significant amounts. Particularly the release of N₂O to the atmosphere may cause considerable environmental problems with respect to ozone depletion and global warming effects (IPCC, 2007). Nitrate in the environment is subject to two major pathways, assimilatory nitrate reduction and denitrification (see below).

Assimilatory nitrate reduction

Assimilatory nitrate reduction is defined as the reduction of nitrate \((\text{NO}_3^-)\) and assimilation (uptake) of the nitrate-derived nitrogen into biomass. This pathway is somewhat more energy-expensive than ammonia assimilation, and therefore, it is prevalent when reduced nitrogen, i.e., ammonium, is low in supply, such as in aerated soils or in the oxic water column of aquatic environments. Assimilatory nitrate reduction is a primary nitrogen input of many microorganisms. Plants can commonly assimilate both ammonium and nitrate.

Ammonification

Ammonification refers to the breakdown of organic nitrogen into ammonium \((\text{NH}_4^+)\). Strictly speaking, it is a two-step process (Libes, 1992). In the initial stage, particular organic nitrogen (PON) compounds are broken down to smaller dissolved molecules, i.e., dissolved organic nitrogen (DON). The DON is then further processed by heterotrophic bacteria to form ammonium. This process is actually called “ammonification,” and involves the enzymatically driven hydrolysis of peptide bonds of proteins to form amino acids, and the subsequent cleavage of the amino groups from the molecules. The resulting ammonium can be assimilated or microbially oxidized in the nitrification process, as outlined above.

Denitrification

Denitrification is the biologically facilitated reduction of nitrate \((\text{NO}_3^-)\) to N₂ and other gaseous intermediates, mostly N₂O, which may return to the atmosphere (Bashkin, 2002). In denitrification, heterotrophic bacteria, such as members of the genera Bacillus, Paracoccus, and Pseudomonas (Madigan and Martinko, 2006), and some archaea (Cabello et al., 2004) oxidize organic matter by using nitrate as their electron acceptor:

\[
5\text{CH}_2\text{O} + 4\text{NO}_3^- \rightarrow 2\text{N}_2 + 5\text{HCO}_3^- + \text{H}^+ + \text{H}_2\text{O}
\]

Recent work has also shown that nitrate reduction can be coupled to methane oxidation (Raghoebarsing et al., 2006). Furthermore, several reactions of nitrate with reduced inorganic species have been reported that may also lead to the reduction of nitrate, whether biologically facilitated (Fe(II); Muehe et al., 2009) or not (MnO, “chemodenitrification”; Brandes et al., 2007). Denitrification leads to an overall loss of organic nitrogen (Hulth et al., 2005). It is the most energetically favorable anaerobic respiration process, with an energy yield only slightly
less than that of aerobic (O₂) respiration. During denitrification, some of the nitrate is not assimilated into biomass but reduced sequentially involving the following reduction steps (canonical denitrification):

\[
\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2
\]

(4)

and the overall reaction

\[
2\text{NO}_3^- + 10e^- + 12\text{H}^+ \rightarrow \text{N}_2 + 6\text{H}_2\text{O}
\]

(5)

Denitrification is usually thought to prevail mainly in soils, waters, and sediments under suboxic or anoxic conditions (Libes, 1992), because O₂ acts as a competing electron acceptor for nitrate, and key enzymes of the denitrification pathways are inhibited by O₂. However, it has recently been reported that denitrification is also proceeding in oxic marine sediments (Gao et al., 2010). Evidently, denitrification is not necessarily inhibited in the presence of substantial oxygen concentrations but rather the co-respiration of O₂ and nitrate occurs. It is as yet unknown, whether this co-metabolism takes place in a single organism, or in different populations within the microbial community.

Like in the nitrification process, denitrification produces gaseous intermediates that cause environmental problems with respect to ozone depletion and global warming effects (Bateman and Baggs, 2005). N₂O is the quantitatively most important of these intermediates. It becomes particularly abundant under acidic conditions of denitrification, and may account for 0–20% of the gaseous nitrogen released (N₂: 80–100%; Bashkin, 2002). In agriculture, denitrification is an undesired process because it removes nitrate, a nitrogen source amenable for plants, from the soil, thus decreasing soil fertility.

Anammox

Anammox, the anaerobic oxidation of ammonium, has only recently been recognized as an important route in the marine nitrogen cycle (Ward, 2003; Dalsgaard et al., 2005). In oxygen-deficient systems, anammox bacteria remove ammonium by reacting it with nitrite (NO₂⁻) to form N₂:

\[
\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + 2\text{H}_2\text{O}
\]

(6)

The anammox bacteria are highly specialized and belong to the order Planctomycetales (Schmid et al., 2005). Anammox was recognized as a major sink for fixed inorganic nitrogen in coastal sediments, in anoxic waters of the Black Sea, and in oxygen minimum zones in upwelling systems (Kuypers et al., 2003, 2005). In isotope labeling studies on surface sediments, anammox was found to account for 4–79% of the total N₂ production (Engström et al., 2005). On a global scale, it was estimated that anammox is responsible for up to 50% of the removal of fixed nitrogen from the world’s oceans (Ward, 2003; Dalsgaard et al., 2005).

Geological and evolutionary aspects

Being a major constituent of the precursor biomass, organic nitrogen is generally found in fossil organic matter, e.g., kerogen, coal, thermogenic natural gas, and crude oils. However, organic nitrogen tends to become more and more depleted over geological times, as a result of preferential loss of N-bearing functional groups from organic matter during burial (e.g., desamination).

Whereas nitrogen is abundant in the atmospheric and biological pools, nitrogen-containing rocks and minerals are relatively scarce. Nitrogen occurs naturally only in a few relatively rare minerals, of which the nitrate salts saltpeter (KNO₃) and Chile saltpeter (NaNO₃) are quantitatively most abundant. Due to the extremely high solubility, these salts are stable only in hyperarid (precipitation <5 mm/year) settings such as the Atacama Desert in Chile. Here, the Chile saltpeter ores, also known as “caliche,” are soil-hosted (NaNO₃ content 3% to >13%, reserves ca. 500,000,000 t). The sources and formation mechanisms of the Atacama nitrate ores have long been debated (Ericksen, 1983). Recent isotope studies support an abiotic origin (Böhlke et al., 1997; Michalski et al., 2004). According to these studies, the main source of the Chilean saltpeter deposits is the precipitation of nitrate produced by photochemical reactions in the atmosphere, and the long-term accumulation in the absence of leaching and biological activity. It has also been suggested that volcanic eruptions from the close-by 70,000 km² Altiplano-Puna volcanic plateau may have fueled the thermal and electric (lightning-induced) fixation of atmospheric nitrogen as nitrate.

Apart from the formation of the actual nitrogen minerals, the adsorption of ammonium and its exchange for alkali metals in clay minerals are important and ubiquitous processes that decrease the bioavailability of nitrogen in sediments and soils, with major consequences for nitrogen turnover rates and soil fertility (Rosenfeld, 1979; Mamo et al., 1993). Moreover, studies revealed evidence that exchange reactions between potassium and ammonium are the dominant control of N₂ generation at depth, and the storage, release, and migration of nitrogen gas in sedimentary rocks. As a consequence, nitrogen frequently occurs as an undesired component natural gas that may potentially reduce the quality of hydrocarbon gas reservoirs (Krooss et al., 2006; Mingram et al., 2005).

The modern biogeochemical cycle of nitrogen depends on two obligately aerobic steps related to nitrification, i.e., ammonium oxidation to nitrite, and nitrite oxidation to nitrate. Hence, closure of the nitrogen cycle, as it is proceeding today, could be achieved only when free oxygen became available after the “Great Oxidation Event” ca. 2.3 billion years ago and promoted the evolution of aerobic ammonia and nitrite oxidizers (see entry Critical Intervals in Earth History). It is unknown whether electron acceptors other than oxygen, such as Fe³⁺, could have been used as electron acceptors fueling the nitrogen cycle prior to rise of oxygen (Bashkin, 2002).
 Likewise, the different routes for nitrogen fixation and assimilation require a portfolio of specialized biochemical pathways operating under different environmental conditions. Because a number of metalloenzymes are necessary for nitrogen fixation and assimilation, it is likely that the availability of transition metals has been a major control on the evolution of nitrogen assimilation pathways over Earth history (Zerkle et al., 2006; Glass et al., 2009). As an example, Fe and Mo are essential elemental components of the nitrogen-fixing enzyme, nitrogenase, and in enzymes responsible for the assimilation of inorganic nitrogen. Whereas Fe is scarce in the well-oxygenated modern oceans, it was abundant in anoxic Archean surface and deep oceans. Similar changes must have occurred in the abundance of Mo but with an opposite sense of direction, because Mo is more soluble in oxygenated than in reducing waters (Zerkle et al., 2006; Anbar, 2008; Scott et al., 2008). It was therefore hypothesized that photosynthesis-derived O₂ in late Archean oceans, particularly the “Great Oxidation Event,” mobilized Mo and enabled the evolution of Mo-based N₂ fixation and the Mo-dependent enzymes for assimilation and denitrification by prokaryotes (Glass et al., 2009).

Summary

Nitrogen is one of the “biological elements” that constitutes organic compounds and its biogeochemical cycle is of utmost importance in the biosphere. The nitrogen cycle is mainly driven by prokaryotes and involves different nitrogen species, mainly N₉, NH₄⁺, N₂, N₂O, NO₂⁻, and NO₃⁻. Nitrogen mostly occurs as dinitrogen (N₂) in the atmosphere and, in a dissolved form, in aquatic systems. Reductive conversion of nitrous oxide (N₂O) into biologically accessible forms (organic compounds, NH₃) is an energy and resource-expensive process that requires specific enzymes and conditions (enzymes). Further reactions used either for assimilatory purposes or in respiratory pathways involve anaerobic reductive processes (ammonia oxidation, NH₃), anaerobic/aerobic reductions (denitrification, NO₃⁻ → N₂), and aerobic oxidations (nitrification; NH₃ → NO₂⁻ → NO₃⁻) of the various nitrogen species. It is unknown whether and how the aerobic steps closing the modern nitrogen cycle were substituted early in Earth history, i.e., before the rise of oxygen. In the geological realm, nitrogen rarely occurs in a few highly soluble minerals such as saltwater (KNO₃, NaNO₃). In addition, it is a constituent element of iron minerals (ammonium), sedimentary organic matter, including kerogen, coal, natural gas, and crude oil, but its reservoir sizes and the modes of occurrence and formation are as yet not well constrained.

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**Cross-references**

Archaea

Bacteria

Critical Intervals in Earth History

Cyanobacteria

Nitrogen Fixation

Thiotrophic Bacteria

**NITROGEN FIXATION**

Nitrogen fixation is the conversion of diatomic nitrogen (N₂) into ammonia (NH₃), which is then used for biosynthesis of organic compounds. See entry “Nitrogen” for further reading.
ORES, MICROBIAL PRECIPITATION AND OXIDATION

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Synonyms
Gossan; Ore deposits; Oxidation zone; Supergene enrichment

Definition
Ore deposits are natural enrichments of chemical elements of economic interest. While all natural elements are present in certain background concentrations in rocks and minerals, they are typically not economically extractable at these levels. Geological processes may lead to enrichments of elements in such a way that orebodies are formed from which large quantities of elements may be extracted at a much lower cost. The formation of orebodies generally occurs in three steps: (1) element extraction from a large volume of rock or melt; (2) transport of elements; (3) deposition of elements in a volume of rock much smaller than the extracted volume. These steps may occur in magmatic melts, hydrothermal systems, diagenetic environments, and at the Earth’s surface. Melts, solutions, gases, and solid phases (minerals) may be involved in these processes. In many cases, ore forming processes occur in magmatic and hydrothermal systems well beyond the temperature limit of microbial growth. However, important ore forming systems are also known within the temperature realm of microbial life (maximum 121°C). These are mainly confined to sedimentary and diagenetic environments and the low-temperature end of hydrothermal systems. A microbial involvement in the different steps of ore formation may be due to direct interaction, i.e., ore precipitation as a result of microbial activity. On the other hand, indirect influences of life may include the conditioning of the environment (e.g., oxidizing atmosphere), allowing transport of certain elements in a soluble form, and so influencing ore forming processes in systems where microbes cannot live. During the weathering of ore deposits under near-surface conditions, microbes may also play an important role. In general, the direct involvement of microbes in ore formation is one of catalyzing redox reactions such as the oxidation of Fe and Mn and the reduction of S, Cr, and U. The role of low-temperature redox fronts as sites of ore formation and availability of energy for microbes have been reviewed by Hofmann (1999).

The possible role of microbes in ore precipitation has long been recognized (Macqueen and Coope, 1985; Southam and Saunders, 2005). Precipitation of ore minerals as a direct result of microbial activity can be inferred from the fact that many microbes can harvest energy from redox reactions, leading to immobilization of elements. Such reactions include the reduction of U(VI), Cr(VI), and Au(I) (Kashefi and Lovley, 2000; Kashefi et al., 2001; Labrenz et al., 2000; Moreau et al., 2004), the reduction of As and Se (Stolz and Oremland, 1999), and possibly also the indirect reduction of oxidized elements by H2S (Mohagheghi, 1985).

Arsenic is an important element in ore deposits. Reduced arsenic (As-I) leads to the precipitation of Ni, Co, Fe, and other elements as arsenides. Native As, As(0), also is relatively common. Native As of distinct low-temperature origin occurs in a stratigraphically well-defined horizon over approximately 300 km² in the Middle Triassic of southern Germany/N Switzerland (Hofmann, 1989a; Hofmann and Gehlen, 1993). While the microbial reduction of As(V) to As(III) is well known
An involvement of microbes in the reduction of As to lower valence states is also known (Bentley and Chasteen, 2002) and may possibly play a role in the precipitation of arsenides and native As.

Oxidation reactions leading to ore mineral precipitation are particularly significant in the case of Fe (Konhauser, 1998; Konhauser et al., 2002; Widdel et al., 1993) and Mn (Tebo et al., 1997).

There are still other possible mechanisms of microbial involvement in ore precipitation such as the microbial destruction of complexants, e.g., of thiosulfate in the case of gold and possibly silver (Lengke and Southham, 2005). Similar processes may apply for cyanate.

Other iron deposits. Phanerozoic sedimentary iron deposits commonly display oolithic or pisolithic textures and are of syngenetic–diagenetic origin. Dahanayake and Krumbein (1986) proposed a biogenic involvement in ore precipitation based on the presence of filaments in ooids.

Sedimentary manganese deposits including ferromanganese nodules. The coincidence of environmental oxidation in the Proterozoic with the precipitation of the Kalahari manganese deposits, the world’s largest manganese orefield, is taken as evidence that deposit formation was induced by biological oxygen production (Kirschvink et al., 2000; Schaefer et al., 2001).

Sandstone-hosted uranium deposits: Major sandstone-hosted uranium deposits most commonly occur in Mesozoic and Cenozoic rocks in the western USA, but also in Australia, southern France, and the former Soviet Union. A summary of uranium deposit types is found in (Dahlkamp, 1993). The sandstone-hosted deposits are often formed where detrital terrestrial organic matter produced local reducing environments. Sandstone-hosted uranium deposits also occur in Proterozoic rocks of the Franceville Basin of Gabon, where the reducing traps are related to hydrocarbon-bearing faults. Phanerozoic deposits exist in tabular and roll-type (Figure 2) geometries. Closely related are redox-boundary controlled uranium deposits in altered phonolitic rocks at Pocos de Caldas, Brazil (Figure 3, see Waber et al., 1992). Both for roll-type deposits (Goldhaber et al., 1978; Reynolds et al., 1982; Warren, 1972) and tabular U-V deposits (Goldhaber et al., 1990; Meunier et al., 1987; Mohagheghi et al., 1985; Wanty et al., 1990) biogenic versus abiogenic reduction processes have been discussed. Considering that these deposits formed in near-surface environments at close to ambient temperatures that they do contain sulfide minerals indicating low-T sulfate reduction, and that they represented redox fronts providing a lot of chemical energy, the absence of a microbial involvement would be surprising. However, no clear biosignatures have been described. Light sulfur isotopes from these deposits have been interpreted biologically (Jensen, 1958; Rackley, 1972), but also nonbiologically (Warren, 1971). The possible involvement of intermediate-valence sulfur species (e.g., thiosulfate), through biological (Bak and Cypionka, 1987) or nonbiological pathways, seem possible.

Unconformity-related uranium deposits: For this major type of uranium deposits (mainly occurring in the Proterozoic of Canada and Australia) a microbial involvement appears generally unlikely due to high formation temperatures of 100–250°C (Suzuki and Banfield, 1999). An involvement of microbial activity has been postulated by Donnelly and Ferguson (1980). There is an interesting paragenetic similarity (predominance of U, Co, Ni, Bi,
Ag) with reduction spheroids, for which a microbial involvement appears likely.

**Deep-sea hydrothermal vents**: Sulfide-rich ore deposits originating from the venting of metal-rich hydrothermal fluids in the deep sea are dominated by Fe–Cu-sulfides (with Au, Pb–Zn-rich in back-arc and sedimentary settings). Based on sulfur isotope data, a part of the sulfide sulfur is of microbial origin (Fallick et al., 2001; Taylor, 2004). In case of Irish-type carbonate-hosted massive sulfide deposits, similar evidence has been provided by Wilkinson et al. (2005).

**Mississippi-Valley-Type (MVT) and related Pb–Zn deposits**. MVT deposits are formed not only from basinal brines, mainly in carbonate rocks, but also in sandstones at the edge of sedimentary basins. Ore deposition is due to cooling, wall-rock reactions, fluid mixing, or a combination thereof (Burstein et al., 1993; Leach et al., 1996). The possible role of microbial sulfate reduction in the precipitation of ores is controversial. Reports on fossilized microbes (Kucha et al., 2005) and sulfur isotopic variations have been interpreted as proof of biogenic involvement, e.g., in salt caprock-associated (Saunders and Swann, 1994) and alpine type deposits (Schroll, 1996). Features of thermochemical sulfate reduction (TSR) as compared with biogenic sulfate reduction (BSR) are discussed by (Machel, 2001), concluding that most MVT deposits are due to TSR, while BSR produces iron-dominated sulfide accumulations. The range of MVT deposits is very large and ore textures range from coarsely crystalline to submicroscopic. This variability, also including sulfur isotopes, and the near-surface origin of a part of the deposits, may indicate that both TSR and
BSR are involved in MVT genesis. Clear criteria for a differentiation have yet to be found.

Low-temperature hydrothermal ore deposits. Low-temperature (epithermal) ore deposits are formed in a temperature range that may overlap with possible microbial growth. Fluctuating systems may provide conditions at least temporarily suitable for microbes. Reports of microbial activity in such occurrences are rather scarce. Hofmann (1989b) reported filamentous forms from the Krunkelbach uranium deposit in (Germany, Figures 4 and 5) and similar features were observed in other Schwarzwald occurrences (Reitner, 2004). Based on morphological similarities, a microbiological origin of features described as “stalactites” from Creede, Colorado (Campbell and Barton, 1996; Hofmann, 1989b; Hofmann et al., 1998; Reitner, 2004), may be ascribed to be due to mineralized microbial fabrics (Hofmann et al., 2008).

Reduction spheroids. Small-scale reduction phenomenon in red beds with accumulations of numerous elements (U, V, Co, Ni, As). Due to geochemical similarities with sandstone-hosted U-V deposits and certain low-T hydrothermal veins, these features of probable microbial origin may present an important genetic link see Reduction Spheroids.

Black shales. Sediments deposited under oxygen-poor to oxygen-free conditions, with low sedimentation rates, are typically enriched in redox-sensitive elements such as U, V, and Mo (Lehmann et al., 2007; Leventhal, 1998). Trace element-rich black shales are a result of the activity of sulfate-reducing bacteria in the water column and/or at the water–sediment interface. Typically, black shales are of subeconomic grade, e.g., the U-enriched Cambrian Alum Shale of Scandinavia. Under conditions of extremely low sedimentation rates, economic ore grades may be reached as in case of the early Cambrian of China (Lehmann et al., 2007). Secondary processes such as deep diagenetic redox fronts (Kupferschiefer of Germany and Poland) or supergene enrichment, e.g., at Ronneburg and Marsberg, Germany (Stribny and Puchelt, 1991), may enrich black shale protores to economic grades.

Weathering and supergene enrichment of ore deposits
Long after the formation of orebodies, interactions with their environment may take part; most commonly observed are weathering and supergene enrichment. In certain cases the processes of weathering lead to a secondary, near-surface enrichment of the ore (supergene enrichment) along a redox front between unaltered

Ores, Microbial Precipitation and Oxidation, Figure 3 Uraninite-rich nodule in altered phonolite from the reduced side of the redox front at Poços de Caldas, Minas Gerais, Brazil. A microbial origin of this local uranium enrichment appears possible, based on its isolated occurrence, low-T origin, and geometry. Field of view is 17 mm.

Ores, Microbial Precipitation and Oxidation, Figure 4 Banded quartz mineralized with pyrite and uraninite, encrusting silicified granite. Krunkelbach uranium deposit, Schwarzwald, Germany. Low-temperature hydrothermal precipitation with possible involvement of microorganisms. Note the change in mineralization in banded quartz at bend. Sample width 3 cm.

Ores, Microbial Precipitation and Oxidation, Figure 5 Filamentous forms preserved as uraninite in low-T hydrothermal quartz from the Krunkelbach uranium deposit, Schwarzwald, Germany (compare Figure 4). Field of view 350 μm. Automontage image.
and oxidized ores. Supergene enrichment may be critical for economic extraction. An involvement of microorganisms in the processes of ore oxidation and supergene enrichment has been reported in many cases from terrestrial (Sillitoe et al., 1996) and marine settings (Glynn et al., 2006). Microbial sulfide oxidation is well studied, mainly with reference to biomining and acid rock drainage.

Probably the most significant microbial process in the oxidation zone is the oxidation of sulfide (Nordstrom and Southam, 1997) and ferrous minerals. Subsurface filamentous fabrics SFF likely of microbial origin are very common in oxidation zone mineral assemblages (Figures 6 and 7). Such morphological evidence of microbial activity in the oxidation zone of ore deposits is corroborated by stable isotopic data (Rainbow et al., 2006; Melchiorre and Williams, 2001; Melchiorre et al., 2001).

In the zone of supergene enrichment, oxidized fluids from near-surface parts of the ore interact with reduced, unaltered ore (commonly sulfides). A microbial involvement in such processes of supergene enrichment has been demonstrated in the Morenci copper mine, Arizona (Enders et al., 2006), and in the case of ore-grade supergene sphalerite from Nevada (Bawden et al., 2003). Based on nannobacteria-like forms, Sillitoe et al. (1996) assumed a microbial origin of cementative copper ore at Chuquicamata, Chile.

Conclusions
It is a fact that microorganisms do perform transformations (mainly redox changes) for a number of key elements in ore deposits. In many cases such microbial transactions are advocated to explain the genesis of orebodies, and in other cases they are excluded. A critical review of the existing literature indicates, however, that proofs of microbial involvements often are weak, while the probability of microbial involvement, based on knowledge in microbial ecology, is highly likely. Clearly, the range of arguments to prove or disprove biological interactions in ore deposit formation needs to be extended.

Bibliography


Cross-references

Acid Rock Drainage
Banded Iron Formations
Biomining (Mineral Bioleaching, Mineral Biooxidation)
Biosignatures in Rocks
Black Shales
Carbon (Organic, Degradation)
Extreme Environments
Hydrogen
Hydrothermal Environments, Fossil Nan(0)obacteria
Origin of Life
Radioactivity (Natural)
Reduction Spheroids
Subsurface Filamentous Fabrics
Sulfate-Reducing Bacteria
Sulfide Mineral Oxidation

ORGANIC CARBON

Carbon that occurs in materials of ultimately biological origin that can be oxidized. Organic matter is material that contains organic carbon. See entries “Carbon (Organic, Cycling)” and “Carbon (Organic, Degradation)” for further reading.

ORGANOMINERALIZATION

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Synonyms

Organomineral formation
Definition

Organomineralization is a process of mineral formation mediated by organic matter (OM), independent of the living organisms which the OM derives from. The organic compounds may be excretion products or detached parts of living organisms, or relics and by-products of dead organisms that have been released into waters or incorporated into soils, sediments, or rocks. Abiotic OM may also induce the formation of minerals in nature or in the laboratory.

Organominerals are the mineralic products of organomineralization. They should be distinguished from minerals precipitated through inorganic processes, among which some may contain OM entombed or complexed during crystal growth, from organic minerals such as graphite and from biominerals produced by living organisms.

Genesis of the organomineralization concept

The term organomineralization was introduced in 1993 by Trichet and Défarge (1995) at the 7th International Symposium on Bionmineralization. It aimed to denote the processes of mineral formation that are similar to biomineral formation by living organisms, because induced by organic templates, compartments and compounds (Bionmineralization, qv), but distinct because occurring in waters, soils, sediments or rocks, independently of the living organisms which the mineralizing OM derives from. The substantive organomineral was also introduced at the same symposium to refer to the mineral products of organomineralization, to be distinguished from the biominerals produced by living organisms (Défarge and Trichet, 1995). These definitions were supported by illustrations of organomineral formation processes including (Défarge and Trichet, 1995; Trichet and Défarge, 1995) diagenetic calcification of gastropod fecal pellets; postmortem encrustation of cyanobacterial filaments, fish organs, and soft tissues of trilobites by calcium carbonate (CC), calcium salts of fatty acids, or pyrite; formation of CC bodies and phosphate pellets under the influence of sedimentary OM; intervention of soil organic compounds in the crystallization of aluminum and iron oxides and hydroxides; precipitation of metal oxides and sulfides following the demixion of organometallic complexes subjected to geothermal heating; and laboratory formation of CC ooids in the presence of OM rich in acidic groups.

The first hypotheses on the role of sedimentary OM in the precipitation of minerals in natural environments, believed to be analogous to that played by organic templates in biomineralization, were published in the 1960s in papers presented on the formation of calcareous microbial deposits, oolites, and coprolites (Trichet, 1967, 1968; Mitterer, 1968; for later references see Morse et al., 2007). These hypotheses were founded on the similarities in composition of the organic fraction of these carbonate bodies with that of biominal matrices, which are both rich in acidic amino acids, and on earlier work evidencing that dissolved organic compounds can favor CC precipitation from waters (see references in Mitterer, 1968; Trichet, 1968; or Trichet and Défarge, 1995).

A decisive step in this research and in the genesis of the concept of organomineralization was made, thanks to studies on carbonate formation in the modern lacustrine microbialites (qv) of Pacific atolls (Défarge and Trichet, 1995): The mineral fraction of these sediments was shown to be principally composed of magnesian calcite micrite clusters that had precipitated within the alveoli, on the wall surfaces of an organic honeycomb-like network formed by the reorganization of extracellular polymeric substances (EPS, qv) secreted by cyanobacteria (qv). These organic templates of precipitation thus had the same origin as those of CC biomineralization in cyanobacteria, which is associated with the cell sheath (Merz-Preiß, 2000). In addition, it was demonstrated that this OM fixed divalent metallic cations at acidic sites, whose affinities with calcium ions explain why they act as nucleation sites for CC biomineralization (Addadi and Weiner, 1992; Gilbert et al., 2005). When they can be individually observed, the magnesian calcite crystals were shown to grow with their c-axis perpendicular to the organic template (e.g., Défarge et al., 1996, Figure 5c), as is frequently observed in epitaxial CC biomineralization (Addadi and Weiner, 1992), e.g., on algal cell surfaces (Weiner and Dove, 2003). This process of micrite precipitation thus appeared analogous to carbonate biomineralization, although distinct because of being mediated by nonliving, sedimentary OM. These micrite organominerals were accompanied by tubular ones, resulting from the postmortem encrustation of cyanobacterial filaments, which thus controlled the final mineral shape, and by biomineralized cyanobacterial sheaths (Défarge and Trichet, 1995). These carbonate microbialites thus revealed a continuum of mineralization–organic–mineral interactions in nature, from biomineralization through organomineralization processes (Défarge and Trichet, 1995; Défarge et al., 1996).

Organomineralization versus biomineralization

The existence of such continua among biominalization processes sensu stricto had already been established: Lowenstam and Weiner (1989) distinguished “biologically controlled mineralization”, characterized by cellular controls on crystal nucleation and growth, delineated spaces of mineral formation, and specific habits of the biominerals produced, and “biologically induced mineralization”, which designates biotic processes that are not specifically designed for mineralization but result in mineral being formed. However, in these latter cases too, organisms exercised some control on mineral formation (Lowenstam and Weiner, 1989; Weiner and Dove, 2003).

Another continuum bridges biologically induced mineralization occurring through crystal nucleation on organic secretions outside of organisms (see Weiner and Dove, 2003, Figure 4), and organomineralization on sedimentary organic substrates derived from EPS described by...
Défarge and Trichet (1995). The distinction between the two processes, however, is justified, as nucleating organic substrates may act remote in space and time from the organisms which they derive from, as in the examples documented by Trichet and Défarge (1995) for carbonate sediments or for metal minerals formed through geothermal heating of organometallic complexes.

In all cases of organomineralization, biotic processes (such as photosynthesis or sulfate reduction in the case of CC; Dupraz and Visscher, 2005) may also participate in solution supersaturation leading to crystal formation on the nonliving organic templates. However, the global process cannot be simply described as “biologically induced” when these nonliving substrates appear to play a necessary role in crystallization, in particular crystal heterogeneous nucleation, or cessation or confinement of crystal growth. In introducing the concept of organomineralization, Trichet and Défarge (1995) aimed to point out that organic matrices and compounds inherited from living organisms may retain mineralizing properties after they have been released into waters and incorporated into soils, sediments, and rocks.

**Evolution of the organomineralization domain**

The same year in which Trichet and Défarge (1995) introduced the term organomineralization at the 7th International Symposium on Biominalerization (1993), Reitner (1993) and then Reitner et al. (1995) at the same conference, hypothesized that organic matrix-mediated processes were responsible for carbonate automicrite and peloid formation in modern microbialites and sponge tissues from reef caves. These processes appeared analogous to those postulated for the precipitation of CC organominerals in Polynesian microbialites (Défarge and Trichet, 1995; Trichet and Défarge, 1995), and have therefore been considered organomineralization processes (Neuweiler et al., 1999; Reitner, 2004). Thereafter, a number of papers have documented CC organomineralization in natural environments or in the laboratory (see review in Défarge et al., 2009). New illustrations included for example, automicrite formation in Lower Cretaceous carbonate mud mounds (qv; Neuweiler et al., 1999) or calcification of the housing tubes of modern and fossil polychaete worms (Fischer et al., 2000). Riding (2000) and Schlager (2003) identified organomineralization as one of the main processes of carbonate precipitation in microbial mats (qv) and biofilms (qv), or more widely in benthic marine sediments. Many in vitro studies continued to demonstrate the control exerted by biological compounds on the crystallization of various mineral species (Davies et al., 2003). Thanks to experiments that induced the precipitation of carbonate bodies in the presence of OM extracted from the Murchison CM2 meteorite, Reitner (2004) pointed out that abiotic OM may also induce organomineral formation.

Recently, Dupraz and Visscher (2005) proposed distinguishing carbonate organomineralization mediated by crystal nucleation on recomposed EPS, as in the former examples documented by Défarge and Trichet (1995) in Polynesian microbialites, from carbonate precipitation following bacterial degradation of EPS: Bacterial degradation liberates EPS-bound ions such as Ca\(^{2+}\), Mg\(^{2+}\), or HCO\(_3^\)-, thus leading to outside solution supersaturation for Ca and Mg carbonates. The latter process is not considered by Dupraz and Visscher (2005) to be organomineralization sensu Trichet and Défarge (1995). However, sedimentary EPS may also concentrate ions independent of their living producers (Trichet and Défarge, 1995; Westall et al., 2000). In the case of carbonate precipitation induced by degradation of that sedimentary OM, since ion binding by EPS is a prerequisite for mineralization, this process should be considered a subcategory of organomineralization. The distinction between organomineralization induced by the liberation in solution of ions previously concentrated by sedimentary EPS and organomineralization involving a nucleating role of EPS corresponds to the distinction originally introduced by Trichet and Défarge (1995) between “organically induced” and “organically supported” organomineralization.

**Geobiological consequences of organomineralization**

**Impact on present-day and past geological processes**

Although difficult to evaluate, because it is frequently hard to distinguish from the direct role of biotic activity, and neglected by most geobiologists, the impact of organomineralization on sediment and soil formation is likely to be far from negligible. The works cited above (reviewed by Défarge et al., 2009) have already documented occurrences of organomineralization processes in the principal environments at the surface of the Earth, from deep cold water to shallow warm marine, through hyposaline to alkaline lakes, and terrestrial soils. They are involved in the formation of sedimentary calcareous buildups such as microbialites, mud mounds, and reefs (qv). Organomineral products include micrites, ooids, peloids, lithified cyanobacterial sheaths, worm housing tubes, animal tissues, and fecal pellets. The rapid authigenic phosphatization of tissues after death, leading to the preservation of soft-bodied fossils, likely involves matrix-mediated organomineralization processes (see Briggs, 2003, p. 292). The principal mineral species forming biominerals (Weiner and Dove, 2003) are also represented by organominerals: Ca carbonates and phosphates, and metal sulfides, oxides, and hydroxides; the silicification of EPS that occurs in biofilms independent of those of the microorganisms (Westall et al., 2000) may also be regarded as an organomineralization.

Défarge et al. (1996) showed that organomineralization processes are probably commonly involved in microbialite calcification; this was then confirmed in numerous cases (Défarge et al., 2009). The process of carbonate–fluorapatite precipitation mediated by dead
bacterial cells, proposed by Sanchez-Navas and Martin-Algarra (2001), in phosphate stromatolites (qv) that are representative of microbial structures frequently encountered in pelagic sediments from Mesozoic Alpine-Mediterranean paleomargins, may also be considered an organomineralization. The involvement of mineralizing OM remains the most plausible hypothesis for explaining the formation of magnesian calcite radial ooids composing numerous shallow marine carbonate sands (Morse et al., 2007). Neuweiler et al. (1999) provided evidence for the involvement of micrite organomineralization in the building of Lower Cretaceous mud mounds that are significant examples of calcareous buildups, possibly originating in the Neoproterozoic and common during the Phanerozoic. As EPS are essential components of microbial biofilms, which are widespread in nature and persistent throughout the history of life on the Earth (Westall et al., 2000), EPS-mediated organomineralization has probably been active at least since the appearance of living organisms, and may represent a geologically early process of mineral production coexisting with, or even preexisting, biomineralization.

Implications for astrobiological interpretations
Organominerals formed through the mediation of biologically derived OM may serve as biosignatures (qv) in the search for evidence of life in the geological rock record or on extraterrestrial bodies devoid of fossils or bioconstructions. Westall et al. (2000) stressed that mineralized EPS could be used as markers of the presence of bacteria in terrestrial or extraterrestrial materials. However, these biotic EPS need to be distinguished from potential prebiotic EPS that might have the same abilities for fossilization (Westall et al., 2000). Laboratory experiments by Reitner (2004) have shown that OM, likely abiotic, extracted from the Murchinson CM2 meteorite, was able to precipitate CC organominerals similar to those known from terrestrial microbial sediments. More generally, organic molecules were proved to be able to precipitate, in sterile conditions, Ca carbonate or phosphate mineral products resembling biologically induced minerals, in particular those interpreted as fossilized “nan(n)obacteria” (qv; Kirkland et al., 1999; Reitner, 2004; see other references in Défarge et al., 2009). Astrobiological studies for the search and the origin of life should keep in mind this spectrum of potential prebiotic, abiotic, or biologically derived organominerals in nature.

Potential engineering applications
Biomineralization processes have already inspired the design of new organic matrix-mediated materials by synthetic analogues of the matrices of natural biominerals (Davies et al., 2003). Such manufactured materials are artificial organominerals, although they can also be regarded, from a global geobiological perspective, as biominerals, because they are formed under direct biological, i.e., human, control. Similarly, organomineralization sensu stricto might, in the future, inspire further engineering processes based on the precipitation of minerals or the extraction of ions from solutions in sterile conditions, applied, for example, to water and wastewater treatment, soil remediation and improvement, protection and restoration of building stones, etc.

Summary and perspectives
The direct role of life in the functioning and the evolution of our Planet is beginning to be fully recognized; witness is this encyclopedia. The geobiological impact of OM, however, which is in part included in the indirect role of biotic activity but may also be due to prebiotic or abiotic processes, is far from being equally well acknowledged and studied. Since the mineralizing role of OM has now been proved in various environments and geological periods, it should be searched for and identified in other case studies, in particular in carbonate formation that took place before the rise of calcareous organisms. While organomineralization research has mainly been devoted until now to EPS calcification, studies on organomineralization processes leading to the formation of other common biomineral species, such as phosphates, metal sulfides and oxihydroxides, or silica, should be continued, and the case of other species such as sulfates might be considered. The role of other types of sedimentary and soil OM also needs to be investigated. The possible involvement of abiotic OM in organomineral formation should be taken into account in astrobiological studies for the search of life on the primitive Earth and on extraterrestrial bodies. In addition to materials applications based on biomineralization processes, other studies might be devoted, in the future, to organomineralization-inspired engineering.

Bibliography


**Cross-references**

- Biofilms
- Biosignatures in Rocks
- Cyanobacteria
- Extracellular Polymeric Substances (EPS)
- Microbial Biomineralization
- Microbial Mats
- Microbials, Modern
- Mud Mounds
- Nan(n)obacteria
- Reefs
- Stromatolites

**ORIGIN OF LIFE**

**Definition**

Life exploits free energy potentials to produce effluent at much reduced value. Put another way, it takes a low entropy feed, transforming it to high entropy waste. At the root or origin of the evolutionary tree as well as at the base of the food chain, life essentially draws down carbon dioxide by reducing it with hydrogen from a variety of sources, aided by a diversity of other aqueous solutes. Thus, life is a coded and organized entropy generator that can search and evolve to find further appropriate potential energies and materials.

**Approaches to the origin of life**

Origin of life theory has yet to reach a stage where it can take its place as the introductory chapter in textbooks in the life sciences. Why is this? Many researchers have concentrated on what life is rather than what life does, and have cast around for organic molecules that might have constituted the “building blocks” for the first cells – nucleic and amino acids, carbohydrates, and lipids. Thus comets (Ehrenfreund et al., 2006; see Chapter *Asteroid and Comet Impacts*), carbonaceous meteorites (Deamer, 1985), cosmic dust particles (Maurette, 1998), the products of lightning in a reduced atmosphere (Miller, 1953; Miller and Urey, 1959), and Fischer–Tropsch reactions within the Earth’s crust (Holm and Charlou, 2001) have all been appealed to in what may be called organogenic or heterogenic theories of origin. But research taking this tack has had little experimental success. Nor does it suggest an evolutionary link between such “prebiotic chemistry” and biochemistry. However, if instead of approaching life in terms of its constituents and where they might come from, but rather ask “what does life do,” it can be apprehended rather as a processor of energy and a generator of entropy, so inviting consideration of a thermodynamic, geochemical, and evolutionary context for the emergence of life. This approach has led to the autogenic theories for life’s origin, theories that view as...
paramount consideration of the conditions upon the early Earth that drove life into being that see the emergence of life as a geological issue.

Therefore, the method applied in this entry is broadly evolutionary, autogenic, and mechanistic. We consider the onset of life as a natural and inevitable outcome of the well-established far-from-equilibrium conditions obtaining on our planet more than 4 billion years ago, especially the potential between hydrothermal hydrogen and atmospheric carbon dioxide. However, more reduced molecules bearing one carbon atom such as formate (CHOO\(^-\)), and methyl thiolate (CH\(_3\)S\(^-\)) may be required to get life going initially. Moreover, electrochemical energy is also seen to be needed in the form of the protonmotive force. Indeed, the first microbial vehicles were "hybrids," driven into being by chemical potential and steep pH, redox, and temperature gradients across their membranes. So, first looking at what life does, we then investigate how life, as an energy and materials processor, may have begun. Only after metabolism’s engine is ticking over could genetic regulation and then replication emerge.

**Life now**

At root life takes molecules that are out of equilibrium at or near the surface of the Earth, reacts them together (i.e., processes or metabolizes them) within cells that can grow, reproduce, and evolve. None of this can happen without cell mortality and the excretion and disposal of relatively disordered waste products. In human metabolism the disequilibrium is between hydrogen released from foodstuffs (by dehydrogenases to nicotinamide dinucleotide (NAD) to NADH)) and atmospheric oxygen (Williams and Ramsden, 2007). The waste products are carbon dioxide and water from energy production and the excreted disordered (higher entropy) organic molecules of lower energy rejected or degenerated during biosynthesis. But we live off others. At the base of the food chain are organisms that directly exploit the disequilibrium between hydrogen released from water in some manner, and from the carbon dioxide gleaned from the atmosphere. The obvious examples of the food-chain's base are the plants and the cyanobacteria (see Chapter *Cyanobacteria*). They split hydrogen from water into four protons and four electrons during oxygenic photosynthesis using, amongst other mechanisms, a complex enzyme known as photosystem 2 (PS2) (Blankenship, 2002); oxygen is released as waste:

\[
2\text{H}_2\text{O} + \text{light} + \text{PS2}_{\text{oxidized}} \rightarrow 4\text{H}^+ + 4\text{e}^- + \text{PS2}_{\text{excited}} + \text{O}_2 \uparrow
\] (1)

The protons and electrons are then "fed" to the carbon dioxide, which with further enzymatic assistance, is thereby reduced to cellular carbon and some waste water:

\[
4\text{H}^+ + 4\text{e}^- + \text{CO}_2 \rightarrow (\text{CH}_2\text{O})_{\text{life}} + \text{H}_2\text{O}
\] (2)

However, there is a still more basic metabolism whereby prokaryotes use the hydrogen emanating naturally from hot springs in a geochemical, rather than a photosynthetic, reaction (Spear et al., 2005; see Chapter *Hydrogen*). The hydrogen is released during hydrothermal convection as the water oxidizes the ferrous iron in minerals such as fayalite olivine (Fe\(_2\)SiO\(_4\)) in the Earth’s crust:

\[
3\text{Fe}_2\text{SiO}_4 + 2\text{H}_2\text{O} \rightarrow 2\text{SiO}_2 + 2\text{Fe}_2\text{O}_4 + 4\text{H}_2 \uparrow
\] (3)

This hydrogen can reduce carbon dioxide in certain prokaryotes through the involvement of nicotinamide dinucleotide (NAD), chemiosmosis, iron-nickel hydrogenases, and iron-sulfur proteins (Daniel and Danson, 1995; Volbeda and Fontecilla-Camps, 2005a; Williams and Ramsden, 2007; McGlynn et al., 2009; see Chapters Hydrothermal Environments, Marine and Nickel, Biology). In such cases the carbon wastes are either acetic acid as in the homoacetogens (see Chapter *Acetogens*):

\[
2\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_3 \cdot \text{COOH} + 2\text{H}_2\text{O}
\] (4)

or methane as in the methanogens (see Chapter *Methane, Origin*):

\[
\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}
\] (5)

Based on the uniformitarian principle (that the present is the key to the past) we expect today’s food chain to recapitulate the evolutionary tree of metabolic pathways, thereby giving us a strong indication of what the earliest biochemistry was like. From this knowledge we might enquire whether there is a match between such a biochemical expectation and the very slow aqueous geochemical reactions arising from the initial conditions on our planet four or more billion years ago – reactions that could have been quickened through the emergence of life. A positive response to the question has led to a hydrothermal theory of the origin of life that at first sight seems to lie strictly in the autogenic camp – a theory that expects that the first metabolism was based on acetogenesis and methanogenesis (see Chapters *Acetogens* and *Methane, Origin*).

**Autogenic theory**

The class of theory that invokes the simplest of components such as carbon dioxide and ammonia to explain the emergence of life was first termed “autogenic” by Haeckel (1870, p. 302; 1892, p. 414). Mereschkowsky (1910, p. 360), Harvey (1924), and Corliss et al. (1981) thought the same way. Because *appropriate* organic molecules were not to be had on the early Earth, an origin of life based on minerals and their component clusters was generally assumed (Leduc, 1911; Goldschmidt, 1952; Hall et al., 1971). A modern autogenic version of the theory considers the early Earth as having surface geochemistries that fed into the overall reactions as identified in Equations 2, 4, or 5 (Shock 1992; Russell and Arndt, 2005; Russell and Hall, 2006). Of these, what is the most
likely first reaction and thereby the most plausible pathway to life? Obviously, if hydrogen were to have been provided freely to emergent life, the putative first organism is hardly likely to have managed (or bothered) to develop such a complicated system of accessing it from water near the turbulent ocean’s surface in the face of destructive ultraviolet radiation. That leaves reaction 4 as the likely energizer of life and source substrate for its structures. Reaction 5 could only supply energy. However, the generation of the intermediate radicals formyl (–HCO), methenyl (–CH’), methylene (–CH2), and methyl (–CH3) in both reactions 4 and 5 are kinetically (Shock, 1990) and in the early steps, thermodynamically (Maden, 2000) challenging, requiring both catalysis and energy for their resolution, (Figure 1). Yet these intermediates are themselves points of departure for biosynthesis (Maden, 2000). The kinetic barrier is higher for methane synthesis because the addition of a carbon-bearing radical (–COX) to a methyl (–CH3) group to produce acetate (H3C–COO–) faces less of an obstacle than does the addition of a hydride (H–) to make methane (CH4) (Maden, 2000). Thus a pathway to acetate was probably the first to develop (Fuchs, 1989; Crabtree, 1997). Exploiting such a metabolism is the task of the acetogenic bacteria via what is known as the acetyl coenzyme-A pathway (Russell and Martin, 2004; see Chapter Acetogens):

\[
2\text{CO}_2 + 8\text{[H]} + \text{co} - \text{A.SH} \\
\rightarrow \text{co} - \text{A} \cdot \text{S} \cdot \text{OC} \cdot \text{CH}_3 + 3\text{H}_2\text{O}
\]

However, the path to methane production has much in common with the acetogenic pathway and could not have been far behind in evolutionary terms (Martin and Russell, 2007). Nevertheless, the microbes that excrete methane are so different from the acetogens that they have been assigned to a different domain of life, viz., the archaea (Woese et al., 1990, see Chapter Archaea). In this entry we will limit ourselves to an explication of the emergence of autotrophic acetogenesis and methanogenesis as biochemistry’s first essay into processing chemical and electrochemical energy and generating cellular carbon. Given these qualifications, is there a likely context for where these reactions might have been promoted on the early Earth? To answer this we need to take a look at the conditions on our planet around 4 billion years ago.

**Conditions at ~4 Ga**

Presently taking up ~70% of the planet’s surface, the Earth 4 billion years ago was probably completely covered with water. Radiogenic, gravitational, and tidal heat rendered any continental massifs differentiated by then so hot as to be too pliable and plastic to break the surface of the Hadean Ocean (Russell and Arndt, 2005). Subject to gales and hurricanes and given to high tides on a planet where the day lasted a few hours, this ocean was in a state of constant turmoil. There was certainly no place near the UV-saturated chaotic surface where organic molecules had the peace to organize into anything more complex than small ephemeral slicks. And the atmosphere was dominated by CO2, mainly from a myriad of highly active volcanoes derived from the Earth’s mantle that had lost its native iron through rapid gravitation to the Earth’s core (Wood et al., 1990; Delano, 2001; Boyet and Carlson, 2005; Wood and Halliday, 2005). Also exhaled from volcanoes, pyrophosphate rained into the ocean (Yamagata et al., 1991; Hagan et al., 2007). Any reduced carbon molecules were photo-oxidized to more CO2. The carbonic ocean was acidic (pH 5–6) (Macleod et al., 1994; Lowell and Keller, 2003; Halevy et al., 2007). Acidic too were (and are) the 400°C springs exhaling at ocean spreading centers and above mantle plumes (see Chapter Hydrothermal Environments, Marine). Because the Fe:H2S ratio was >>1 in these very hot fluids emanating into the Hadean Ocean, transition metals exhaled directly into the reservoir of that acidic ocean (Kump and Seyfried, 2005; Konhauser et al., 2009). These metals (e.g., Fe, Mn, Zn, Ni, Co, V, Mo, and W) are just the ones that contribute to metalloenzymes, particularly important to the autotrophic prokaryotes (Cody, 2004; Volbeda and Fontecilla-Camps 2006; McGlynn et al., 2009; see Chapter Metalloenzymes). In contrast to the acidic hot springs, a large number of alkaline springs would have emanated at some distance from the oceanic spreading centers, similar in many ways to those found today at Lost City, 15 km from the

![Origin of Life, Figure 1](image-url)
Mid-Atlantic Ridge (Kelley et al., 2001). The Lost City fluids exhale at moderate temperatures (<90°C), are alkaline (pH ≤ 11) and contain up to 15 mmol/kg of dissolved hydrogen (Kelley et al., 2001; Martin et al., 2008; and see Macleod et al., 1994). They have exhaled without a break for at least 35,000 years (Früh-Green et al., 2003). Apart from hydrogen, other reduced entities likely delivered by moderate temperature alkaline springs on the early Earth were formate (HCOO$^-\$), ammonia (NH$_3$), hydrosulfide (HS$^-\$), methanol (CH$_3$OH), methane thiol (CH$_3$S$^-\$), and methane (CH$_4$) (Schulte and Shock, 1995; Russell and Hall, 1997; Shock et al., 1998; McCollom and Seewald, 2003). Metals of potential biochemical interest carried in these moderate temperature fluids are molybdenum and tungsten. These solutes from alkaline springs – when juxtaposed with the carbon dioxide, transition metals and protons in the Hadean Ocean – provide all the basic nutrient and appropriate energy requirements for chemolithotrophic life as outlined below (Figure 2).

The theory of the onset of life at an alkaline hydrothermal vent

The hydrogenations of atmospheric carbon dioxide on the early Earth to either acetate or methane (Equations 4 and 5), as well as of the organic by-products of these reactions (and their intermediates) that comprise living cells, are faced with both thermodynamic and kinetic barriers (Figure 1). This is the theoretical challenge to an evolutionary theory of the emergence of life. To conquer kinetic barriers, there must be common naturally occurring minerals that not only have the appropriate catalytic propensities but also are likely to be easily sequestered by organic molecules that they might evolve to protoenzymes. To override the thermodynamic barriers at the early stages of reduction (Figure 1), extra electrochemical energy is required to drive reactions 4 and 5 toward completion; energy supplied through chemiosmosis and the protonmotive force as alluded to below (Mitchell, 1967, 1979; Kell, 1988).

A plausible context where the necessary thermodynamic/electrochemical drive and natural catalysts comes into play is at a long-lasting hydrothermal vent. Here such alkaline solutions feed springs of constant temperature (<100°C) and pH (<11) that exhale at a distance from ocean-floor spreading centers into a somewhat acidic ancient ocean (Shock, 1992; Russell et al., 1994) (Figure 2). As this alkaline solution titrates with the metal-bearing acidulous ocean, porous and semi-permeable hydrothermal mounds form through precipitation of the metals as sulfides, carbonates, clays, and oxyhydroxides (green and white rust) on the ocean floor. Evidence for the porous nature of mound structure derives from modern examples (Marteinsson et al., 2001; Kelley et al., 2005) as well as from 350 million year old fossil representatives at mineral deposits in Ireland. These latter structures formed from similar titrations to those defined above, though where the pH of the two solutions was inverted (Figure 3) (Russell and Hall, 1997). Like these...
morphologies, the growing surface of Hadean mounds probably comprised bubbles and compartments made of iron sulfide (FeS) and hydroxide gels with subordinate nickel, wherever the alkaline solutions were particularly sulfidic. Microcavities within initially colloidal iron monosulfide and hydroxide precipitates acted as the original chemically and electrochemically driven catalytic culture chambers for early metabolism and embryonic life (Russell et al., 1994). Thus the hydrothermal mound takes on the attributes of a continually renewed catalytic flow reactor and fractionation column, fed by hydrogen from within, and protons from without (Russell and Hall, 1997; Stone and Goldstein, 2004; Martin and Russell, 2007). Here, fresh catalytic iron sulfide and iron hydroxide nanocrystalline surfaces are continually offered as catalytic reaction sites between hydrogen (separated into electrons and protons) plus formate in the hydrothermal solution, and the carbon dioxide dissolved in the ancient ocean. The sulfides are mainly mackinawite (FeS) and greigite (Fe3S4) (see Chapter Iron Sulfide Formation) – sulfides which accommodate nickel, an effective and common catalyst. The atomic lattices of these sulfides have affinities with those of the active centers of enzymes involved in the acetyl coenzyme-A pathway mentioned above (Figure 4) (see Chapter Metalloenzymes).

Mackinawite (FeS) comprises rhomboids of FeSMS (where M = Fe, Ni or Co and S = sulfur) whereas in the rather more oxidized greigite [NiFe3S8] these rhombs associate to form Fe3S4 cuboids with a distal iron or nickel atom ligated through two sulfur atoms (Figure 4a). Further oxidation (sulfidation) to pyrite (FeS2) is prevented by formaldehyde (HCHO) (Rickard et al., 2001), significant because a formyl (–CHO) group is an early intermediate produced along the acetyl coenzyme-A pathway (Figure 1) (see Chapter Acetogens). The mackinawite rhomboids have a similar topology to the active centers of Fe–Fe and Fe–Ni hydrogenases involved in acetogenesis and methanogenesis (McGlynn et al., 2009). The greigite structure (Figure 4a), by contrast, is topologically similar to that of the [4Fe–4S]W+/centers of the ferredoxins (proteins that act as electron transfer agents) (Figure 4b); to the active center of carbon monoxide dehydrogenase (CODH) (e.g., [Fe3S4]S–Ni or [Fe3NiS4]Fe) (Figure 4c) where CO2 is reduced to CO; and to the [Fe3S4]cys-Ni-cys2-Ni+ center of acetyl coenzyme-A synthase (ACS) where the acetyl group (CH3CO−/CO) is assembled (Figure 4d) (Vaughan and Ridout, 1971; Rickard and Luther, 2007; Drennan et al., 2001; Drennan et al., 2001; Volbeda and Fontecilla-Camps, 2005a, b). (An animation of the mechanism by which an Fe–Ni hydrogenase, with the involvement of two 4Fe–4S centers and a 3Fe–4S center, splits hydrogen into 2 protons and 2 electrons, is beautifully illustrated at http://www.kcl.ac.uk/ip/richardcammack/H2/animation/movie1.html).

Origin of Life, Figure 4 The molecular structure of the mineral greigite (a) is very similar to that of the thiocubane unit (b) of the ferredoxin protein, as well as to the cuboidal complex (c) in the active site of the enzyme acetyl-CoA synthase/carbon monoxide dehydrogenase (shown in schematic form). The X-ray crystal structure (d) for the so-called A cluster of the latter confirms this similarity. Atoms are colored as follows: iron, red; sulfur, yellow; nickel, green; carbon, gray; nitrogen, blue. R signifies links through sulfur to the remainder of the protein. Part (d) is modified from Darnault et al. (2003) (and see Fontecilla-Camps and Ragsdale, 1999). (From Russell 2006, with permission.)
Is there experimental evidence to favor catalytic involvement of such iron-nickel sulfides or of nickel in the synthesis of acetate as we might expect from Equation 4 and in the conditions outlined above? No. The stumbling block appears to be the reduction of carbon dioxide to carbon monoxide in the analogy with a reversed “eastern branch” of the acetyl coenzyme-A pathway (see Ragsdale, 1997) (Figure 1). However, though yields are low, Heinen and Lauwers (1996) have reduced carbon dioxide to methyl sulfide ($\text{CH}_3\text{S}^-$) (in a short-cut through a reversed “western branch”) irreversibly from hydrogen sulfide and carbon dioxide in acidic conditions in the presence of ferrous sulfide where pyrite was apparently also produced at the liquid–vapor boundary:

$$7\text{FeS} + 8\text{HCl} + \text{CO}_2 \rightarrow 4\text{FeCl}_2 + 3\text{FeS}_2 + \text{CH}_3\text{SH} + 2\text{H}_2\text{O}$$ (7)

Though a much simpler molecule, methylsulfide ($\text{CH}_3\cdot\text{SH}$) is comparable to the thiol acetyl-coenzyme-A (Co-A · SH) that expedites the leaving of activated acetate from ACS. In theory methane thiol activities would rise 1000-fold when generated from H$_2$ and CO (rather than from ACS. In theory methane thiol activities would rise 1000-fold when generated from H$_2$ and CO (rather than from ACS). However, apart from the thiol, they also used carbon monoxide gas rather than the much more stable dioxide (Huber and Wächtershäuser, 1997):

$$\text{CO} + 2\text{CH}_3\text{SH} \rightarrow \text{CH}_3 \cdot \text{COSCH}_3 + \text{H}_2\text{S}$$ (8)

In a further departure from the biochemical pathway it should be noted that carbon monoxide is not stable in alkaline solution. However, a single carbonyl radical (=C=O) could perhaps be produced locally at each nickel site in greigite, dissociated from hydrothermal formate in the mound in a reaction driven to the right by the high CO$_2$ and H$_2$ partial pressures obtaining at an alkaline vent:

$$\text{CO}_2 + \text{H}_2 \Leftrightarrow \text{HCOO}^- + \text{H}^+ \Leftrightarrow \text{CO} + \text{H}_2\text{O}$$ (9)

However, the Huber–Wächtershäuser reaction (8) is comparable to the addition of a carbonyl group to the methyl sulfide facilitated by acetyl coenzyme-A synthase (ACS) (Figure 4d) (Crabtree, 1997; Schink, 1997; Amend and Shock, 2001). The synthesis works best when catalyzed by NiS in acidic or alkaline conditions or with nickel sulfate in alkaline conditions – a result to be expected from knowledge of the biochemical reaction, the hydrothermal context, and also the role of nickel in the active site of ACS (Figure 4d) (Ragsdale, 2004). As we shall see, this energetic thioester ($\text{CH}_3 \cdot \text{COSCH}_3$) could be variously carboxylated, condensed, aminated, or used to make pyrophosphate. In the latter case, acetate is the waste product as it is of the acetogens. We turn now to energy requirements for further biosynthetic reactions that are also thermodynamically uphill. Where might this energy have come from at the emergence of life?

**Pyrophosphate**

Life requires measures whereby energy can be stored. Now it employs various pyrophosphates for the job, notably adenosine triphosphate (ATP) produced either from substrate reactions (Gottschalk, 1985) or via the protonmotive force (Mitchell, 1967, 1979). ATP is too complex a molecule to be considered a geochemical product. It must be a product of early biological evolution. Thus we need to consider a precursor. One possibility is inorganic pyrophosphate (Westheimer, 1987; Baltcheffsky et al., 1999). And, seeing that acetate is one likely early product of emergent biochemistry, the first organic phosphate may have been acetyl phosphate ($\text{CH}_3 \cdot \text{CO} \cdot \text{PO}_4^{2-}$), perhaps produced by substrate phosphorylation of the acetyl thioester (from Equation 8) (de Duve, 1991):

$$\text{CH}_3\text{COSCH}_3 + \text{HPO}_4^{2-} \rightarrow \text{CH}_3\text{SH} + \text{CH}_3\text{COPO}_4^{2-}$$ (10)

Reacting this acetyl phosphate product with inorganic phosphate ($\text{HPO}_4^{2-}$) in the presence of Fe$^{II}$ minerals, de Zwart et al. (2004) have generated pyrophosphate with a yield of 25% at ~40°C,

$$\text{HPO}_4^{2-} + \text{CH}_3 \cdot \text{CO} \cdot \text{PO}_4^{2-} \rightarrow \text{HP}_2\text{O}_7^{3-} + \text{CH}_3 \cdot \text{COO}^-$$ (11)

comparable to the way ATP is produced by the phosphorylation of adenosine diphosphate (ADP) in the acetyl coenzyme-A pathway. FeS was found to strongly retard hydrolysis of, and thus preserve, the pyrophosphate (de Zwart et al., 2004). Either the acetyl phosphate or the resulting pyrophosphate (PPi) (Equation 10) could have provided a proportion of the energy to drive hydrogenations at the formate-to-formyl step along the acetyl coenzyme-A pathway (for the acetogens in Figure 1) (Romero et al., 1991; Baltcheffsky et al., 1999). It might also have polymerized amino acids. For example, an overall condensation of glycin ($\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{COO}^-$) with pyrophosphate on a mineral surface could theoretically produce diglycine:

$$\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{COO}^- + \text{HP}_2\text{O}_7^{3-} + \text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{COO}^- \rightarrow \text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{COO}^- + 2\text{HPO}_4^{2-} + \text{H}^+$$ (12)

However, this amount of pyrophosphate is a small proportion of the energy required for biosynthesis (Amend and Shock, 2001; Martin and Russell, 2007). The extra energy
could only be supplied by protons, via the protonmotive force as detailed next.

At the origin of life the iron sulfide/hydroxide precipitates do more than merely provide catalytic surfaces. They also act as compartment walls, tending to trap organic products that become involved in further reactions, while permitting the transfer of anions across the membrane driven by proton flow (Russell et al., 1994). This natural protonmotive force, a consequence of the pH gradient operating across these inorganic membranes, is also the energy with the potential to drive pyrophosphate synthesis and thereby further hydrogenations and condensations (Mitchell, 1979). If the fluid inside the compartments has a pH of \( \approx 10 \) and the ocean has a pH of \( \approx 5 \), then the “protonic” potential approximates 300 mV; enough geochemical energy to have driven metabolism on early Earth (Russell et al., 2003). That the pH-dependent boundary between the mono- and di-phosphate fields intersects the (primarily) Eh-dependent iron sulfide fields demonstrates how “proticity” (a proton current) could have driven the condensation of inorganic phosphate (Pi) to pyrophosphate (PPI) (Figure 5). So in theory the proton gradient could have been simply coupled through the membrane to this dehydration or condensation of monophosphate on nanocrystalline surfaces within the inorganic membrane (Russell and Hall, 2006):

\[
H^+ + 2HPO_4^{2-} \rightarrow HP_2O_7^{3-} + H_2O \tag{13}
\]

In biochemical energetics the process is known as “oxidative phosphorylation,” a reference to the fact that protons in living systems are initially driven to the outside of the membrane (to maintain charge balance with outflowing electrons to an acceptor) before returning to recharge the phosphate (Mitchell, 1967). The protonmotive force is the power behind metabolism and is therefore indispensable to life. So the fact that this ambient force is a feature of the redox and acid-to-alkaline interface separated by a semiconducting inorganic membrane suggests that the emergence of life was driven by similar energetic gradients as obtain in modern living systems. Nevertheless, the “ATPase” responsible for the conversion in today’s organisms is a complex rotating turbo-motor driven by a flow of protons from the membrane’s exterior (Elston et al., 1998). We assume that phosphate adhering to the mineral surfaces comprising the inorganic membrane was sequestered by short chains of amino acids (peptides) in a prototype of the phosphate-binding loop (the so-called P-loop) to form a pyrophosphatase as explained in a later section (Milner-White and Russell, 2005, 2008). This eventually evolved to the relatively simple, static \( H^+ \)-pyrophosphatase (Baltscheffsky et al., 1999; Hirono et al., 2007). That the amino acids and the subsequent heterochiral peptides required for such sequestering can be generated in an alkaline hydrothermal environment at moderate temperatures has been demonstrated by Wächtershäuser and his collaborators (Huber and Wächtershäuser, 1998; Huber et al., 2003).

### Amino acid and peptide synthesis

Huber and Wächtershäuser (2003) have successfully aminated \( \alpha \)-keto acids (like the pyruvate synthesized hydrothermally by Cody et al., 2000), but only in alkaline conditions. Amino acid yields are highest around pH 9, close to the pK\(_a\) of ammonium at 9.25 (Huber and Wächtershäuser, 2003). In these experiments Fe(OH)\(_2\) (“white rust”; a constituent, along with FeS, of the precipitate membranes) was shown to be just as effective a catalyst as iron monosulfide.

Similarly, alkaline (pH ~ 9) conditions are required in the condensation of amino acids to short peptides, though in these experiments an iron-nickel sulfide slurry was used as catalyst and carbon monoxide employed as condensing agent (Huber and Wächtershäuser, 1998, 2003). Of significance to the debate on the origin of chirality, leptotyrosine was found to racemize on condensation to give alternating L and D residues (Huber and Wächtershäuser, 1998, 2003). Leman et al. (2004) also condensed amino acids at similar alkaline pH, though using carbonyl sulfide in place of CO (Figure 5). The formation of peptides heralded a new stage in complexity at the emergence of life as we shall see. However, before this stage we must consider how organic molecules produced in these kinds of reactions might have been concentrated in the inorganic compartments comprising the mound.

### Organic concentration in inorganic compartments

At the origin of life the iron-nickel precipitates did more than merely provide catalytic surfaces. They also acted as compartment walls, tending to trap organic products that became involved in further reactions. Yet they likely...
permitted the transfer of anions across the membrane driven by the flow of protons from the acidulous Hadean Ocean through to the alkaline interior (Russell et al., 1994). And the protons had the potential to drive the condensation of phosphate to pyrophosphate; an example of ambient chemiosmosis. Evidence that such compartment walls could have existed in such an environment is afforded by what appear to be hollow but contiguous iron sulfide bubbles found at the 350 million year old Tynagh metal sulfide deposit in Ireland (Russell and Hall, 1997) (Figure 3). This ephemeral trapping of the rising hydrothermal solution, and its interfacing with the carbonic ocean across the inorganic membrane, also placed it at the mercy of physical as well as chemical gradients in an environment that could cause crowding of product (cf. Ellis, 2001).

Concentration through thermal diffusion
Quantities of the organic intermediates and further products of geochemical acetogenesis and methanogenesis, such as amino acids and short peptides, will tend to be trapped in solution within the inorganic compartments comprising the hydrothermal mound. Noting that the hydrothermal mound could act as an affinity column, some of the organic anions will be concentrated on the compartment wall. However, Braun and Libchaber (2004) demonstrate in vitro how thermophoresis (thermal diffusion) accumulates charged polymers, with the potential to drive them to high concentrations in the cooler, stagnant portions of individual reaction microchambers. Such a process would be most effective on the outer margins of a hydrothermal mound where the temperature gradient was high and ranged between 50 and 100°C; just the kind of gradient to be expected in the envisaged mounds growing at off-ridge hydrothermal springs and seepages. Such “crowding” of newly generated macromolecules would decrease the activity of water and thence inhibit hydrolysis and thus further enhance the assembly of oligomeric structures and their subsequent folding into functional peptides. It would also quicken the rates of reaction along metabolic pathways; a prelude to an organic takeover from mineral catalysis and compartmentation.

An organic takeover
Protoenzymes and coenzymes
The consanguinity between the first structural and catalytic sulfide precipitates (Figure 4) and pyrophosphate with early enzymes (Russell and Hall, 1997; Beinert et al., 1997; Baymann et al., 2003; McGlynn et al., 2009) suggests that these structures were sequestered by the short heterochiral peptides similar to those produced in the Huber–Wächtershäuser experiments. Eck and Dayhoff (1966), pointing to the likely ubiquity and catalytic propensity of FeS on the early Earth, made the suggestion that the ferredoxins had the longest pedigree of all enzymes. Even before genetic control similar structures may have formed spontaneously. For example, Bonomi et al. (1983) demonstrate the synthesis in water of iron sulfide clusters ligated to organic sulfides (e.g., thioethanol) to produce [Fe₄S₄][RS]₄⁺ anions in aqueous solution. Mildly hydrophobic short peptides are attracted to these anionic complexes by virtue of the δ charge on the amino group nitrogen atoms along the backbone of the peptide chain (N^δ⁺ H · CH₂ · CO^−; N^δ⁺ H · CH₂ · CO^−). These bend and bow to satisfy the negatively charged clusters (Figure 6). Cosseted in such “peptide nests,” the active centers (“eggs”) would be partially protected from dissolution, nucleation, and crystallization. Also, given the high surface-to-volume ratio of the clusters, they would remain highly active, yet spaced at distances appropriate for electron tunneling that allowed multi-electron bond-forming and breaking catalysis at high rates (Milner-White and Russell, 2005, 2008; Moser et al., 2006; Hengeveld and Fedonkin, 2007).

While these protoferredoxins were involved in electron transfer, phosphate anions were required for the storage of energy used in biosynthesis. These anions could also be sequestered by the backbones of short peptides. Indeed, a similar structure – the P-loop – is still a protein-motif of the phosphatases in prokaryotes to this day, the conformation now permitted by the one or more of the non-chiral amino acid glycine residues featured in the structure (Milner-White and Russell, 2008) (Figure 6). The “ready-made” aspect of these catalytically active molecular clusters is at one with the view that there was an evolutionary continuum between hydrothermal chemistry on the early Earth and emergent biochemistry (Russell and Hall, 1997; Martin and Russell, 2007). However, the short peptides may have had another important role as we see below.

Amyloid as the first organic membrane
Although FeS-bounded “cells” are plausible candidates for the hatcheries of life, they are flimsy, leaky, and poor
insulators. In the absence of lipids, which appear to be a late microbiological invention (Koga et al., 1998), peptidic membranes and walls to protocols offer several advantages to a metabolizing system before the advent of a genetic control. For example, sticky amyloid peptide would have made protective insulating layers and thus may have constituted the first organic membranes and cell walls (Zhang et al., 1993; Chernoff, 2004; Carny and Gazit, 2005; Liu et al., 2008). Some amyloid strands are like the nests described above but are flatter (Carny and Gazit, 2005; Liu et al., 2008). Some amyloid blocks are juxtaposed such that their charges are the same, then the natural repulsion between, for example the δ of the carbonyl oxygens of one peptide (\( \cdot N^{\delta\text{+}}H \cdot CH_2 \cdot CO^{\delta\text{–}} \cdot N^{\delta\text{+}}H \cdot CH_2 \cdot CO^{\delta\text{–}} \)) will interact electrostatically with the δ of the nitrogen atoms along the backbone of the next. Thus sheets of amyloid are formed that rapidly aggregate to blocks. Composites of this type of amyloid might coat the inside of the iron sulfide compartments, and eventually take over from it entirely. However, the amyloid is not limited to merely acting as an inert and impermeable membrane. In cases where the amyloid blocks are juxtaposed such that their charges are the same, then the natural repulsion between, for example the δ of the carbonyl groups will generate a natural channel that could allow transmission of particular ions, e.g., H\(^{\text{+}}\) and K\(^{\text{–}}\) (Milner-White and Russell, 2008).

Because this type of amyloid is composed of alpha-sheet, it could truss clusters such as the [Fe\(_2\)S\(_4\)][RS]\(_{4}\)\(^{2\text{–}}\) anionic centers within its structure as stabilizers and electron transfer agents (Figure 6). It could also sequester the phosphates and pyrophosphates [\( HP_2O_4^{3\text{–}} \)] required for early biosynthesis. Moreover, as glycine comprised much of the first peptide (Hennet et al., 2001), tetracyclines could sequester single atoms of Ni, Co and other transition elements as reduction- and group-transfer enzymes, as do the tetrapyroles in acetogenesis and methanogenesis (Eschenmoser, 1988; Thauer, 1998; Milner-White and Russell, 2008).

The emergence of the code

We have seen how the mound plausibly acts as the organic-molecule factory, i.e., as a compartmentalized flow reactor. Acetate, amino acids, and short peptides are synthesized in it. And natural iron-nickel sulfide catalysts, and phosphate condensing agents, are sequestered by short peptides to produce proteoenzymes and coenzymes. The inorganic, compartmentalizing membrane may then be superseded by a peptidic/amyloid membrane. The hydrothermal feed of hydrogen, formate, ammonia, and sulfide continues without interruption and meets the carbon dioxide, ferrous iron and phosphate in the acidic Hadean Ocean. Unlimited energy in the form hydrothermal hydrogen and of protons and electron acceptors in the ocean guarantees continual synthesis of organic molecules. What we do not have is replication – a reliable regulator, translation, or memory – we do not have genes.

Yet at first no special place in this decentralized “unintentional” world should be given to RNA beyond it being metabolically and homeostatically useful and thereby a surviving molecule (Hengeveld and Fedonkin, 2007). Prior to the involvement of nucleic acids in RNA and DNA, the nucleosides had metabolic roles, for example as pyrophosphate-bearing molecules like ATP and GTP (White, 1976). In time, and as polymers, they took over as information molecules, helping to regulate metabolic interactions, replicating and coding for propagation. But how were the ribose bases first assembled?

Although an unstable entity, once formed RNA would be less mutable when secured upon a mineral surface, especially in the presence of highly reduced fluids. Nevertheless, the synthesis of nucleic acids, composed of a phosphorylated ribose sugar attached to one of four possible bases, is a problem more daunting than that of the amino and carboxylic acids. As a start we note that pyrophosphate, introduced through volcanoes to the early oceans, remained in solution in the relatively acidic ocean, although some would have been precipitated as vivianite (\( Fe_2(PO_4)_2 \cdot 8H_2O \)) and as a condensed pyrophosphate on mixing with moderate temperature alkaline fluids at the hydrothermal mound (Rouse et al., 1988; Yamagata et al., 1991; Russell and Hall, 1997; de Zwart et al., 2004; Hagan et al., 2007). An explanation of how RNA bases were first synthesized in the mound still escapes us. One possibility is that they were constructed upon mineral surfaces in a sequence not very different from the way they are generated in the cell, for example with the simple entities; aspartate, glutamine, glycine, formyl phosphate, ammonia, and carbon dioxide (Martin and Russell, 2007). The synthesis of ribose phosphate, the particular pentose sugar attached to the bases, is also difficult to understand but may have been produced from phosphoenolpyruvate itself a product of the acetyl coenzyme-A pathway (Martin and Russell, 2007). Anyway, apart from the easily synthesized adenine, only small concentrations of the rest were needed because, as we shall see, RNA polymers were the “moulds” that may have produced a myriad of peptide “casts.”

Emergence of the RNA world

In living cells amino acids are sequenced and polymerized in a process centered on the ribosome, composed essentially of RNA that ratchets along messenger RNA (Ban et al., 2000). Such a complex process must have evolved from a simpler system. Just what this was is the most challenging problem facing origin of life research. What follows is a speculative attempt to point to research directions. Neglecting their side chains, amino acids comprise a negatively charged carboxyl group (\( –COO^- \)) and a positively charged amino group (\( –NH_3^+ \)). Amino acids...
are polymerized by the loss of the constituents of water, OH\textsuperscript{-} from the carboxyl of one amino acid and of H\textsuperscript{+} from the next. Though experiments show that condensation may be driven by carbon monoxide, carbonyl sulfide, or by drying (Ferris et al., 1996; Huber and Wächtershäuser, 1998; Huber et al., 2003; Leman et al., 2004), pyrophosphate phosphorylation is a possibility more in line with an alkaline hydrothermal origin though in conditions where water activity was low (see Baltscheffsky et al., 1999; Baaske et al., 2007) (Equation 18). While this may have happened in the first membranes, a polymerase would have been more efficient.

Woese (1967) suggested that genetic information was first transferred to protein sequences directly by selection through a somewhat indiscriminate “codon-amino acid pairing.” This relied upon the affinity between the shape and charge of the codon (a triplet of three nucleic acids) with the shape and charge of the amino acid and especially of its side chain (Woese, 1967, pp. 174–175). Thus, what is known as the peptidyl transferase reaction of an RNA molecule might have evolved via direct translation on a protoribosome. Although somewhat indiscriminate, triplets of side chains of RNA facilitating polymerization tended to favor particular amino acid side chains to the polymerizing amino acid sequence. Mellersh (1993) emphasized that RNA triplets offered a cleft-like (tridentate) conformation to attract amino acids in this way when adhering to a solid phase such as clay. The rows of RNA triplets then gripped and juxtaposed amino acid monomers in such a way as to offer the carboxyl group of one amino acid to the amino group of the next for bonding (Mellersh, 1993). At the same time the affinity between the clefts of RNA and the side chains of the amino acids would happen to effect a crude selection by the RNA polymer determines the decision of whether it catalyzed the polymerization of D- or L-amino acids into peptides (Mellersh, 1993). To achieve a low energy state, as with mineral growth we might expect RNA to tend to lengthen while preserving either left or right chiralities, i.e., a favorable packing arrangement (Joyce et al., 1984). Were a monomer with the opposite stereochemistry to be added to a growing chain, growth would be thwarted (Sanders, 2003). That filter would have been sufficient to tip the holochirality scale since, despite the presence of a racemic mixture of amino acids in the microcavities only amino acids of the same \(\alpha\)-carbon configuration (similar stereochemistry) would preferentially have ended up in peptides, to yield a population of distinctly handed peptides. The rest are eluted to the Hadean Ocean. Some of the retained peptides are eventually fed back in a hypercyclic manner to favor the syntheses of their “stereochemically appropriate” polymerizing template.

Eventually the more robust but less reactive DNA (deoxyribonucleic acid) molecules took over from RNA and thence survived. Braun and Libchaber (2004) demonstrate that secondary convection and thermophoresis, driven by temperature gradients within microcavities in a hydrothermal mound, could concentrate, elongate, and drive the replication of DNA. It remains to be seen if RNA can be elongated and replicated by the same process.

However, during the organic takeover the protoribosome required another surface in place of a mineral such as a peptide sequence rich in positively charged side chains. Such a peptide would have attracted the phosphates of RNA that they might polymerize and still offer the triplet clefts. With amino groups on their side chains, lysine, arginine, and ornithine are equally useful in such a peptide. Mellersh and Wilkinson (2000) demonstrated that poly-adenosine, which includes the clefts AAA expected to have affinity for lysine, does stereoselectively bind L-lysine from dilute aqueous solution of L-amino acids. Moreover, about half the amount of L-arginine and L-ornithine also was found to bind with poly-adenosine. As adenosine was likely the most common of the nucleic acids, and lysine and probably ornithine can be made abiotically, then we have the makings of a feedback cycle that involves the transfer of information.

In this scenario the chance stereochemistry of the short RNA polymer determines the decision of whether it catalyzed the polymerization of D- or L-amino acids into peptides (Mellersh, 1993). To achieve a low energy state, as with mineral growth we might expect RNA to tend to lengthen while preserving either left or right chiralities, i.e., a favorable packing arrangement (Joyce et al., 1984). Were a monomer with the opposite stereochemistry to be added to a growing chain, growth would be thwarted (Sanders, 2003). That filter would have been sufficient to tip the holochirality scale since, despite the presence of a racemic mixture of amino acids in the microcavities only amino acids of the same \(\alpha\)-carbon configuration (similar stereochemistry) would preferentially have ended up in peptides, to yield a population of distinctly handed peptides. The rest are eluted to the Hadean Ocean. Some of the retained peptides are eventually fed back in a hypercyclic manner to favor the syntheses of their “stereochemically appropriate” polymerizing template.
Of course, all this begs the question as to how RNA itself was first generated.

**Replication by a natural PCR (polymerase chain reaction) in the mound**

According to Koonin and Martin (2005) replication of genetic information and protein coding was probably expedited by populations of virus-like RNA molecules operating within the system of contiguous iron sulfide compartments. Braun and Libchaber (2004) demonstrated in vitro how laminar thermal convection can drive DNA replication and how thermophoresis (thermal diffusion) accumulates the charged polymers (Baaske et al., 2007; Koonin, 2007). These authors point out that such systematic evolution of ligands by exponential amplification is likely to have progressed naturally in porous hydrothermal mounds where the temperature gradient was high and ranged between 50°C and 100°C. This is just the kind of gradient to be expected in off-ridge hydrothermal springs and seepages like Lost City, an alkaline spring that has been operating at least for the last 35,000 years (Früh-Green et al., 2003). While convective temperature cycling encourages the polymerase chain reaction, thermophoresis concentrates DNA and other polymers in cool stagnant zones of the reaction chamber or pore space which act as a thermogravitational microcolumn (Baaske et al., 2007). These first “genes” would stabilize selection. Such “crowding” of newly generated macromolecules would further enhance the assembly of oligomeric structures and their subsequent folding into functional proteins and speed up the rates of reaction along metabolic pathways (Ellis, 2001; Spitzer and Poole, 2009).

**Early evolution and the common ancestral community**

So far we have seen how geochemical processes may have evolved into biochemical processes. We now enter an evolutionary realm with an empirical base. The universal ancestor of life probably comprised a community of single-celled organisms still housed within its hydrothermal hatchery that possessed all of the attributes common to all bacteria and archaea: the genetic code, the ribosome; DNA; a supporting core and intermediate metabolism needed to supply the constituents of its reproduction; replication; compartmentation from the environment; redox chemistry; and the use of a proton gradient. This last common common community (the LCC) occupied the mound within which life emerged. It is argued that the first acetogens are the root organisms of the Bacterial domain and that the first methanogens evolved into the Archaeal domain (the methanogens are found only amongst the archaea). Escape from the vent of these first microbes down into the contiguous ocean floor was only possible when genetically encoded lipid synthesis and cell wall synthesis had been achieved, and when autogenous formyl pterin synthesis as well as ab initio ion-pumping mechanisms had been developed for bioenergetic reasons relating to energy conservation efficiency, but in independent lineages of energetically sustainable and genetically replicating ensembles within the network of FeS-bearing compartments (Koonin and Martin, 2005; Russell and Hall, 2006; Martin and Russell, 2007). Here anaerobic microbial communities could be both autotrophic and heterotrophic, the autotrophs utilizing H₂ and formate as substrate while the first heterotrophs could use nitric oxide, elemental sulfur and ferric iron (FeIII) as electron acceptors (Vargas et al., 1998; Philippot et al., 2007; Ducluzeau et al., 2009; see Chapters Geobacter and Shewanella).

Evolution in the mound extended beyond mere optimization of the acetate and methane reactions (Martin and Russell, 2003). A next step was adaptation that exploited the reduced carbon and energy to be found in waste products and dead cells:

\[
\begin{align*}
\text{CH}_3 \cdot \text{COO}^- + \text{Fe}^{III} + 4\text{H}_2\text{O} & \rightarrow 8\text{Fe}^{2+} + 2\text{HCO}_3^- + 9\text{H}^+ \\
\end{align*}
\]

Ferric iron (Fe^{3+}), as an electron acceptor, provided a means of such respiration (oxidative metabolism) (Liu et al., 1997; Vargas et al., 1998), as did elemental sulfur (Philippot et al., 2007) and nitric oxide and its close derivatives (Ducluzeau et al., 2009).

The last common ancestral community occupied the very hatchery in which life first emerged. The acetogenic proto-bacteria and the methanogenic proto-archaea emerged at moderate temperatures (40–50°C) (Brochier and Philippe, 2002) (Figures 1 and 7). A period of high
ambient temperature, caused either by a meteorite impact or a carbon dioxide greenhouse (Kasting and Ackerman, 1986; Kasting and Brown, 1998; Nisbet and Sleep, 2001) could explain why the last common community, residing in the ocean crust contiguous with the mound where life originated, may have been thermophilic, perhaps living at 50–60°C (Gaucher et al., 2003; Schwartzman and Lineweaver, 2004; see Chapter Basalt (Glass, Endoliths)).

Conclusions
The onset of life is assumed here to be an inevitable evolutionary outcome of the far-from-equilibrium conditions obtaining on a planet such as ours. These geophysical and geochemical states of the Earth 4 billion years ago are well-established. Life emerged as the ever-renewing catalyst that resolved the potential between carbon dioxide in the atmosphere/ocean and hydrogen in hydrothermal solution. Its hatchery may have been in iron-sulfide-bearing pores within a submarine mound precipitated above a hydrogen-rich alkaline spring. A natural proton motive force, a consequence of a proton gradient across the inorganic walls of the pores spaces from the acid exterior to the alkaline interior, provided much of the energy for biosynthesis. The autotrophic acetyl coenzyme-A pathway was the first metabolic process to develop within this natural hydrothermal reactor. One variant of the autotrophic pathway produced acetate as waste, the other produced methane. This differentiation between the first microbial processors may have had a profound evolutionary consequence. The protoacetogens perhaps were the forerunners of the bacterial domain, while the protomethanogens evolved into the archaeal domain.

Acknowledgments
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Origins of the Metazoa

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Synonyms
Animals

Definition
Metazoans – heterotrophic, multicellular organisms that possess eukaryotic cells. Heterotrophs – organisms that must ingest organic carbon to support growth, unlike plants that are autotrophic and can use light to photosynthesize organic compounds.

Evolutionary concepts
A major event in the time line of Earth’s history was the evolution of multicellular animal life, known scientifically as the Metazoa (Knoll and Carroll, 1999; Knoll et al., 2006; Budd, 2008; Love et al., 2009; Xiao and Laflamme, 2009). Metazoans possess eukaryotic cells which contain a distinct membrane bound nucleus, nuclear material (DNA) packaged into multiple linear chromosomes, membrane bound organelles, and modes of cellular division distinct from that of Bacteria and Archaea (Cooper and Hausman, 2009). Metazoans are primarily set apart from plants (also multicellular eukaryotes) in being heterotrophic, and in lacking a rigid cell wall (Campbell et al., 2008).

While all other forms of life (Bacteria, Archaea, and plants) display extensive genetic and metabolic diversity (Venter et al., 2004), the metazoans through 600+ million years of evolution (Peterson et al., 2008) display the greatest variety of locomotory habits, behaviors, and morphologies. Indeed, a fundamental feature of the Metazoa is the morphological diversity displayed by the 35+ (Collins and Valentine, 2001) major groups of animals that constitute the Kingdom Animalia (Carroll, 2001).

Metazoan morphological diversity is essentially generated by two means. Firstly through an ability of a collection of cells to accurately communicate with one another during the process of development, and secondly for members of that collection of cells to adopt fates with distinct functions. This is most clearly conceptualized when considering the process of metazoan development. Typically, unicellular gametes (sperm and egg cells) fuse to form a zygote (a fertilized egg), which then divides to produce a multicelld embryo and eventually a reproductively mature adult (Gilbert, 2006). During this process, undifferentiated embryonic cells become progressively specified to a particular cell fate (e.g., muscle, neuron, skin, gonad, and eye). Without cell fate specification, tissues (e.g., muscle), organs (e.g., hearts), and organ systems (e.g., digestive system) and the variety of functions that these collections of cells fulfill would not develop (Arendt, 2008).

Evolutionary changes to the metazoan developmental program are ultimately responsible for generating diversity in the adult phenotype. This realization has fuelled the growth of the field of evolutionary-developmental biology (Carroll, 2005). Comparisons of the genes that regulate different metazoan developmental programs now holds the exciting promise of explaining on a molecular level how metazoan morphological diversity is generated (Martindale et al., 2003; Matus et al., 2006). Additionally, recent developments in high-throughput DNA sequencing technologies have seen the completion of a variety of metazoan genomes (King et al., 2008; Putnam et al., 2008; Chapman et al., 2010). These large-scale datasets allow exhaustive comparisons to be made between disparate animal phyla, and deepen our understanding of the genetic innovations that accompanied the diversification of metazoan life.

Related to these efforts are attempts to understand how metazoan life first arose. This requires that we accurately understand how metazoan and pre-metazoan groups are evolutionarily related, and calls on the field of

Origins of the Metazoa, Figure 1 A phylogenetic tree represents a hypothesis regarding the evolutionary relationships of a given set of organisms. Speciation events (indicated by an arrow) generate new lineages of organisms that in turn speciate to generate new “sublineages” thereby increasing diversity. This simplified phylogenetic tree summarizes a recently proposed hypothesis (Pick et al., 2010) that suggests the last common ancestor of the Metazoa (indicated by the red circle) was a sponge-like animal that was descended from a choanoflagellate-like organism.
Phylogenetics (Felsenstein, 2004). Although phylogenetics was arguably born over 150 years ago with Darwin’s publication of the *Origin of Species* (Darwin, 1859), our understanding of metazoan relationships is far from settled, and remains an exciting and dynamic field of scientific research which now benefits from modern techniques in molecular biology (high-throughput DNA sequencing) and super computing (Stamatakis et al., 2008). Arguably, the current and most widely accepted theory of the evolutionary origins of the Metazoa (based on a recent meta-analysis of DNA sequence data from a large collection of metazoan groups), maintains that sea sponges (phylum Porifera) are the most ancestral-like metazoan, with choanoflagellates (a primarily unicellular eukaryotic organism) representing the closest non-metazoan cousin (Figure 1; Pick et al., 2010). This theory is attractive because it is conceivable that a choanoflagellate ancestor once formed a colony of...
individuals (modern choanoflagellates are known to do this), which then evolved into a primitive sponge, the first metazoan. However, because the Metazoa has origins dating back 600–1 billion years thus obscuring many of the phylogenetic signals used to infer kinship, and our understanding of the ways in which the mechanisms of molecular evolution operate are still being developed, competing theories should also be considered (Dunn et al., 2008; Schierwater et al., 2008).


Cross-references
- Critical Intervals in Earth History
- Ediacaran Biota
- Early Precambrian Eukaryotes
- Ediacaran Biota
- Silica Biomineralization
- Sponges (Porifera) and Sponge Microbes
PARASITISM

Parasitism is a form of symbiosis, in which one organism benefits but the other is harmed. See entry “Symbiosis” for further reading.

PEDOGENIC CARBONATES

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Synonyms
Secondary terrestrial carbonates; Soil carbonates; Vadose zone carbonates

Definition
Definition and mineralogy
Pedogenic carbonates are authigenic (or secondary) carbonate deposits precipitated in soils (Lal et al., 2000). When found in the soil but directly inherited from parent materials, carbonates are defined as detrital or lithogenic (Kraimer et al., 2005). Pedogenic carbonates are characterized by a wide diversity of shapes, from secondary diffuse micritic (micron-size) crystals inside the soil matrix to coalescent hardened layers, sometimes capping whole landscapes. They are mainly composed of calcium carbonate as calcite and rarely as aragonite (Schaetzl and Anderson, 2005), of magnesian calcite, dolomite (Whipkey et al., 2002), and occasionally of iron-rich carbonate such as ferroan calcite, siderite, and ankerite (Table 1). Iron-rich carbonates are generally found in organic-rich soils and soils under the influence of water-table fluctuations with active oxidation–reduction processes.

Origin
Carbonate physicochemical equilibria
Various processes are involved in pedogenic carbonate formation. The first significant process is related to the leaching of carbonate through the soil profile. In the upper horizons, inherited calcium carbonate is dissolved by carbon dioxide enriched waters and displaced in solution toward deeper horizons where it precipitates. This precipitation is supposed to occur if the concentration in \( \text{Ca}^{2+} \) increases, the \( \text{pCO}_2 \) decreases, or if soil solutions concentrate due to water loss (evaporation). In other words, all these processes are driven by the following general equations when lithogenic (detrital) carbonates are involved:

\[
\begin{align*}
\text{CO}_2 + \text{H}_2\text{O} & \rightarrow \text{H}_2\text{CO}_3 \quad \text{(formation of carbonic acid with carbon dioxide and water)} \\
\text{H}_2\text{CO}_3 & \rightarrow \text{H}^+ + \text{HCO}_3^- \quad \text{(carbonic acid is a weak acid)} \\
\text{CaCO}_3 + \text{H}^+ & \rightarrow \text{Ca}^{2+} + \text{HCO}_3^- \quad \text{(dissolution of detrital carbonates in the upper horizons)} \\
\text{Ca}^{2+} + 2\text{HCO}_3^- & \rightarrow \text{CaCO}_3 + \text{H}_2\text{O} + \text{CO}_2 \quad \text{(reprecipitation of calcium carbonate in the lower horizons)}
\end{align*}
\]

A second process is related to the transformation, in surficial environments, of primary minerals which can also lead to the precipitation of pedogenic carbonates. For
example, weathering of alkaline feldspar (plagioclase) by water enriched in carbon dioxide leads to the solubilization of calcium ions as well as hydrogen carbonate (i.e., bicarbonate) ions according to the equation:

$$\text{CaAl}_2\text{Si}_2\text{O}_8 + 2\text{CO}_2 + 3\text{H}_2\text{O} \rightarrow \text{Ca}^{2+} + 2\text{HCO}_3^- + \text{Al}_2\text{Si}_3\text{O}_5(\text{OH})_4$$
(5)

The presence of calcium and hydrogen carbonate ions can lead to pedogenic carbonates, according to equation (4) if, for example, appropriate pH conditions are reached. Such pedogenic carbonates include carbonate cements, calcitic coatings associated with pores (mostly related to soil desiccation and/or root suction), and matrix impregnation by carbonate crystals of various sizes, from sparite (>20 μm) to micrite (<2 μm), during displacement of equilibrium in soil solution (Deutz et al., 2001).

Although often presented as the engine of main pedogenic carbonate accumulations, these physicochemical equilibria remain fairly theoretical and do not incorporate all the complexities in the field. Processes in nature cannot be reduced to a simple set of equilibrium equations. First, kinetics of the carbonate system depend on many factors such as CO₂ diffusion as well as relationships between soil pores and water or the presence of specific ions such as Mg²⁺ or phosphorus, making the system much more complex than it seems. Second, the role of life is barely taken into account in such equations. Indeed, pedogenic carbonates are probably much more related to biomineralization processes (directly or indirectly) rather than simple displacement of physicochemical equilibria. To illustrate this point, just consider the distribution of the pCO₂ in a soil. The deeper the horizon, the higher the pCO₂. So why are pedogenic carbonates precipitating in the solum where the conditions seem to be the least favorable, that is, in the deeper horizons? The explanation can be found when the contribution of life is explored.

The role of microorganisms

Bacteria undoubtedly play an important role in the formation of pedogenic carbonates. It has been demonstrated that precipitation of CaCO₃ could be the reaction of heterotrophic bacterial guilds to an increase in soil organic matter. Another example is given by the oxalate–carbonate pathway (Cailleau et al., 2005). In soils enriched in oxalate by plants and/or fungi, oxalotrophic bacteria can use oxalic acid and/or calcium oxalate as an electron donor and a carbon source. The result is a change in soil pH as well as the production of hydrogen carbonate ions. Calcium carbonate can precipitate when pH is high enough and the carbonate species and calcium are in appropriate concentrations, even in acidic environments such as tropical soils. Therefore, bacterial activity not only contributes to pedogenic carbonates but also changes chemical soil properties. In soils where nitrate and sulfate minerals are available, heterotrophic reducing bacteria can use these substrates as electron donors to produce CaCO₃ (as long as calcium is present). When iron is present with calcium sulfate, bacterial activity can lead to the concomitant precipitation of calcium carbonate and iron sulfide. This process assumes some soil redox conditions. Another example is given by methanotrophic bacteria which can transform soluble organic matter from the soil solution percolating in deep horizons. This transformation occurs in the phreatic zone, under soils enriched in organic matter or peats, that is, in a distinctly anoxic environment. Secondary calcium carbonate appears as coatings in cracks of the parent rock. In conclusion, bacteria influence the kinetics and the reactions involved in pedogenic carbonates, even if their direct role in crystal nucleation is still difficult to prove beyond doubt. In any case, bacteria influence the composition of the soil solution and the biogeochemical cycles of major elements.

Fungi are also important factors in the formation of pedogenic carbonates. Their growth is related to the development of mycelia which can form a network in the soil. In their need to absorb Ca²⁺ for their metabolism, filamentous fungi developed the ability to concentrate these ions in their thallus as calcium oxalate or calcium carbonate salts, inside or outside their membrane. In addition, fungi excrete large quantities of organic acids, especially oxalic acid. Two common fungal features in pedogenic carbonates can be observed: calcified filaments and hyphae associated with needle fiber calcite (called NFC; Figure 1a and b). Calcified filaments are fungi covered with oxalate crystals (Figure 1c and d). The way in which fungi can form such accumulations of calcium oxalate around their cell walls is still unclear, although the excretion of oxalic acid must play a role in the formation of calcium oxalate.

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**Pedogenic Carbonates, Table 1** Mineralogy and occurrence of pedogenic carbonates

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Formula</th>
<th>Occurrence of pedogenic carbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite</td>
<td>CaCO₃</td>
<td>All latitudes</td>
</tr>
<tr>
<td>Aragonite</td>
<td>CaCO₃</td>
<td>Detected in some temperate carbonate soils</td>
</tr>
<tr>
<td>Magneisian calcite</td>
<td>(Ca₀.₉₀−₀.₉₅, Mg₀.₁₀−₀.₁₀)CO₃</td>
<td>Detected in carbonate soils from evaporitic and tropical environments</td>
</tr>
<tr>
<td>Dolomite</td>
<td>(Ca, Mg)(CO₃)₂</td>
<td>Detected in carbonate soils from evaporitic or paralic environments</td>
</tr>
<tr>
<td>Ferroan calcite</td>
<td>Ca[Fe(III)]CO₃</td>
<td>In carbonate soils with an active iron cycle</td>
</tr>
<tr>
<td>Siderite</td>
<td>Fe(III)(Ca, Mg)CO₃</td>
<td>In carbonate soils with active oxidation–reduction processes and organic matter</td>
</tr>
<tr>
<td>Ankerite</td>
<td><a href="CO%E2%82%83">Ca, Fe(III)(Mg, Mn)</a>₂</td>
<td>In carbonate soils with active oxidation–reduction processes and organic matter</td>
</tr>
</tbody>
</table>
as well as in the dissolution of carbonate parent rocks. It is likely that part of the dissolution products diffuse inside the pores of the matrix and reprecipitate as secondary calcium carbonate a bit further away, contributing to soil matrix induration. Regarding the oxalates, they constitute a carbon and an electron source for oxalotrophic bacteria. As stipulated above, this oxalotrophic pathway also contributes to secondary calcium carbonate accumulations in soils. The origin of needle fiber calcite is also controversial (Verrecchia and Verrecchia, 1994; Cailleau et al., 2009b), often interpreted as a purely physicochemical product related to plant suction or other specific soil conditions. In fact, NFC seems to be mainly related to fungal activity, as it is always associated with fungal filaments (rhizomorphs). NFC crystallographic properties show that the crystals are complex, often polygenic, and undergo early diagenetic evolution, such as epitactic growth, inside the soil pores. Finally, some authors have observed nanorods associated with fungal filaments. These features are often considered either calcitic nanocrystals, a former stage of NFC, or mineralized bacteria. Recent research seems to demonstrate that these nanorods are decayed cell wall fibrous material from fungal rhizomorphs (Birdschedler et al., 2010). This material acts as a template for calcium carbonate precipitation, leading to a mineralized mesh covering soil aggregates (Cailleau et al., 2009a). In conclusion, microorganisms play a prominent role in pedogenic carbonates by changing the biogeochemical conditions in microenvironments, by their direct influence on the precipitation of calcium carbonate, as well as their potential role to serve as a template for CaCO₃ crystal nucleation.

The role of the rhizosphere
The impact of the rhizosphere in the formation of pedogenic carbonates is related to two main processes: dissolution of carbonate parent material, leading to the redistribution of calcium carbonate in the soil matrix, and indirect and direct precipitation of carbonate due to root activity (Becze-Deak et al., 1997). The dissolution of carbonate parent rock by roots increases the soil porosity as well as the saturation index of the soil solution in carbonates. Although dissolution seems to be a destructive process, it is associated with a redistribution of soil carbonates leading to soil induration. Indeed, when organic acids are excreted by roots, dissolution of lithogenic carbonate occurs, increasing the concentration of dissolved carbonate inside the soil solution. On the other hand, when conditions are dry, roots need water: they pump...
water from the soil solution, consequently increasing the concentration of calcium and carbonate ions in the solution around the root, leading to periporal precipitation of secondary calcium carbonate. Simple soil desiccation through the root channel may produce the same results. Such a cementation process, i.e., impregnation by secondary calcium carbonate of the soil matrix around root traces, is at the origin of rhizoliths (pedogenic carbonates having the shape of roots, Figure 2a and b). Not only do roots excrete large quantities of organic acids, protons, and even hydrogen carbonates, but they also absorb potassium, nitrogen, water, and calcium. Some plants have the ability to accumulate CaCO$_3$ in their vacuome in order to detoxify a possible excess of Ca$^{2+}$. These cellular biomineralizations are called calcified root cells (Jaillard et al., 1991; Figure 2c) and they are pedogenic carbonates directly related to the interaction of plant roots with soil solution. Finally, regarding pedogenic carbonate nodules found in present-day soils as well as paleosols, they probably result from the complex interactions between microorganisms, plant roots, and the composition of soil phases (soil solution and gas).

Animals may also play an important role in pedogenic carbonate formation inside the rhizosphere. For example, termites both mine carbonate from deep parent rock (bioturbation) and biomineralize the calcite in their body (Liu et al., 2007). Earthworms contribute to calcium carbonate accumulation in soils (Canti and Pierce, 2003). They excrete calcitic biospheroids in their casts (Figure 2d). The biospheroid formation occurs in the calciferous cells of the epithelium that secrete amorphous calcium carbonate, crystallizing in the lumen of the esophageal pouch. These spheroliths of calcite are usually up to 2 mm in diameter with an average weight of 1.5 mg. They indicate the presence of earthworms. When, through diagenesis, obvious traces of earthworms are missing in buried paleosols, the presence of biospheroids provides good arguments for relative stability of the soil-sedimentary environment, i.e., no rapid burial and no drastic prolonged water stress.

**Calcretes**

The term “calcrete” should only be used for complex terrestrial carbonate accumulations, often hardened and showing specific features such as nodular, lamellar, massive, and laminar horizons. However, calcretes are characterized microscopically by many features similar and/or related to pedogenic carbonates (root traces, calcified root cells, needle fiber calcite, matrix nodulization, calcitic coatings), which explains why the term has been inappropriately used as an equivalent of pedogenic carbonates in many studies (Nash and McLaren, 2007). The origin of vadose calcretes is still controversial. Soil scientists consider calcretes as soils: calcium carbonate is leached from the upper horizon toward the lower horizon, until CaCO$_3$ infills all the pores leading to a plugged horizon. Sedimentologists interpret calcrete as complex (polygenic) sedimentary formations where pedogenesis, biogenic processes, and sedimentation episodes interact. In this case,
calcrites record a long history of landscape evolution whereas, in the soil model, calcrites are only related to a single process of dissolution and reprecipitation in a relatively stable environment. For example, for soil scientists, mature calcrites are characterized by a laminar horizon resulting from cementation inside a carbonate-rich perched water table, in which the soil solution is unable to percolate further down because it is stopped by the plugged horizon. This horizon is therefore posterior to the upper soft soil. Sedimentologists consider this laminar horizon as a surficial deposit (in contact with the atmosphere) related to cyanobacterial activity and sedimentary processes (weak runoff, eolian dust incorporation). The upper soft soil, where present, is therefore posterior to the laminar crust.

**Pedogenic carbonates as a carbon sink?**

The question of a potential role of pedogenic carbonates as a carbon sink is often asked (Lal et al., 2000; Landi et al., 2003; Cailleau et al., 2004). Researchers consider that the residence time of carbon in pedogenic carbonates is 3–4 orders of magnitude higher than that of soil organic matter. In order to answer this question, the origin of calcium (Ca) has to be considered (Van der Hoven and Quade, 2002). There are two possible origins for the Ca in pedogenic carbonates. Ca can be inherited from weathering of preexisting CaCO₃ (e.g., dust or parent rock limestone). Recrystallization of pedogenic CaCO₃ allows atmospheric CO₂ catchment but, in contrast, fossil CO₂ has been released during weathering, leading to a zero net balance for the carbon cycle. Thus, this process has to be considered only a molecular CO₂ substitution from fossil limestone to pedogenic carbonates. A second possible origin of Ca is when released from the weathering of noncarbonate minerals (see e.g., Equation 5) and precipitated as CaCO₃. In this case, the catchment of atmospheric CO₂ by the released Ca into authigenic pedogenic carbonates acts as a carbon sink.

**Summary**

Pedogenic carbonates are authigenic deposits found in soils. Their origin is related to biological activity involving bacteria, fungi, roots, and soil animals, as well as complex kinetics between minerals and soil solution. Consequently, their biogeochemical signature as a proxy should be used with caution to reconstruct paleoenvironments (Quast et al., 2006). On the other hand, they can constitute a carbon sink if the cations originate from noncarbonate substratum.

**Bibliography**


**Cross-references**

Calcite Precipitation, Microbially Induced Carbon Cycle Carbonates Fungi and Lichens Microbial Degradation Soils
PERMAFROST MICROBIOLOGY

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Synonyms
Microbiology of perennially frozen ground

Definition
Permafrost is the ground (soil or rock) beneath the surface of the earth that remains at or below 0°C for at least two consecutive years. Permafrost is defined on the basis of temperature. It is not necessarily frozen; moisture in the ice form may or may not be present (van Everdingen, 1998). For microbiology is important the usage of permafrost that requires also the presence the moisture in the ice form. In other cases the following terms are used: (a) overcooled, because the freezing point of the included water may be depressed several degrees below 0°C; (b) frosty, if the moisture content is close to zero (Basic foundation of geocryology, 1959).

Introduction: Why permafrost?
Because the occurrence of a viable Cenozoic generation of microorganisms within the permafrost is intriguing as a window into microbial life as it was before the impact of humans (Tiedje et al., 1994) and as a storage system and ancient biota became easily involved anew in present-day ecological processes.

Because permafrost represents a wide range of possible habitats and inhabitants on the planets without obvious surface ice such as in Mars, and if life existed during the early stages of development on this Earth-like planet, then, similar to the Earth, remnants of primitive forms may be found within frozen material.

Because permafrost thawing due to anthropogenic or natural processes exposes ancient life to modern ecosystems and ancient biota became easily involved anew in present-day ecological processes.

Only these problems demonstrate the significance and perspectives of permafrost microbiology.

Cryobiosphere
The terrestrial Cryosphere consists of two parts: Glaciosphere (snow and ice) and Cryolithosphere, the frozen part of the Lithosphere, containing long-term and seasonally cryogenic formations with ancient and recent viable microorganisms, respectively. The comparison of number of the cells survived over geological time at temperature above 0°C in halite (Lipman, 1928; Dombrowski, 1963; Norton et al., 1993; Denner et al., 1994; Vreeland et al., 2000; Stan-Lotter et al., 2003) and amber (Galippe, 1920; Cano and Borucki, 1995; Lambert et al., 1998; Greenblatt et al., 1999) and at temperature below 0°C in Cryosphere, shows that the latter is the most spread and rich terrestial depository of ancient viable microorganisms. Cryosphere that is found between the upper atmospheric layers (Wainwright et al., 2003), including overcooled cloud droplets (Sattler et al., 2001) and the bottom of Antarctic Ice Sheet, represents a significant part of Biosphere, the Cryobiosphere, where life is confined in ice and permafrost.

Icy world
Biota of Greenland Ice Sheet, 120,000-years old, and Antarctic Ice Sheet, ~400 Kyr have been widely studied to the depth more than 3 km (Abyzov, 1993; Kapitsa et al., 1996; Priscu et al., 1998; Karl et al., 1999; Petit et al., 1999; Skidmore et al., 2000; Deming, 2002; Miteva et al., 2004; Miteva and Brenchley, 2005). The age of the oldest glacial ice as well as immured bacteria are still under discussion: >500,000 years at Guliya ice cap on Tibetan Plateau (Thompson et al., 1997; Christner et al., 2003), ~2 million years at the bottom of Vostok ice core (Salamat et al., 2004) or even ~8.1 million years (Sadgen et al., 1996; Bidle et al., 2007) in Beacon Valley, Antarctica. Microbial population and traces of life in ice sheets are interpreted to be the Earth’s most representative models of extraterrestrial icy habitats (like Jupiter’s Ice-covered moon Europa, or Enceladus – Icy moon in Saturn system).

The initial number of mostly air-borne microorganisms isolated from snow and seasonally ice covers is not great (10⁵ cells per ml) and of the same order as viable cells within the ancient ice sheets core’s. Such fact could be interpreted as absence of reduction of microbial population once immured in ice for thousand years and could be explained, for example, by the near zero background radiation in the ice. The ultra small (<µ3) cells dominated in its population (Miteva and Brenchley, 2005) and the number of viable cells in ice cores increases sharply in the presence of dust particles (Abyzov, 1993). Unlike they occupying the narrow liquid veins (Price et al., 2004) only, because data from Vostok cores showed that the most abundant are the upper layers in spite of extremely low temperatures (~50°C). Distribution of cells along the hole indicates that the populace of viable cells in Antarctic Ice Sheet decrease with the increasing age of embedding ice.

Greenland ice cores also indicate a good preservation of relatively young, ~2–4 Kyr, genomic DNA (Willerslev et al., 1999), as well as bacterial and plant viruses varied in age 0.5–100,000 years (Castello et al., 2005). Therefore, freezing prompts viral endurance. Unfortunately, this relates to human viruses too – in Arctic, RNA of Influenza
A is preserved in high concentrations in lake’s seasonally ice (Shoham, 2005; Zhang et al., 2006).

The last review of icy Biosphere made by Priscu and Christner (2004) and the recent level of the problem are shown in the book Life in Ancient Ice Castello and Rogers (2005).

Cryolithosphere

However, the most inhabited part of Cryosphere, represented by Cryolithosphere or permafrost, underlie about a quarter of the Earth’s land surface. Permafrost is the integral part of Arctic (the northern reaches of North America and Eurasia) and ice-free regions of Antarctica and Greenland. The so-called, Alpine permafrost is wide spread in the high mountains of all continents: Europe, western China, and both Americas, as well as offshore permafrost occupied the polar shelf surrounding Arctic and, probably, Antarctica.

This considerable frozen mass, up to several hundreds of meters deep, where microorganisms are adsorbed on organic or mineral particles, harbors a numerous (up to dozen millions cells per 1 g of dry soil – gdw) of various ecological and morphological viable microbial groups survived under permafrost conditions since the time of its formation. They have been isolated from frozen cores with permanently constant ground temperatures (−1)−(−2) °C near the south border of permafrost in Siberia and down to lowest in Arctic: −17°C, on 80°N, Ellesmere Island – the most northern latitude (Steven et al., 2007) and in Antarctica: −27°C on 78°S, Dry Valleys – the most southern latitude (Gilibinksy et al., 2007a). Viable microorganisms were found down to 400-m depth in Mackenzie Delta (Gilibinksy, 2002) and up to 4700-m elevation in Qinghai-Tibet Plateau (Zhang et al., 2007). The age of the isolates corresponds to the longevity of the permanently frozen state of the sediments and dates back from few thousand to 2–3 million years in northeastern Siberia and to 5–8 million years and probably older in Antarctica (Gilibinksy et al., 2007a). This great mass of living matter is peculiar to permafrost only, and taken into account the thickness of Cryolithosphere (50–1000 m), it is easy to conclude that permafrost contain a total biomass of cold adapted microorganisms many times higher than that of the modern soil cover.

In contrast to the ice sheets, where the overwhelming majority of discovered microorganisms came from the atmosphere, microorganisms isolated from permafrost are similar to those of surface and aquatic cold ecosystems and habitats. The total cell number counted by epifluorescence microscopy reached 10^7 –10^8 cells/gdw in Arctic (Siberian and Canadian) permafrost (Vorobyeva et al., 1997; Steven et al., 2007) and 10^5–10^6 cells/gdw in Antarctic permafrost (Gilibinksy et al., 2007a). This indicates the initial low level of microbial population in Antarctic Dry Valley environment in comparison with Arctic. Antarctic permafrost also is several orders of magnitude lower in the number of viable cells, and has less microbial diversity, than Arctic one. The fraction of the total bacterial population that could be recovered from frozen samples (the number of cells that grow on nutrient media) varied 10^3–10^5 cells/gdw, ranging 0.01–1.5% of the total amount counted by epifluorescence microscopy (Vishnivetskaya et al., 2000) and this conclusion is supported by experimental data from different cores and regions. Note that cold adapted organisms of the microbial community within the permafrost has the optimal growth at room temperature, i.e., according to the growth temperature classification of Morita (1975, 1997), are not psychrophilyc, or restricted to permanently cold habitats (Gounot, 1986), but are predominantly psychrotrophic.

Permafrost is not only bacterial (aerobic and anaerobic, spore-forming and spore less, Gram-positive and Gram-negative) archival depository, but also contain viable cyanobacteria and green alga (Vishnivetskaya, 2009), yeast (Dmitriev et al., 1997; Faizutdinova et al., 2005), actino- and micromicetes (Kochkina et al., 2001), as well as their metabolic-end products: biologically active macromolecules – free intra- and extracellular enzymes (invertase, catalase, protease and amylase) (Vorobyeva et al., 1996), pigments (chlorophyll a, b, and pheophytin) (Erokhina et al., 2004), and biogenic gases, such as methane (Rivkina et al., 2007) and even biological objects of higher level: mosses (Gilibinksy et al., 2001), seeds (Porsild et al., 1967; Yashina et al., 2002), and protozoa (amoeba,infusorium and flagellates) (Shatilovich et al., 2005).

This is why permafrost could be considered as a unique subsurface complex, where the long-term impact of constant physicochemical regimes, including subzero temperatures, should be regarded not as the extreme and limiting but rather as a stabilizing factor. The frozen environment is characterized by solid and liquid water phases. The solid phase (ice) makes up 93–98% of total water volume and serves as a cryoprotector, a reagent favored for conservation of biological objects at temperatures below 0°C. At subzero temperatures the rates of biochemical reactions and biological processes become extremely low, and ensure preservation of biological system. Concomitant with the solid phase, the remaining few percent of the water is in an unfrozen state and occurs most commonly as thin films enveloping soil particles and, occasionally, as brine pockets. In contrast to ice, the unfrozen water serves as a cryoprotector and plays the leading role in the preservation of microorganisms. These films, by coating the organic and mineral particles, protect the viable cells sorbed onto their surface from mechanical destruction by growing ice crystals and make possible the mass transfer of microbial metabolic end-products within the permafrost, preventing the cell’s biochemical death. This is why the unfrozen water serves as a main ecological niche for microorganisms (Gilibinsky et al., 1995).

The quantity of the unfrozen water and the thickness of its films are independent of the total ice content, but decrease with subzero temperature decreasing (Nersesova and Tsytovich, 1966; Anderson, 1967). Given these
characteristics of Arctic permafrost at temperatures between $-3$ to $-12^\circ$C, the amount of unfrozen water can be estimated as 3–8% by weight, which depends upon the ion content and texture. In the coarse Antarctic sands, because of the low temperatures, the unfrozen water values are so small that the instrumental methods fail to record them. Unfrozen water in Antarctic permafrost must, therefore, only be firmly bound “liquid” water with binding molecules, which indicates a “biologically dry” permafrost environment.

**Life below freezing point**

The subzero temperature environments themselves are not the limiting factor, and microorganisms are able to grow at these temperatures if the media is not frozen, at least as low as $-10^\circ$C for one year in the presence of glycerol as cryoprotector (Gilichinsky et al., 1993). This correlates with the lower temperature limit for microorganisms growth (Russell and Hamamoto, 1998), but the conditions of experiments are too far from the permafrost environment in situ. Such conditions (temperatures down to $-10^\circ$C and up to 200 g/L salt concentrations) are present within the lenses of cryopeg – overcooled water brines in permafrost, where metabolic activity without cell division was shown using resazurin reduction as indicator (Bakermans et al., 2003) and uptake of $\delta$-[14C] glucose into the bacteria biomass (Gilichinsky et al., 2003, 2005). Hypersaline waters are defined as having salt concentrations greater than that of sea water (3.5% w/v) while the salinity of cryopeg lenses reaches 15–20%.

The unfrozen water films, high in salts, represent the same microcryopegs, and most investigators indicate that at least part of permafrost community (20%, according to Steven et al., 2006) growth at temperatures between $-2$ and $-10^\circ$C (Shcherbakova et al., 2004, 2005; Ponder et al., 2006; Rodrigues et al., 2006; Bakermans et al., 2006), while 95% of the isolated cells of aerobic bacteria did not grow at temperatures above 30°C (Gilichinsky et al., 1995).

Biotic survival in the late Cenozoic frozen and overcooled high-salt aquatic environments and Permian-Triassic salt deposits (Stan-Lotter et al., 2002, etc.) indicates unknown bacterial adaptations. It remains to be determined whether the salt tolerance of these cells may also be associated with cold tolerance on a geological scale. Experiments showed that under subzero temperatures halophilic bacteria survive better than non-halophilic and remain viable at $-80^\circ$C in the presence of 25% NaCl and extreme halophiles require NaCl concentrations above 15.6% (w/v) for growth (Rothschild and Mancinelli, 2001; Mancinelli et al., 2004).

The permafrost, mostly psycho-halo-tolerant microbial community, has been described as “a community of survivors” (Friedmann, 1994), with the starvation–survival lifestyle as the normal physiological state (Morita, 2000). We can conclude that this community represents the only the selected members of buried surface or aquatic ecosystems adapted to permafrost conditions and have efficient repair mechanisms that allow for these and not, for example, other tundra organisms to continue to live in permafrost (Ponder et al., 2006). This is why from one hand, permafrost bacteria have been previously isolated from Arctic tundra soils (Bab’eva and Sizova, 1983), and from the another one – many types of bacteria present in the Arctic soils, were not isolated from permafrost samples (Shi et al., 1997).

The preservation of a viable microbial community in permafrost over geological times at subzero temperatures raises the significant possibility of metabolic reactions for these conditions. The following facts provide indirect evidence that ancient microorganisms can be active in the permafrost nutritional-temperature regime and allows speculation concerning the possibility of biogeochemical processes in situ: the ability of immobilized enzymes in permafrost to become instantly activated (Vorobyova et al., 1996); the presence of usually metastable ferrous sulfides (Siegert, 1987) and nitrates (Jansen and Bock, 1994); the ability of cells to grow on media after being exposed to ground radiation during up to millions of years. This ability implies their capacity to repair DNA damage even in the frozen environment, i.e., at the stable rate of damage accumulation a comparable rate of repair must also exist.

However, the question about the metabolic state of microorganisms, microbiological and biogeochemical processes, and life forms within permafrost still remains open to debate. For recent microbial communities measurements show that Antarctic lichens may be active at a temperature of $-17^\circ$C (Kappen et al., 1996) and evidence was obtained of low rates of bacterial DNA and protein synthesis at subzero temperatures (–12 to $-17^\circ$C), which indicates that the organisms divided in snow at the South Pole (Carpenter et al., 2000). For ancient microorganisms to the moment we can refer only to results of few experiments, suggesting that permafrost microorganisms are not, as previously thought, in a frozen resting state. The incorporation of 14C-labeled acetate into lipids shows that in permafrost 2–3 million years old, at temperatures down to $-20^\circ$C, anabolic metabolism is possible, resulting in the formation of bacterial lipids with the minimum 160 days doubling time (Rivkina et al., 2000). Also, using the 14C-labeled substrates (H14CO3 and 14CH3CO2), hydrogenotrophic and acetoclastic methanogenesis was measured in the late Pliocene to Holocene permafrost samples at temperatures down to $-16.5^\circ$C (minimal permafrost temperature in Arctic) and even to $-28^\circ$C (permafrost temperature in Antarctica), indicating the energetic metabolism – the ability of microorganisms to carry out redox reactions after thousands to millions years of existence in permafrost (Rivkina et al., 2004, 2005, 2007; Gilichinsky et al., 2007).

From the biological point of view, taking into account the natural radiation background of 1–2 mGy/year, the dose from radio nuclides diffused through the permafrost is far from sufficient for complete sterilization even the
oldest microbial complexes, i.e., it is not fatal to viable cells, but it is highly enough to cause selection effect and to destroy the DNA of ancient cells. The calculated data correlate with the number of viable cells in permafrost of different age and with experimental results. The cell viability and growth on media implies a high capacity for DNA repair and we can conclude that DNA repair occurs in the frozen environment, i.e., at the stable rate of damage accumulation, while a comparable or lower rate of reparation also exists (Gilichinsky et al., 2007b). The same suggestion made Johnson et al. (2007). Using a combination of molecular biology techniques and direct measurement of CO2 production from permafrost of different age and with experimental results. The cell viability and growth on media implies a high capacity for DNA repair and we can conclude that DNA repair occurs in the frozen environment, i.e., at the stable rate of damage accumulation, while a comparable or lower rate of reparation also exists (Gilichinsky et al., 2007b). The same suggestion made Johnson et al. (2007). Using a combination of molecular biology techniques and direct measurement of CO2 production from permanently frozen samples they find strong evidence that long-term survival is closely tied to cellular metabolic activity and DNA repair as a mechanism in sustaining bacteria viability.

The DNA preservation and reparation in permafrost is a huge area of important problems. Microbial communities began now characterized by newly emerging culture-independent molecular techniques – PCR amplification of diverse environmental 16S rRNA genes as well as the diversity of fungi – by 18S rRNA genes. Long-term persistence of bacterial, archaean, and fungal DNA were found in relatively young, late Pleistocene-Holocene, Canadian (Steven et al., 2007) and Tibetan (Zhang et al., 2006) permafrost and within late Pliocene–Pleistocene Siberian (Shi et al., 1997; Lydolph et al., 2005; Vishnivetskaya et al., 2006) and Antarctic (Spirina et al., 2003, Gilichinsky et al., 2007a). These studies show that DNA, usually degraded rapidly in most environments, survived within permafrost over geological time and the question is how long the DNA could be preserved. Series papers (Willerslev et al., 2003, 2004; Hansen et al., 2006; Johnson et al., 2007) on the basis of observed rates of DNA degradation indicate a limit of ca. 400–600,000 years beyond which PCR amplifications are prevented. This conclusion is still under discussion because based on the same few samples only and has not the statistical support and even its authors agree that studied organisms maintain a metabolic activity to repair DNA damages. Also because in another studies the viable microorganisms were isolated and DNA was extracted from permafrost samples up to 3–5-million-years old.

Conclusion

Microbiological and molecular studies of paleobiological objects in permafrost expand our knowledge about spatio-temporal limits of Biosphere and form the basis for new knowledge in various scientific multidisciplinary (geo/bio/cryo) fields: quaternary geology, geocryology, bacterial paleontology, geo-cryo-, paleo-, and exobiology. Also, the advances in biotechnology evidently indicate that in the visible future the genetic recourses will be even more important than geological one, and the permafrost microorganisms, as well as their metabolic-end products might be of potential value for industrial application, for example, on the base of abilities to operate at subzero temperatures.

Acknowledgments

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Bibliography


Cross-references
Algae (Eukaryotic)
Archaea
Astrobiology
Bacteria
Chemolithotrophy
Cyanobacteria
Extreme Environments
Methanogens
Microbial Communities, Structure, and Function
Microbial Ecology of Submarine Caves
Protozoa (Heterotroph, Eukaryotic)
Snowball Earth
Soils

PHOSPHORUS, PHOSPHORITES

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Definition
Phosphorus is a chemical element (P) with atomic number 15, which occurs mainly in the form of phosphate (PO₄³⁻). P is essential for life, but may negatively impact existing ecosystems when present in excess. The P cycle is tightly coupled with the carbon cycle through photosynthetic and biogeochemical weathering processes.

Introduction
Phosphorus (P) occurs most widely in the form of phosphate (PO₄³⁻) and plays – as an essential and often limiting nutrient – an important role in the overall functioning of the biosphere and its environment. It is in this capacity that this element is heavily recycled within the biosphere, and the most important source is its release from preexisting stocks within the biosphere. Its primordial source, however, is related to the biochemical weathering of P-containing rocks and the consequent transfer into the biosphere (Lerman et al., 1975; Froelich et al., 1982; Compton et al., 2000; Ruttenberg, 2005).

P exerts important interactions with the carbon cycle through the interfaces of photosynthesis and biogeochemical weathering. In the first case, P may drive the transformation of atmospheric CO₂ into organic C in those ecosystems where P is limiting, and in the second case, the amount of atmospheric CO₂ determines the amount of P liberated from its host rock and transferred to the biosphere (Mackenzie et al., 1993).

The study of the present-day P cycle on a worldwide scale together with the reconstruction of temporal changes in the P cycle during the Earth’s history is essential in the reconstruction of global environmental change at present and in the past (Cook and McElhinny, 1979; Bentor, 1980; Arthur and Jenkyns, 1981; Filippelli and Delaney, 1994; Föllmi, 1995, 1996; Trappe, 1998; Slomp and Van Cappellen, 2007).

The global phosphorus cycle
The principal release mechanism of P to the environment consists in the biogeochemical weathering of P-containing crystalline, volcanic, and sedimentary rocks (Froelich et al., 1982; Föllmi et al., 2009). Volcanic and hydrothermal activity provides only minor amounts of P, if at all – except eventually for basaltic volcanism and the formation of P-enriched deposits such as carbonatite and anorthosite (Baturin, 1982; Notholt et al., 1989; Feely et al., 1990).

Once transferred into the biosphere, P predominantly resides in soils, where it becomes part of the organic fraction and may also be included into secondary mineral phases such as iron oxyhydroxides (Walker and Syers, 1976; Frossard et al., 1989). Approximately 3–6% of the total terrestrial P stock is incorporated in vegetation, with organic P:C ratios of 1:500–1:800 (Delwiche and Likens, 1977; Berner et al., 1993).

P is transported to oceans via rivers and wind, where the eolian fraction corresponds to 7–15% of the total preanthropogenic P freight (Graham and Duce, 1979; Compton et al., 2000). Rivers transport P as dissolved inorganic P (DIP), dissolved organic P (DOP) and in particular forms (PIP, POP). PIP transport occurs in the form of detrital P and P absorbed onto iron oxyhydroxides and clay minerals (Meybeck, 1982, 1993; Compton et al., 2000; Seitzinger et al., 2005; Harrison et al., 2005). Recent estimates show that presently approximately 85% of the total P freight to the ocean is transported in particulate form (Beusen et al., 2005).

Once in the ocean, P enters a recycling loop between organic and inorganic forms, and only approximately 1% of the total amount of P used in primary productivity is permanently stored in the sedimentary reservoir per time unit. The P:C ratio in oceanic primary producers is significantly lower than that for terrestrial plants and amounts to 1:106–117 (Redfield, 1958; Anderson et al., 2001). The present-day residence time of oceanic P is estimated as between 10,000 and 17,000 years (Ruttenberg, 1993, 2005; Colman and Holland, 2000).

Recent attempts to quantify P reservoirs and flux rates on a global scale have been published in Mackenzie et al. (1993), Compton et al. (2000), Harrison et al. (2005), Beusen et al. (2005), and Ruttenberg (2005).

Phosphorus, microbes and life
The degree to which P may limit primary productivity in certain ecosystems is judged differently in the literature (Falkowski et al., 1992; Tyrrell, 1999). A vast array of
Phosphorites

Phosphorites are sedimentary deposits of phosphorus minerals, particularly phosphates, which are often rich in other dissolved ions such as calcium, silicon, iron, or manganese. They are found in marine and lacustrine environments and are typically formed in anoxic conditions where the decomposition of organic matter occurs. Phosphorites are important for their high phosphorus content, which can range from 30% to 80% of their dry weight. They have been found in different time periods throughout Earth's history, notably the Paleocene, Eocene, Oligocene, Miocene, and Pliocene periods. Phosphorites are crucial for understanding the history of phosphorus cycling, eutrophication, and the formation of dead zones in both marine and lacustrine environments.

**Phosphogenesis and phosphorites**

The precipitation of phosphate minerals in sediments is termed “phosphogenesis.” The principal mineral containing P in such environments is francolite (Ca$_{10-a}$Na$_a$Mg$_b$(PO$_4$)$_6$·(CO$_3$)$_6$·x(CO$_3$)·y(SO$_4$)·zF$_2$; Jarvis et al., 1994). Phosphogenesis is a process which occurs in the first few top centimeters of marine and lacustrine sediments. Favorable to this process are the sufficient supply of reactive P and overall low sediment accumulation rates. The supply of P may be from internal sources such as the decomposition of trapped organic matter or the desorption of P from iron and manganese compounds, or external sources such as the supply from bottom waters across microbial mats at the sediment–water interface (Krajewski et al., 1994). The concentration of thus formed phosphates into phosphorites, i.e., P-enriched deposits which are sedimentary and generally of marine origin, is largely a mechanical affair, related to bottom-water currents and gravity-flow-related redeposition (Glenn et al., 1994a; Föllmi, 1996).

Presently, phosphorites form in a limited number of marine localities, which are almost all related to oceanic sites where the upwelling of deeper, P-enriched waters occurs (offshore Peru and Chile, Baja California, Namibia, SW India; Veeh et al., 1973; Burnett, 1977; Baturin, 1982; Jahnke et al., 1983; Garrison and Kastner, 1990; Glenn et al., 1994b). Recent phosphorite formation has also been reported from E Australia and Pacific atolls and guyots (O’Brien et al., 1990). Phosphogenesis and the formation of minor amounts of authigenic francolite are, however, phenomena in marine sedimentary environments which are more widespread than previously thought (Ruttenberg 1993, 2005; Delaney, 1998).

**Phosphorus during the Earth’s history**

Periods of formation of exploitable phosphorite deposits are unevenly spread throughout the Earth’s history. Important deposits are known from the following time windows (Cook and McElhinny, 1979): Precambrian–Cambrian boundary (China, Australia); Ordovician (Baltic countries); Permian (Wyoming, Idaho); Jurassic (Siberia, Mexico); late Cretaceous–Eocene (N Africa and Middle East countries); and Miocene (Florida). The unequal occurrence of P-enriched deposits in time suggests important fluctuations in the global P cycle, which are confirmed by other approximations of temporal behavior of the P cycle, such as a detailed analysis of P contents and variations therein in sedimentary cores retrieved during the Deep Sea Drilling Project and Ocean Drilling Program (Cook, 1984; Filippelli and Delaney, 1994; Föllmi, 1995). These temporal changes have been related to changes in global geochemical weathering output, which again is dependent on climate conditions and the creation of relief through orogenetic processes. Global change in the output of reactive, bioavailable P engendered important changes in the environment and life during the Earth’s history, such as shifts from oligotrophic to eutrophic ecosystems, accompanied by the widespread disappearance of reef and carbonate-platform systems and the enrollment of oceanic anoxic episodes (Hallock and Schlager, 1986; Hallock, 1987; Föllmi et al., 1994, 2006; Mort et al., 2007).

**Phosphorus, eutrophication and anthropogenic impact**

Recent estimates suggest that approximately 65% of the total DIP river flux to the ocean is anthropogenically derived (Harrison et al., 2005). The increased load of reactive P has a considerable impact on the environment in that the so-called dead zones without free oxygen develop and expand, and that wider zones with diminished oxygen contents are created, both in lakes as well as in marginal and open marine environments (Joos et al., 2003; Mee, 2006). Furthermore, shifts occur in the composition of ecosystems which may negatively affect oligotrophic ecosystems such as coral reefs (Hallock, 1987; Roberts et al., 2002). Some possible consequences of excessive P availability to the environment are (Föllmi, 1996):

- Development of dysaerobic to anaerobic mid-water and/or bottom-water conditions
- Severe damage to marine oligotrophic ecosystems such as coral reefs
– Limitation of marine and lacustrine benthic communities in dysaerobic bottom-water conditions
– Shorter feeding networks and simplified ecosystems
– Change to opportunistic phytoplankton species
– Increase in the intensity and frequency of “red tides,” i.e., toxic blooms of dinoflagellates and planktonic algae
– Toxic blooms of benthic cyanobacteria
– Higher rates of primary productivity
– Increased burial rates of lacustrine and marine organic matter

Summary

The strong interferences between the element P, life, and the environment render this element of particular interest. From the certainly biased view of a geologist, important recent contributions and potential pathways of future research relate to the following topics:

– The extend of the capacity of P as a limiting nutrient
– Precise quantifications of reservoir and flux rates and of residence times
– Possible interferences between present-day anthropogenic change in the P cycle and the environment
– Coupling between the P cycle and other cycles of biophyle elements and molecules
– Weathering of P-containing rocks and the release of P to the biosphere
– Pathways and conditions leading to P mineralization and accumulation in sedimentary environments
– Temporal changes in the global P cycle during the Earth’s history and their impact on the paleoenvironment.

Bibliography


Photosynthesis is the most important biological process on Earth since it provides the reduced carbon and the molecular oxygen which are essential for almost all life on this planet. It is the basic metabolic reaction of organisms that contain chlorophyll where energy rich organic compounds are synthesized using the energy of sunlight. During the light-dependent reactions oxygen is released as a by-product (Figure 1a).

The net equation for the light reactions of the oxygenic photosynthesis is:

\[
2 \text{H}_2\text{O} + 2 \text{NADP}^+ + 3 \text{ADP} + 3 \text{P}_i + \text{light energy} \rightarrow 2 \text{NADPH} + \text{H}^+ + 3 \text{ATP} + \text{O}_2
\]

Plants and cyanobacteria, the organisms using oxygenic photosynthesis, contain two photosystems: photosystem I (PSI) and photosystem II (PSII). Each photosystem is surrounded by a light harvesting complex comprising a large number of pigments, mainly chlorophylls and carotenoids. The reaction center of PSII contains a pair of chlorophyll \(a\) molecules with an absorption maximum of 680 nm wavelength and is therefore called P680, while the chlorophyll \(a\) of PSI (P700) has an absorption maximum of 700 nm. In cyanobacteria, the photosystems are situated in the plasma membrane which is often folded to stacks called thylakoids. In plants, the thylakoid membrane can be found in the chloroplasts which are derived from cyanobacteria.

When sunlight strikes the light-harvesting complex of PSII, a photon excites an electron of a pigment molecule and the energy is passed on to the next pigment molecule until it reaches the reaction-center of PSII where an electron of the P680 pair of chlorophyll molecules is excited to a higher energy state. This electron is transferred to plastoquinin, the primary electron acceptor, through charge separation. In the oxygen evolving complex, water is split into two electrons, two hydrogen ions and an oxygen atom. The electrons fill the gap in the P680 while the oxygen atom combines with another oxygen atom to form dioxygen. Meanwhile, the electron from the plastoquinin is transported down an electron transport chain consisting of the electron carrier plastoquinone (PQ), a cytochrome complex (Cytb6,}, and the protein plastocyanin (PC). A proton gradient, which is built up when electrons pass through the electron transport chain in thermodynamically favorable direction, is used by the enzyme ATP-synthase for ATP-synthesis via photophosphorylation, where ATP is generated by adding a phosphate group to ADP. In the reaction-center of PSI, an electron of P700 pair of chlorophyll is excited after light energy is transferred from the light-harvesting complex of this photosystem. This electron is passed on to the primary electron acceptor, a special chlorophyll molecule, while the P700 now

Cross-references
Bacteria
Biogeochemical Cycles
Black Shales
Carbon Cycle
Microbial Biomineralization
Sediment Diagenesis – Biologically Controlled
Thiomargarita
Thiotrophic Bacteria

PHOTOSYNTHESIS

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Definition
Process used by photoautotrophic organisms to combine carbon dioxide into carbohydrates utilizing the energy of sunlight.

Introduction
Photosynthesis is the most important biological process on Earth since it provides the reduced carbon and the molecular oxygen which are essential for almost all life on this planet. It is the basic metabolic reaction of organisms that contain chlorophyll where energy rich organic compounds are synthesized using the energy of sunlight. During the light-independent reactions oxygen is released as a by-product (Figure 1a).

The net equation of photosynthesis is:

\[
\text{CO}_2 + \text{H}_2\text{O} + \text{light energy} \rightarrow (\text{CH}_2\text{O}) + \text{O}_2
\]

Oxygenic photosynthesis which releases oxygen is found in green plants and cyanobacteria while anoxygenic photosynthesis is found in purple bacteria, green sulfur bacteria, green nonsulfur bacteria, and heliobacteria.

Photosynthesis is almost as old as life itself. Oxygenic photosynthesis has evolved from anoxygenic photosynthesis, however there are different opinions on when this happened. Oxygenic photosynthesis was responsible for the Great Oxygenation Event about 2.300 million years ago when oxygen was released into the Earth’s atmosphere, but it probably evolved at least several hundred million years earlier (see Björn and Govindjee, 2009 for review).

Oxygenic photosynthesis

During the light-dependent reactions of oxygenic photosynthesis, light energy is absorbed by chlorophyll and converted to chemical energy in the form of ATP (adenosine triphosphate) and NADPH+H\(^+\) (nicotinamide adenine dinucleotide triphosphate) which can then be used in the light-independent reactions to produce sugars from carbon dioxide. During the light reactions oxygen is released as a by-product (Figure 1a).

The net equation for the light reactions of the oxygenic photosynthesis is:

\[
2 \text{H}_2\text{O} + 2 \text{NADP}^+ + 3 \text{ADP} + 3 \text{P}_i + \text{light energy} \rightarrow 2 \text{NADPH} + \text{H}^+ + 3 \text{ATP} + \text{O}_2
\]
accepts the electron that reaches the end of the electron transport chain of PSII. The excited electron of PSI is then transferred along a second electron transport chain and is then used to reduce NADP$^+$ to NADPH$^+$ + H$^+$. This electron transfer requires ferredoxin (Fd) and ferredoxin-NADP$^+$-oxidoreductase (FNR; for more details on photosystems and light reactions, see, e.g., Whitmarsh and Govindjee, 1999; Lawlor, 2001; Renger, 2010).

The Calvin cycle uses the energy of ATP and the reducing power of NADPH$^+$ + H$^+$ which are produced during the light reactions to convert carbon dioxide into carbohydrates. It is independent from sunlight and therefore often called dark reaction. The Calvin cycle takes place in the stroma of the chloroplasts of plants and in the cytoplasm of cyanobacteria.

The net equation for the light-independent reactions of the oxygenic photosynthesis is:

$$3 \text{CO}_2 + 9 \text{ATP} + 6 \text{NADPH} + \text{H}^+ \rightarrow C_3\text{H}_5\text{O}_3\text{P} + 9 \text{ADP} + 8 \text{P}_i + 6 \text{NADP}^+ + 3 \text{H}_2\text{O}$$

Carbon dioxide enters the Calvin cycle during carbon fixation, a process which is catalyzed by ribulose bisphosphate carboxylase (rubisco). Each molecule of carbon dioxide is attached to a molecule of the five-carbon sugar ribulose-1,5-bisphosphate. The resulting unstable six-carbon sugar splits in two 3-phosphoglycerate molecules which then become 1,3-bisphosphoglycerate, receiving phosphate groups from ATP. With the help of NADPH + H$^+$, each molecule of 1,3-bisphosphoglycerate
is then reduced to glyceraldehyde-3-phosphate which can be used to form glucose or other organic compounds. However, only one of six glyceraldehyde-3-phosphate molecules leaves the Calvin cycle while the rest is used for the regeneration of the carbon dioxide acceptor ribulose-1,5-bisphosphate which requires more ATP (Figure 1b; for more details on Calvin cycle, see, e.g., Lawlor, 2001; Heldt, 2003).

Anoxygenic photosynthesis
Anoxygenic photosynthesis, which can be found in purple bacteria, green sulfur bacteria, green non-sulfur bacteria, and heliobacteria, differs from oxygenic photosynthesis in that it requires only one photosystem and, since no splitting of water occurs, no oxygen is produced. The photosystem used by purple bacteria and green nonsulfur bacteria is similar to the PSII of plants and cyanobacteria, but contains bacteriochlorophyll instead of chlorophyll. The electron transport is cyclic since the electrons return to the reaction center after passing through a series of electron carriers. The product of the electron transport in the photosystem is ATP. Purple bacteria and green nonsulfur bacteria must oxidize inorganic or organic compounds such as H2S, H2, succinate, or malate to provide the reduction potential necessary for carbon fixation. By using these compounds as electron donors, NAD+ (nicotinamide adenine dinucleotide) is reduced to NADH+H+ in a process called reverse electron transport. Purple bacteria use the Calvin cycle to fix carbon dioxide while green non-sulfur bacteria use a special process called 3-hydroxypropionate pathway.

Green sulfur bacteria and heliobacteria contain a photosystem that resembles the PSI of oxygenic photosynthetic organisms. It has a cyclic electron flow, but here NAD+ can be reduced directly. Green sulfur bacteria use the reverse Krebs cycle to fix carbon dioxide, while heliobacteria are photoorganotrophs (for more details on anoxygenic photosynthesis, see, e.g., Whitmarsh and Govindjee, 1999; Madigan et al., 2009).

Summary
The light reactions of photosynthesis use the energy of sunlight to provide chemical energy and reducing power in the form of ATP and NADPH+H+, respectively, which can then be used in the light-independent reactions to fix carbon dioxide and to convert it into carbohydrates. During the oxygenic photosynthesis of plants and cyanobacteria, water is split and dioxygen is released, whereas anoxygenic photosynthetic organisms such as purple bacteria, green sulfur bacteria, green nonsulfur bacteria, and heliobacteria do not produce oxygen.

Bibliography

et al., 1981). Subsequently, numerous deep-sea barophilic bacterial strains have been isolated from the water column, sediments, intestinal tracts, and decaying parts of invertebrates in the deep sea, many of which have been characterized physiologically and phylogenetically (e.g., DeLong et al., 1997).

In 1995, Yayanos (Yayanos, 1995) suggested a change in nomenclature of pressure-loving bacteria, from baro-(Greek word, weight-loving) philes to piezo- (Greek word, pressure-loving) philes. Piezophilic bacteria are now defined as those prokaryotes that display optimal growth at pressures greater than 0.1 MPa or that showing a requirement for increased pressure for growth (Yayanos, 1995). Based on their response to pressure, piezophiles can be classified as piezotolerant (growth from 0.1 to 10 MPa), piezophilic (10–50 MPa), and hyperpiezophilic bacteria (>50 MPa) (Fang and Bazylnski, 2008). This classification is based on the optimal growth pressure, not the maximum growth pressure, of a piezophilic bacterium. Note that the relative growth rate of these organisms decreases with pressure. The upper limits of life with respect to pressure are not yet defined. For example, Colwellia strain MT41 grows at pressures greater than 130 MPa, which is more than hydrostatic pressure at any ocean depth (Yayanos, 1986).

**Taxonomy and diversity of deep-sea piezophilic bacteria**

Most known cultured piezophilic bacteria belong to one of the following five genera of the Proteobacteria: Shewanella, Photobacterium, Colwellia, Moritella, and a genus containing strain CNPT3, recently identified as Psychromonas (Nogie et al., 2002). Shewanella includes S. benthica and S. violacea. Shewanella species are particularly widely distributed in the deep sea with a broad range of optimal growth pressure conditions from piezophilic to hyperpiezophilic. Representative species include S. violacea strain DSS12; S. benthica strain PT99; and strains DB1172F, DB1172R, DB21MT-2, DB5501, DB6101, DB6705, DB6906, WHB46, F1A, PT48. Moritella includes M. japonica, M. yayanosii, M. profunda, and M. abyssi. Colwellia includes C. hadaliensis and C. piezophila with two hyperpiezophilic strains MT41 and BNL-1. Photobacterium includes P. profundum. P. profundum strain SS9 is one of the most extensively studied piezophilic bacteria whose genomic sequence has been reported recently (Vegetti et al., 2005). Psychromonas includes P. kaikoi and P. profunda. Overall, it seems that there is a limited diversity of the cultured piezophilic bacteria which may be an artifact of the use of rich, heterotrophic growth media. It is likely that diversity of piezophilic bacteria will increase as the use of different types of growth media increases (DeLong et al., 1997). For a more detailed taxonomic description of piezophilic bacteria, see Fang and Kato (2010) and Fang and Bazylnski (2008).

**Piezophily, piezophysiology and metabolism of piezophilic bacteria**

Temperature (T) and pressure (P) are two interrelated environmental parameters that determine piezophilic bacteria growth over a (P;T)-domain. A bacterial isolate is piezophilic if it has a greater generation time at some high pressure than it does at atmospheric pressure when tested at its habitat temperature (Yayanos, 1998). Generally, deep-sea bacteria show strongest piezophilic response to pressure at their upper temperature for growth (typically 15°C) (Kato et al., 1995). Piezophilic strains become more piezophilic at higher temperatures (Kato et al., 1995). The degree of piezophily increases with increasing collection depth or pressure (Yayanos, 1986). Therefore, each piezophilic isolate would have a single maximum growth rate, \( k_{\text{max}} \) (being able to tolerate wide range of pressures). The temperature range \( (T_{\text{max}} - T_{\text{min}}) \) is roughly 10–20°C (Yayanos, 1986), whereas the pressure range is about 40 MPa for bacteria captured at a depth of less than 3,600 m (Jannasch and Taylor, 1984) and 80 MPa for isolates of depths greater than 5,000 m (e.g., Yayanos and DeLong, 1987).

Deep-sea piezophilic bacteria are stenothermal (Yayanos, 1998) but “eury-piezoic” (being able to tolerate wide range of pressures). The temperature range \( (T_{\text{max}} - T_{\text{min}}) \) is roughly 10–20°C (Yayanos, 1986), whereas the pressure range is about 40 MPa for bacteria captured at a depth of less than 3,600 m (Jannasch and Taylor, 1984) and 80 MPa for isolates of depths greater than 5,000 m (e.g., Yayanos and DeLong, 1987).

Laboratory and field studies based on substrate utilization have confirmed piezophilic bacterial activity in deep-sea sediments. Deming and Colwell (1985) examined microbial activity in box cores and sediment trap samples collected in the Demerara abyssal plain in the South Atlantic Ocean (4,470 and 4,850 m). Samples were incubated with low levels (<10% above natural abundance) of \(^{[14C]}\) glutamate at 3°C and in situ and atmospheric pressures. In both sediment and sinking particulate samples examined, enhanced microbial utilization of \(^{[14C]}\) glutamate was observed at in situ pressure, suggesting that indigenous piezophilic bacteria, not the piezo-sensitive microbes originated in shallow surface waters, were metabolically active and functionally predominant in the cycle of naturally low levels of organic matter in the abyssal sediments (Deming and Colwell, 1985). Similar results were obtained in a study conducted on sediment from the Porcupine Abyssal Plain of the Atlantic Ocean (Eardly et al., 2001). Thus, it is highly likely that piezophilic bacterial activity may be widespread in deep sea. However, the proportion of the metabolically/functionally dominant piezophilic bacterial population over the total microbial population in deep sea is unknown.

It is generally believed that the effect of pressure on metabolic processes is secondary to that of temperature. However, based on thermodynamic calculations, Fang and Bazylnski (2008) demonstrated that pressure does have a significant effect on microbial-mediated redox reactions (Figure 1); high pressures reduce the energy yield of \( \text{O}_2 \) reduction, \( \text{NO}_3^- \) reduction, and sulfate reduction and increase the energy yield of the reduction of \( \text{Fe}_2\text{O}_3 \). The additive effect of temperature and pressure...
has raised the \( p_{eq}^{o} \) values of the first four reactions commonly taking place at more positive redox potentials and lowered the \( p_{eq}^{o} \) values (electron activity at the biological standard state) for other six reactions that take place at more reducing conditions. Thus, redox reactions prevailing at more oxidative condition (\( O_2, NO_3^-/NO_2^- \) reduction, etc.) yield slightly more energy in the deep-sea low temperature–high pressure conditions, whereas those that dominate in more reducing conditions yield relative less energy, compared to surface environments (25°C, 0.1 MPa).

**Lipid biosynthesis and carbon isotope fractionation of piezophilic bacteria**

The most abundant lipids detected in piezophilic bacteria are \( n \)-alkyl, acetogenic lipids (i.e., fatty acids); no polyisoprenoids are detected (Fang, unpublished data). Fatty acids biosynthesized by piezophilic bacteria include \( C_{12-19} \) saturated, monounsaturated, terminal methyl-branched, \( \beta \)-hydroxyl, and cyclopropane fatty acids (Fang et al., 2003; Fang and Bazylinski, 2008). Piezophilic bacteria are unique and distinctive in fatty acid composition in that: (1) they synthesize abundant polyunsaturated fatty acids (PUFA), i.e., eicosapentaenoic acid (EPA, 20:5\( \omega_3 \) cis-5,8,11,14,17) and docosahexaenoic acid (DHA, 22:6\( \omega_3 \) cis-4,7,10,13,16,19) and (2) the abundance of unsaturated fatty acids is high and can comprise up to 70% or more of the total fatty acids.

It is generally believed that the carbon isotopic composition of cell components and lipid biomarkers can provide information on modes of microbial metabolism and physiology. The carbon isotope signature is especially useful in deep-sea geomicrobiology for two reasons (Fang and Kato, 2007). First, environmental conditions (temperature and pressure) of the deep sea may exert a greater influence on carbon isotope fractionation in the biosynthesis of fatty acids and therefore the carbon isotopic ratios of individual

**Piezophilic Bacteria, Figure 1** Sequence of microbially-mediated reduction reactions based on values of electron activity at biological standard state (\( p_{eq}^{o} \)) at 25°C and 1 bar (a) and 2°C and 400 bar (b).
compounds (Fang et al., 2006). Second, the deep-sea piezophilic bacteria possess two simultaneously operating biosynthetic pathways of fatty acids which dictate distinctive carbon isotope signature for the long-chain polyunsaturated fatty acids (Metz et al., 2001).

Recently, Fang et al. (2006) examined carbon isotope fractionation during fatty acid biosynthesis in *Moritella japonica* strain DSK1 and demonstrated that carbon isotope fractionation in biosynthesis of fatty acids of piezophilic bacteria is pressure-dependent. Fatty acids became progressively more depleted in $^{13}$C with growth pressure. There was a strong linear correlation between carbon isotopic fractionation and hydrostatic pressure. Overall, piezophilic bacteria fractionate carbon isotopes significantly (14–18%) more than surface heterotrophic bacteria. Thus, the recycling and re-synthesis of fatty acids by piezophilic bacteria utilizing organic matter originating from primary production will greatly alter the carbon isotope signature of both short-chain bacterial and long-chain planktonic fatty acids in oceanic environments and marine sediments. Further, PUFA had much more negative $^{13}$C values than other short-chain saturated and monounsaturated fatty acids. This was attributed to the operation of two different fatty acid biosynthetic systems in piezophilic bacteria: the FAS (fatty acid synthase)- and PKS (polyketide synthases)-based pathways (Fang and Kato, 2007). The FAS-based pathway is one common to surface bacteria which synthesizes typical bacterial fatty acids. The PKS-based pathway is a fundamentally different pathway which involves polyketide synthases (Metz et al., 2001) which catalyze the biosynthesis of long-chain polyunsaturated fatty acids. The PKS pathway appear to be widely distributed in marine bacteria (Wallis et al., 2002) as genes with high homology to the *Shewanella* EPA gene cluster (*Shewanella* sp. SCRC-2738) (Yazawa, 1996) have been found in *Photobacterium profundum* strain SS9 which synthesizes EPA (Allen et al., 1999) and in *Moritella marina* strain MP-1 which produces DHA (Tanaka et al., 1999). Thus, fatty acids (particularly EPA and DHA) and carbon isotope signatures become more informative in deep-sea geomicrobiology.

**Conclusions**

Piezophilic bacteria are prokaryotes that display optimal growth at pressures greater than 0.1 MPa or that showing a requirement for increased pressure for growth. The deep-sea piezosphere, influenced by hydrostatic pressure, probably represents the largest biotope of the Earth. The evidence obtained thus far suggests that microbial life in the deep sea is diverse and that microbial biomass there can be significant. All of the piezophilic isolates reported thus far are facultatively anaerobic, some demonstrating anaerobic respiration as well as fermentation. It has been shown that the degradation of organic matter by fermentation and anaerobic respiration is the principal energy generation processes in both surface and subsurface marine sediments (Parkes et al., 2005). Thus, marine bacteria must play an important role in the overall ocean carbon cycle. These microorganisms surely influence the surface of the Earth by changing the chemistry of the ocean and by affecting the rate of organic carbon burial, with consequences for both the marine carbon cycle and global climate (Fang and Bazylnski, 2008).

**Bibliography**


**Cross-references**

Bacteria
Biogeochemical Cycles
Biomarkers (Molecular Fossils)
Deep Biosphere of the Oceanic Deep Sea
Isotopes (Methods)
Terrestrial Deep Biosphere

**PORE WATERS**

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**Synonyms**

Interstitial waters

**Definition**

The term *pore water* refers to the water contained in the interstices/pore space of aquatic sediments.

**Introduction**

Extracting pore waters and analyzing various dissolved constituents is a central approach in marine sediment geochemistry, biogeochemistry, and geo(micro)biology. The ensuing pore-water concentration-depth profiles are used (a) to characterize the geochemical environment and redox zonation of aquatic sediments (Froelich et al., 1979; Berner, 1981; Postma and Jakobsen, 1996), (b) to infer and identify early diagenetic and biogeochemical processes by compounds which are either consumed or released by the particular reaction (Reeburgh, 1969; Froelich et al., 1979), (c) to calculate diffusive fluxes of dissolved constituents (Hensen et al., 2006; Zabel and Hensen, 2006), and (d) to model reaction and turnover rates of biogeochemical and diagenetic processes – including mineral dissolution and precipitation (Berner, 1980; Van Cappellen and Wang, 1995; Boudreau, 1996). Furthermore, pore-water profiles are applied to quantify the abundance of gas hydrates in marine deposits (Ussler and Paull, 2001; Bohrmann and Torres, 2006) and to study subsurface fluid flow (Saffer and Sereon, 2003).

Pore-water profiles evolve as a consequence of the complex interplay between successive microbial degradation of organic matter by various electron acceptors and a broad spectrum of secondary diagenetic reactions on the one hand and different transport processes (diffusion, advection, bioturbation, and bioirrigation) on the other hand. Due to the close coupling between geochemistry and microbiology, concentration-depth profiles of dissolved chemical species often point to “new” – i.e., to date unknown – biogeochemical processes and metabolic pathways and pave the way for the discovery of novel microorganisms or consortia of microbes which mediate the particular reactions. A well-known example is the process of anaerobic oxidation of methane by sulfate (AOM) which was already postulated to occur on the base of pore-water profiles in the late 1960s (Reeburgh, 1969). Only about 30 years later, new and advanced techniques in molecular biology ultimately were able to prove the existence of microbial consortia of archaea and sulfate-reducing bacteria mediating this process (Boetius et al., 2000; Orphan et al., 2001).

**Extraction and analyses of pore waters**

The classical ways of extracting pore waters are (a) by squeezers where pressure is applied either by an inert gas (e.g., nitrogen, argon) or with a hydraulic press, or (b) by centrifuging and subsequent decanting (Gieskes et al., 1991; Schlüter, 1990; De Lange et al., 1992; for a review see Schulz, 2006, and references therein). In recent years, a new technique for a rapid and more or less nondestructive collection of pore waters from marine sediments has been developed and established – the so-called rhizon sampling (Seeberg-Elverfeldt et al., 2005; Dickens et al., 2007). Rhizon samplers are thin tubes of hydrophilic porous polymer which have a minimum pore size of 0.1 µm. They are inserted into the sediment and are connected to either vacuum vials, spring-loaded syringes, or operated with peristaltic pumps which allow to
extract interstitial fluids without seriously altering the sediment structure.

Due to the often limited amount of pore water extractable – on average only a few milliliters – the analyses of individual dissolved constituents have to be performed on small sample volumes. Therefore, common methods of seawater analyses (Grasshoff et al., 1999) have been modified accordingly to allow the determination of the concentrations of nutrients and other dissolved chemical species from milliliter to submilliliter sample aliquots. A detailed overview of the techniques and instruments used in pore-water retrieval and analyses is available on http://www.geochemie.uni-bremen.de/koelling/index.html. For a concise introduction into the interpretation of pore-water profiles as well as the calculation of diffusive fluxes of dissolved pore-water constituents please refer to Schulz (2006).

Besides the extraction and analysis of pore water, chemical gradients at the sediment/water interface as well as in other environments characterized by a highly condensed geochemical zonation are generally determined by means of microsensors. Typical applications comprise the use of microelectrodes for oxygen, pH, and hydrogen sulfide as well as fiber-optic sensors – i.e., optodes and planar optodes – for oxygen to study and quantify transport processes as well as biogeochemical reactions in surface sediments (Archer et al., 1989; Jorgensen, 2001; Kühl and Revsbech, 2001; Wenzhöfer et al., 2001). The advantage of microelectrodes and optodes compared to the above-described pore-water sampling approaches is that they are sufficiently small to resolve steep chemical gradients and thus allow to accurately measure concentration differences and to calculate diffusive fluxes and model reaction rates. Determining chemical gradients at a high spatial resolution is of prime importance in geo(micro)biology in order to identify microenvironments and other small-scale geochemical heterogeneities as well as to determine steep concentration gradients as they typically exist in and around microbial mats and biofilms as well as at and around mineral and rock surfaces (De Beer and Kuhl, 2001; Kuhl, 2005; Krüger et al., 2008).

Mathematical modeling of pore-water profiles
Due to the fact that pore-water profiles are influenced by the sum of all reactions, process rates directly calculated from pore-water profiles may be highly inaccurate and always represent net rates. The development of numerical models that couple transport processes and (bio)geochemical reactions has therefore been a major advance that allows to more comprehensively interpret sediment pore-water profiles (Berner, 1980; Van Cappellen and Wang, 1995, 1996; Boudreau, 1996, 1997; Soetaert et al., 1996). Such early diagenetic reaction-transport models have been applied on various sediment depth scales, reaching from the simulation of processes close to the sediment surface like calcite dissolution and the cycling of iron and manganese (Van Cappellen and Wang, 1995, 1996; Adler et al., 2001; Pfeifer et al., 2002; Jourabchi et al., 2008) down to reactions occurring at and below the sulfate/methane transition zone in more deeply buried deposits (Riedinger et al., 2005; Wallmann et al., 2006). Reaction-transport models generally include a network of primary and secondary redox reactions as well as different transport processes and are fitted to the measured pore-water profiles. In this

Biogeochemical processes and redox zonation
Particulate organic matter that is deposited on the seafloor is attacked by a broad range of organisms that all contribute to its degradation and gradual mineralization. At the sediment surface, macrofauna play a particularly important role by mechanically disintegrating the detritus and mixing and irrigating the upper sediment layer so that organic material becomes repeatedly exposed to oxygen. After the microbial fermentation/depolymerization of larger organic compounds into smaller organic molecules like, e.g., acetate, formate, and hydrogen microorganisms utilize a suite of external oxidants (electron acceptors) to mineralize these fermentation products (Froelich et al., 1979; Jorgensen, 2006). The different dissolved and solid phase oxidants are used in the following order of decreasing energy yield: oxygen, nitrate, manganese (oxyhydr) oxides, iron (oxyhydr)oxides, and sulfate (Froelich et al., 1979). This sequence of oxidants used in the degradation of organic matter as well as the manifold secondary and abiotic redox processes lead to the establishment of a typical redox or biogeochemical zonation (Froelich et al., 1979; Berner, 1981) which is shown schematically in Figure 1 together with the characteristic concentration-depth profiles of dissolved pore-water constituents. Besides marine sediments a similar segregation of electron-accepting processes in separate zones during the degradation of organic material is also found in lacustrine deposits as well as in groundwater environments.

It should be emphasized that the scheme depicted in Figure 1 is of course simplified and that in natural aquatic sediments the different redox processes in the degradation of organic matter can considerably overlap or the redox zones can even be reversed depending on the availability, abundance, and reactivity of the particular electron acceptors (Postma and Jakobsen, 1996). In any case, the evolving biogeochemical zonation – being itself significantly shaped by microbial metabolism (and activity of macrofauna at the sediment surface) – at the same time exerts a strong control on a broad spectrum of processes like (a) colonization by macro- and microorganisms, (b) different degrees of selective degradation and preservation of organic compounds (including paleoceanographic proxies) under oxic, anoxic or sulfidic conditions (Versteegh and Zonneveld, 2000), as well as (c) processes of mineral dissolution and precipitation (Torres et al., 1996; Peckmann et al., 2001; Kasten et al., 2003).
way, reaction rates can be obtained which can, however, differ from gross reactions rates.

In the case of sulfate reduction, gross rates can be measured by experimental incubation of sediment samples after addition of radioactively labeled sulfate ($^{35}$SO$_4^{2-}$) [for reviews see Jørgensen (2006) and Jørgensen and Kasten (2006)]. While this experimental technique is optimal to determine process rates in shallow and highly dynamic surface sediments, a modeling approach is generally preferred in more deeply buried sediments (Jørgensen, 2006).

**Conclusion**
Pore-water profiles represent a powerful tool to determine the variations in redox and biogeochemical conditions over sediment depth which is a prerequisite for the identification and quantification of early diagenetic influences on the geological record and for an accurate interpretation of paleoceanographic proxies. Furthermore, chemical gradients in the pore water of aquatic sediments are used to infer and identify biogeochemical reactions and to model process rates. However, it should be emphasized that due to the fact that pore-water profiles result from the interactive effects of production and consumption of multiple species during biogeochemical processes certain reaction products are not always detectable in pore water, although the particular reaction proceeds at high rates. For the same reason care has to be taken when process rates are derived from modeling of pore-water profiles. In general, numerical modeling has its strength in deep sediments while experimental measurements are more suitable to determine gross process rates in surface sediments.

**Bibliography**

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**Pore Waters, Figure 1** Schematic representation of the biogeochemical zonation in marine sediments. The names of the main zones were proposed by Froelich et al. (1979) and Berner (1981, in parenthesis). The depth scale is quasi-logarithmic; the exact depths, however, vary strongly and increase from the shelf to the deep sea. The pore-water chemistry shows relevant dissolved species. Peak heights and concentration scales are arbitrary. The chemical profiles reflect the depth sequence of the dominant mineralization processes through which organic matter is oxidized to CO$_2$. (Modified from Froelich et al., 1979; Jørgensen and Kasten, 2006, with permission.)


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**Cross-references**

- Alkalinity
- Anaerobic Oxidation of Methane with Sulfate
- Carbon Cycle
- Carbon (Organic, Degradation)
- Microbial Degradation (of Organic Matter)
- Microsensors for Sediments, Microbial Mats, and Biofilms
- Sediment Diagenesis – Biologically Controlled
- Sulfate-Reducing Bacteria
- Sulfur Cycle

**PROTOZOA (HETEROTROPH, EUKARYOTIC)**

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**Synonyms**

Protist (pro parte); Protactista (pro parte)

**Definition**

Protozoa (historical) Animals that can be reduced to the status of one cell. Protozoa (modern) All eukaryotes after exclusion of the derived kingdoms such as Animalia, Fungi, Plantae, and Chromista.

**Introduction**

Protozoa are a loose grouping of organisms with similar (usually unicellular) organization and heterotrophic mode of nutrition. They are found in most soils, fresh water, and oceans. As components of the micro- and meiofauna, Protozoa are important consumers of bacteria and an important food source for microinvertebrates. While most are solitary individuals, various colonial forms exist. The taxonomic relationships of protozoans to one another and to other protists continue to be revised. Most are free-living, but some are commensalistic, mutualistic, or parasitic. Pathogenic Protozoa include species of *Plasmodium*, the cause of human malaria, *Trypanosoma gambiense*, the cause of African trypanosomiasis, and *Entamoeba histolytica*, the cause of amebic dysentery. The smallest known protozoans are tiny blood parasites less than 2 microns long; the largest may be 16 mm long and visible to the naked eye. The cells show all typical features of an eukaryotic cell. Protozoan shapes vary, but all share such eukaryotic features as lipid–protein membranes and membrane-enclosed vacuoles and organelles. Ultrastructure as well as biochemical cell composition varies a lot due to the broad diversity of these organisms. They also show wide variation in modes of movement, nutrition, and reproduction. Reproduction is usually asexual, occurring mostly by cell division, or binary fission; some forms reproduce asexually by budding or by the formation of spores (reproductive cells that give rise to a new organism without fertilization). In several groups sexual reproduction also occurs.

The number of protozoan species is controversial. Only less than 10% of the species are described and the theoretical basis, that is, the species concept, is also controversial. The most widely used biological species concept is hardly applicable to Protozoa as reproductive isolation, which is the basis of the biological species concept, is rarely investigated for most taxa. In practice, protozoan species were largely defined based on morphological characteristics. However, the degree of molecular similarity between sexual conspecifics is increasingly used as a yardstick for asexual ones from comparable evolutionary lineages. For an increasing number of morphologically described species (morphospecies) molecular data demonstrate an insufficient taxon resolution. Consequently, the species number estimates are increasing. Current estimates of species numbers range from several hundreds of thousands to several million with around 70,000 described species. Further, environmental molecular surveys mostly based on the 18S rRNA gene demonstrate that only a minority of sequences is known and the species number estimates based on morphological analyses may underestimate the diversity by orders of magnitude.

**Megasystematics and nomenclature**

During the period of protistology, that is, from the first descriptions of microorganisms by van Leeuwenhoek in 1674 to date, the classification of Protozoa changed several times depending on the available methodology. Motility was the main or only criterion for the first observers to place these organisms within the animal kingdom; any signs of internal organization were also considered sufficient proof of the animal nature of these organisms. From the mid-nineteenth century onward, the mode of nutrition, that is, heterotrophy and specifically particulate food uptake versus autotrophy, became the dominating criterion in differentiating Protozoa (animals) from Protophyta (plants). The systematic position of the Protozoa and related organisms remained and still remains unclear for many lineages. In consequence, alternative concepts arose, which shall be briefly introduced, starting with the historic context of the
definitions of the terms Protozoa, Protista, and Protocista, which are often synonymously used despite the different conceptual background (Scarmadella, 1999).

Based on the german term “Urthiere” (von Oken, 1805) Georg August Goldfuß introduced the class Protozoa (first, or early animals) in 1820 for a class of organisms within Kingdom Animalia that consisted of Infusoria (called ciliates today), Lithozoa (corals), Phytozoa (e.g., Cryptomonas), and Medusinae. The term implies that these organisms are animals or animal-like and have been separated from the plant-like protophytes. After the formulation of the cell theory, Carl Theodor von Siebold defined in 1845 the Protozoa as animals that can be reduced to the status of a cell and subdivided his Phylum into the classes Infusoria (ciliates) and Rhizopoda (amoebae, foraminifera). In 1858, Richard Owen referred to this grouping as Kingdom Protozoa and defined the group by their lack of a certain number of characters, or “superadditions,” of true plants and animals. Since molecular systematics increasingly demonstrates a close relationship between phototrophic and phagotrophic microeukaryotes the term Protozoa was increasingly replaced by Protista (see below). Still, the term Protozoa is used by some scientists as a paraphyletic basal kingdom, that is, comprising the Amoebozoa, Choanoflagellates, Rhizaria, Excavata, and Alveolata (Cavalier-Smith, 2003).

Based on the problems to distinguish the Protozoa from the Protophyta, John Hogg proposed the term Protocista in 1861 as a grouping of organisms having the common characters of both plants and animals. Hogg’s term “Protocista” was expressive of organisms that came first in evolutionary time. What is significant in Hogg’s depiction of the Protocista is that plants and animals share a common ancestry from the Protocista. Protocista are those microscopic and macroscopic eukaryotes that remain after exclusion of all animals developing from a blastula, all plants developing from embryonic stages, and all higher fungi without a flagellate stage in their life cycles (Margulis et al., 1990).

Agreeing with the erratic separation of Protozoa and Protophyta but disagreeing with Hogg’s view of Protocista being the evolutionary first beings, Haeckel considered that these organisms evolved independent of the lineages of the animal and plant kingdoms. In 1866, he proposed the term Protista as a boundary kingdom intermediate between the animal and vegetable kingdoms containing organisms neither animals nor plants. According to the various definitions given by Haeckel himself, bacteria were Protista as well but Infusoria (ciliates) were placed in Kingdom Animalia as he (and other investigators) considered them multicellular animals. In the modern sense, the Protista embraces all eukaryotes that lack tissues, that is, a loose grouping of 30 or 40 disparate phyla with diverse combinations of trophic modes, mechanisms of motility, cell coverings, and life cycles.

In an ecological or functional context, Protozoa (≈ phagotrophic protists) still is a meaningful term but due to the conceptual problems increasingly replaced by the term protists or by applying narrower functional groups such as heterotrophic flagellates, amoebae, and ciliates. The major lineages of phagotrophic protists are (see Adl et al., 2005):

- Amoebozoa Lühe, 1913, emend. Cavalier-Smith, 1998, that is, organisms with amoeboid locomotion generally with noneruptive morphologically variable pseudopodia (lobopodia). Flagellate stages may sometimes occur usually with a single flagellum. Note that several groups of amoeboid protists do not belong to the Amoebozoa.
- Choanomonada Kent, 1880 (choanoflagellates), that is, phagotrophic with collar of microvilli around a single flagellum. This group is traditionally divided into three groups based on the presence or absence of a cellulose theca or lorica of siliceous strips.
- Rhizaria Cavalier-Smith, 2002, that is, cells with fine pseudopodia (filopodia) varying as simple, branching, anastomosing, or supported by microtubules (axopodia). This group includes ecologically important benthic and soil protists such as the usually amoeboid and/or biflagellated Cercozoa and well as the palaeoecologically important Foraminifera and Radiolaria.
- Alveolata Cavalier-Smith, 1991, that is, protists with cortical alveolae. This group comprises the ciliates (Ciliophora Doflein 1901), the parasitic Apicomplexa including the malaria-parasite Plasmodium, and the Dinoflagellates.
- Several linages within the Stramenopiles (→ algae), which secondarily lost the plastid or reduced the plastid to a leucoplast. Important lineages are, for instance, the Bicosoecida and the chrysomonads (= chrysophytes). These lineages are closely related to major algal groups such as the (see entry Diatoms) (Bacillariophyceae) and the brown algae (Phaeophyceae).
- Cryptophyceae Pascher 1913 (= Cryptomonads), again a group with pigmented phototrophic members and colorless phagotrophic members.

Ecology, microbial food web, and functional groups

In aquatic environments, protist abundances usually range between 10² mL⁻¹ and 10⁴ mL⁻¹. The dominant taxonomic groups among heterotrophic protist communities within different marine, brackish, and limnetic pelagic communities (heterokont taxa, dinoflagellates, choanoflagellates, kathablepharids, and ciliates) and benthic communities (euglenids, bodonids, thaumatomonads, apusomonads, cercomonads, and ciliates) are relatively similar. Soil samples contain between 10⁴ and 10⁸ active protist individuals per gram soil and litter, with flagellates being the most dominant group followed by gymnamoebae, testate amoebae, and ciliates. Numbers decrease with increasing soil depth and are usually lower in unplanted soil. Bacterivorous protists are the most important among secondary saprotrophs; the main bacterivores belong to the bodonids and the cercomonads,
which comprise most soil flagellates, the colorless chrysonomonad Oikomonas, the Vahlkampfiidae, the gymnamoebae, Testacealobosia, and dictyostelids (Amoebozoa), the filose testate amoebae, and the ciliates. Soil protists may be responsible for up to 70% of the soil animal respiration and for 14–66% and 20–40% of the mineralization of C and N, respectively.

In protozoan feeding, either phagotrophy or osmotrophy predominate in particular species. In addition, chlorophyll-bearing flagellates profit from photosynthesis. Some Protozoa, specifically small flagellatea and some filter-feeding ciliates are among the most important consumers of bacteria whereas many of the larger Protozoa prefer algae and smaller Protozoa as food. Life observations on food uptake provided early insights in these functional differences. The more recent research foci on the plant–animal–fungi–protist distinction and the bacterial ingestion as basic in the serial endosymbiosis theory further extended this research direction. This line of thinking based on functional groups culminated in the 1980s in the formulation of the microbial loop concept with heterotrophic phagotrophic flagellates and ciliates as consumers of bacteria (Figure 1). In general, phagotrophic protists (≈ Protozoa) are a major component in any ecosystem and are responsible for the majority of eukaryotic respiration and production whereas their autotrophic counterparts are important primary producers. Protists are key mediators in the enhancement of nutrient flow by regulating decomposition rates and specific metabolic pathways to the benefit of plants and microorganisms in soil and aquatic habitats. Bacterial production is stimulated by protists excreting secondary metabolites, excess nitrogen, and phosphorous in the form of ammonium, inorganic phosphorous, or organic compounds. They channel carbon and nutrient flow from one of the largest standing stocks of living biomass, that is, bacteria (see entry Bacteria) and archaea (see entry Archaea), to higher trophic levels. Thus, with respect to global biogeochemical cycles (see entry Biogeochemical Cycles), phagotrophic protists are a major force that shape the movement and fate of microbial biomass in any ecosystem. Selective grazing has already been demonstrated with respect to prey size, cell wall chemistry, nutritional value, activity, and the ability to produce inhibitory compounds.

Protozoa (Heterotroph, Eukaryotic), Figure 1 The microbial food web. A substantial portion of the primary production is channeled through the microbial food web. Dissolved organic carbon is utilized by bacteria, which are grazed by protozoa.
Fossil record
Protozoa are important in reconstructing past ecological conditions as several protozoan taxa form microfossils. The fossil remains of Protozoa are naturally confined to those classes or orders, which are shell-producing during life. They are most common in deposits of marine environments, but also occur in brackish water, fresh water, and terrestrial sedimentary deposits.

Protozoa (Heterotroph, Eukaryotic), Figure 2 (a–c) Cercozoa are abundant protozoa in soils and sediments. (a) Cercomonas braziliensis; (b) Paracercomonas sp. (c) Filoreta marina; (d–g) Unpigmented chrysomonad flagellates have been regarded as protozoa but are now placed within the Chromista. (d, e) Chrysomonad (=chrysophyceen) resting stages (stomatocysts); (f) Surface scales of chrysophytes; (g) Resting stage covered in surface scales. Figures 2a, b and c are courtesy of David Bass, The Natural History Museum, London, UK.
Radiolarians (see entry Radiolarians) build mostly tests from opal (hydrated silica), some from silica and organic material, and some from strontium sulphate. They are important index fossils and a source of marine silica cements, chert (see entry Cherts), etc. Foraminifers (see entry Foraminifera) have typically multichambered tests, although some are unilocular. Foraminifers are important as biostratigraphic index fossils and as a reliable source of untransported carbonate shell material for stable-isotope analyses. Further, the Paleozoic Acritarchs (nonacid soluble organic structures, potentially cysts) and Chitinozoa (chitinous organic-walled flask-like chambers) potentially are also protozoan microfossils. Besides the fossils of heterotrophic taxa, specifically several Chromist taxa (diatoms, golden algae) build important microfossils such as the diatoms (see entry Diatoms), the Coccolithophorids (see entry Chroococcidiopsis), and the golden algae (Figure 2).

Diversity and biogeography: “everything is everywhere”?

Two main hypotheses about the causes of nonrandom distribution of microorganisms continue to obtain conflicting experimental support: the idea that “everything is everywhere” is based on the assumption that the enormous dispersal capabilities of microorganisms allow them to spread into virtually any habitat. Absence of taxa is caused by unfavorable local conditions that prevent them from getting established everywhere. In a contrasting model, the rate of dispersal of microorganisms is not sufficiently high to overcome historical dispersal limitations and human influence. This allows for existence of endemic taxa many of which remain to be discovered. Adjusting the species concepts is a necessary precondition for drawing conclusions about derived theories such as flagellate biogeography and the “everything is everywhere” debate. The predominant view of a low to moderate number of nanoflagellate taxa, most of which seem to be globally distributed and ubiquitous, is increasingly replaced by the view of a tremendous number of taxa with a much more restricted distribution, both with respect to habitat type and geographic distance.

Summary

Protozoa are a loose grouping of organisms with usually unicellular organization and heterotrophic mode of nutrition. The systematic position of the Protozoa and related organisms remained and still remains unclear for many lineages. Functional groups based on rough morphological characters comprise amoeboid, flagellated, and ciliated organisms. Protozoa are a major component in any ecosystem and are responsible for the majority of eukaryotic respiration and production whereas their autotrophic counterparts are important primary producers. Protists are key mediators in the enhancement of nutrient flow by regulating decomposition rates and specific metabolic pathways to the benefit of plants and microorganisms in soil and aquatic habitats. They build important microfossils both in the marine and in freshwaters.

Bibliography


Cross-references

Acritarchs
Algae (Eukaryotic)
Bacteria
Carbon Cycle
Coccolithophores
Diatoms
Foraminifers
Microbial Ecology of Submarine Caves
Radiolarians
Symbiosis

PYRITE OXIDATION

The most common metal sulfide mineral is pyrite (FeS₂). When exposed to surface weathering, pyrite, as well as other metal sulfides, reacts with the oxygen in air in the presence of water, forming an acidic ferrous sulfate solution:

\[
\text{FeS}_2(s) + \frac{7}{2} \text{O}_2(g) + \text{H}_2\text{O}(l) \rightarrow \text{Fe}^{2+}(aq)
+ 2\text{SO}_4^{2-}(aq) + 2\text{H}^+(aq)
\]

The resulting “Acid Rock Drainage” (see entry) is produced by numerous chemical and microbiological processes within a complex hydrogeological environment. For details, see entry “Sulfide Mineral Oxidation.” In aquatic settings, pyrite oxidation may result in the remobilization and subsequent enrichment of sedimentary sulfide iron. Please refer to entry “Iron Sulfide Formation” for further reading.
RADIOACTIVITY (NATURAL)

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Synonyms
Natural radiation; Terrestrial radiation

Definition
Natural radioactivity (NR) is predominantly due to the decay of $^{238}\text{U}$, $^{235}\text{U}$, $^{232}\text{Th}$ (and their chains of daughter elements), $^{87}\text{Rb}$ (27.8% of natural Rb), and $^{40}\text{K}$ (0.012% of natural K). Early in the Earth’s history $^{244}\text{Pu}$ (half life 82.6 Ma) also was an important radioelement. These are all primordial isotopes formed prior to the origin of the solar system. The list of natural radioisotopes also includes primordial $^{147}\text{Sm}$ and $^{187}\text{Re}$ and short-lived cosmogenic isotopes such as $^{10}\text{Be}$, $^{14}\text{C}$, and $^{26}\text{Al}$. Of all these elements, only U, Th (including daughter elements) Rb, and K represent significant sources of terrestrial natural radioactivity. U, Th, and daughters are emitters of alpha and beta particles and gamma rays; $^{87}\text{Rb}$ emits beta particles, $^{40}\text{K}$ is a beta and gamma emitter. Another source of NR are the cosmic rays, dominantly protons (and minor He nuclei) with a very wide range of energies. The higher energetic ones are of galactic origin, while the sun contributes on the lower energy side of the spectrum. Upon arrival in the atmosphere, a cascade of secondary particles is created (air shower) which ultimately may affect life on the surface. The Earth’s surface is heavily shielded from cosmic rays by both its magnetic field and its atmosphere (Bauer and Lammer, 2004). The contribution of cosmic rays to the natural dose of radioactivity is strongly dependent on altitude and ranges from a minor fraction at sea level to dominant at high altitudes.

The radioactive elements U, Th, Rb, and K are strongly concentrated in the continental crust, and a large part of these elements are relatively evenly distributed with an average concentrations in crustal rocks of 2.7 ppm U, 9.6 ppm Th, 90 ppm Rb, and 2.1% K (2.5 ppm $^{40}\text{K}$). The specific activities, including all daughter elements in secular equilibrium, are 179 Bq/kg for 1 ppm U, 41 Bq/kg for 1 ppm Th, 0.87 Bq/kg for 1 ppm Rb, and 302 Bq/kg for 1% K. Natural enrichment processes can lead to local concentrations much higher than the crustal average. High-grade ores may contain several percent U or Th and up to 52% K (sylvite). The most radioactive mineral is uraninite $\text{UO}_2$ with up to 88% uranium and a specific activity of $1.58\times10^8$ Bq/kg (Figures 1 and 2). Natural chemical or physical separations between U, Th, and their daughter elements can lead to U enrichments lacking daughters (until equilibrium is reached again) or enrichments of daughters unsupported by parent elements. Such disequilibria are of geologically short duration (few half lives) and do occur at sites of high element turnover, e.g., in submarine hot springs (Jolivet et al., 2003) and in the oxidation zone of uranium ores. Radioactive hot spots may form due to accumulation of short-lived daughter elements, e.g., $^{210}\text{Po}$ and $^{210}\text{Pb}$ in hydrothermal vents, and locally cause high doses to fauna (Cherry et al., 1992).

The separation of the short-lived $^{238}\text{U}$ daughter $^{222}\text{Rn}$, a noble gas, and its migration in groundwater and soil gas is of particular importance for human radiation protection.

A special case of NR are the 1.97 Ga natural fission reactors of the Franceville basin, Gabun (Mathieu et al., 2001). During the activity of these natural reactors, they must have been the sites with the highest NR on Earth ever.

The two related actinide elements uranium and thorium show an important difference in chemical behaviour: Thorium only occurs in the tetravalent state Th(IV) and
is nearly immobile (very low solubility) under ambient conditions. Uranium occurs in two major states of oxidation: U(IV) is immobile as thorium, but U(VI), mainly occurring in the form of the uranyl ion UO$_2^{2+}$, is very soluble and the stable form in chemical equilibrium with the Earth’s oxidizing modern atmosphere (Figure 3). Uranium accumulations due to reducing barriers and microbial redox interactions can therefore be expected in the case of uranium, but not for thorium. Uranium thus plays a special role among natural radioactive materials because its minerals have the highest specific activity, and can be formed as a result of low-T redox processes.

There are a range of different interactions, certain and potential, between NR and life on Earth, and, potentially, on other celestial bodies. Such interactions may be direct (affecting organisms) or indirect (changes of the environment or of products of life). The most important interactions between life and NR are summarized here:

(a) NR is a potentially detrimental factor for life, causing gene damage and, at high doses, cell death. The doses of NR are only relevant for gene damage with rare exceptions (Oklo reactors during their activity). For dormant microbial spores unable to perform repair processes, the situation may be drastically different and NR may pose a limiting factor for the long-term survival. NR in the form of cosmic rays is a limiting factor during the actual (manned space missions) and potential presence of life in space, e.g., dormant spores inside a meteoroid being transferred from one planetary body to another in a process called lithopanspermia (Mileikowski et al., 2000).

(b) The low doses of NR on the Earth’s surface are important for evolution in producing a range of mutations as a basis for natural selection.

(c) Microbial life has found ways to adapt to high doses of NR by applying highly efficient DNA repair mechanisms. Such organisms include the bacteria Deinococcus radiodurans and Thermococcus gammatolerans (Jolivet et al., 2003). Apparently in D. radiodurans this mechanism, unlikely to evolve in environments of low NR, was developed as a...
means to deal with desiccation (Mattimore and Battista, 1996).
(d) NR may have played a role in the origin of life through production of reactive organic and inorganic radicals (Parnell, 2004). Natural heavy mineral enrichments (placers) containing radioactive minerals may have been such sites at a time when the specific activity of radioactive minerals was significantly higher than today due to the presence of $^{244}$Pu and higher levels of $^{235}$U.
(e) NR can cause the immobilization of biogenic hydrocarbons during migration. Polymerization of hydrocarbons leads to coatings of immobile organics on radioactive minerals (Rasmussen, 2005; Rasmussen et al., 1993). Such coatings are tracers of hydrocarbon migration, and of life in general (Figure 2). Organic matter in ore deposits generally shows signs of radiation-induced maturation (Hofmann, 2004).
(f) In the near-surface environment of low-shielded planetary bodies (e.g., Mars), destruction of biomolecules (e.g., amino acids) as a result of radiolytic destruction due to cosmic ray bombardment is potentially important over geological time scales (Kminek and Bada, 2006). This puts limits on the detectability of molecular biosignatures.
(g) In certain deep subsurface environments, NR is a key source of chemical energy for microbial life. Through radiolysis of water, kinetically inert reductants (H$_2$) and oxidants (H$_2$O$_2$ as short-term product, sulfate as possible long-term product) may be formed. These and related compounds may then serve as an energy source for microbial growth (Hofmann, 1992; Jørgensen and D’Hondt, 2006; Lin et al., 2005; Lin et al., 2006; Savary and Pagel, 1997).
(h) Generation of local anomalies of NR through direct or indirect microbial reduction of uranium. Both direct enzymatic reduction (Lovley et al., 1991, 1993) and indirect reduction due to microbial production of hydrogen sulphide (Mohagheghi et al., 1985) may be relevant. Microbial cells may become mineralized by uranium phases (Milodowski et al., 1990).
(i) NR is the most important source of geothermal heat and thus a major driver of plate tectonics. This makes NR one of the key influences on geological processes, with which life on Earth is intimately interwoven.

Conclusions
Life on Earth would be different and maybe even nonexistent without natural radioactivity, and the environment would be a totally different one. Natural radioactivity plays only a minor role in the life of single individuals or generations of the bulk of microbial and higher life on Earth, but is a key factor in evolution. In specific extreme environments, like the deep subterranean biosphere, natural radioactivity may provide a crucial energy source.

Bibliography


Cross-references
Biosignatures in Rocks
Carbon (Organic, Degradation)
Extreme Environments
Hydrogen
Ores, Microbial Precipitation and Oxidation
Origin of Life

RADIOLARIA

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Radiolaria are planktonic marine protozoa showing unicellular organization and heterotrophic mode of nutrition. They build tests varying in shape from simple scattered spicules to highly ornamented geometric-shaped shells (Adl et al., 2005). Sizes usually range from hundredths to tenths of millimeters. The tests are made from opal (hydrated silica), some from silica and organic material, and some from strontium sulphate. The name “Radiolaria” derives from the marked radial skeletal spines that characterize many species. Radiolarians are known from the very beginning of the Paleozoic (early Cambrian of the Yangtze Platform; Braun et al., 2007). They are important index fossils and may significantly contribute to marine silica-rich sediments. Radiolarian cherts (radiolarites), a variety of chert (see entry Cherts) composed of radiolarian remains, indicate deep water deposition at depths below which siliceous sediments are stable, but carbonates are dissolved. See entry “Protozoa (Heterotroph, Eukaryotic)” for further reading.

Bibliography


RAMAN MICROSCOPY (CONFOCAL)

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Synonyms
Confocal Raman imaging (CRI)

Definition
Confocal Raman microscopy (CRM) is a nondestructive analytical technique that merges Raman spectroscopy and confocal microscopy for the visualization of molecular information over a defined sample area.

Introduction
Raman spectroscopy is well suited for studies in mineralogy and petrography, as it provides nondestructive mineral identification fast and with high specificity. In addition, Raman spectroscopy allows the characterization of complex organic materials, which makes it particularly useful in biogeo science applications (Hild et al., 2008). This technique has long been applied in geosciences, for example, for the identification and characterization of minerals, or in the observation of mineral phase transitions in high and ultra-high pressure/temperature experiments. In most cases, measurements have been carried out in a micro-Raman set up, i.e., information was obtained from single or multiple points of interest on a sample. This way, little detail on the spatial distribution and association of components or mineral phases, or chemical variation could be observed, even though this information may contribute significantly to the understanding of a sample’s complexity.

By means of CRM, such sample characteristics can be evaluated from large scale scans in the centimeter range to the finest detail with sub-micron resolution. Modern confocal Raman microscopes allow for such measurements with very high sensitivity and spatial as well as spectral resolution. CRM is a tool that not only provides complementary information to data obtained by e.g., electron microprobe (EMP), energy dispersive x-ray analysis (EDX), or secondary ion mass spectrometry (SIMS). In addition to the quantitative and semiquantitative elemental and/or isotopic data acquired by these techniques, CRM contributes the visualization of the distribution for molecular information over a defined sample area. Furthermore, considering that most geomaterials are transparent from the UV (NUV) to VIS and NIR to some degree, this information can be obtained three dimensionally due to the confocal set-up of the microscopes. The following discussion provides background information and examples that shall serve to highlight some key analytical features of this technique for applications in geosciences. A recent and comprehensive summary on the application of CRM in Geoscience can be found in Fries and Steele (2010).
Principles of confocal Raman microscopy

CRM essentially merges two techniques into one. First, Raman spectroscopy, which allows nondestructive chemical analysis; secondly, Confocal microscopy which allows the user to examine samples with diffraction-limited resolution as well as to obtain three-dimensional information from the sample. The theory behind these two techniques will be explained in the following sections, followed by an illustration of how images with chemical sensitivity can be obtained using this combination of techniques.

Raman spectroscopy

When light of a certain wavelength interacts with a molecule, most photons are elastically scattered and therefore have the same energy as the incident photons. However, a very small fraction (approximately 1 in $10^6$ – $10^7$ photons) is in elastically scattered, which means that the energy of the scattered photon is different than the energy of the incident photon.

This is called the “Raman effect”, and it was discovered by Sir Chandrasekhara Raman in 1928 (Raman, 1928; Raman and Krishnan, 1928). Unlike today, he used a filtered beam of sunlight as an excitation source and his eye as a detector for the frequency shifted light. This was long before the development of the first laser by Maiman in 1960. Raman was awarded the Nobel Prize in 1930 for this discovery. The theory behind the Raman effect was derived five years earlier by Smekal (1923).

The tremendous importance of the Raman effect lies in the fact that the energy shift between the exciting and the Raman scattered photon is caused by the excitation (or annihilation) of a molecular vibration. This energy shift is thus characteristic and therefore a fingerprint for the type and coordination of the molecules involved in the scattering process.

Theory

The following section shall provide some basic descriptions and definitions relevant to Raman spectroscopy. Readers interested in a detailed theoretical background are referred to Ibach and Lüth (2003).

In quantum mechanics, the scattering process between a photon and a molecule is described as an excitation of a molecule to a virtual state lower in energy than a real electronic state and the (nearly immediate) de-excitation. The lifetime of the virtual state is extremely short and can be calculated by the Heisenberg uncertainty relation:

$$\Delta t \cdot \Delta E \geq \frac{\hbar}{2} \tag{1}$$

With typical photon energies of 1–2 eV, the lifetime of the excited state is only about $10^{-15}$ s. After this extremely short time, the molecule falls back either to the vibrational ground state or to an excited state (Figure 1). When the initial and final states are identical, the process is called Rayleigh scattering.

If the initial state is the ground and the final state a higher vibrational level, the process is called Stokes scattering, if the initial state is energetically higher than the final state, this is referred to as Anti-Stokes scattering. The difference in energy between the incident and the Raman scattered photon is equal to the energy of a vibration quantum of the scattering molecule. A plot of intensity of scattered light versus energy difference is called a Raman spectrum.

The position of a Raman line is usually given in relative wavenumbers (1/cm), which is the energy shift relative to the excitation line:

$$\nu = \frac{1}{\lambda_{\text{incident}}} - \frac{1}{\lambda_{\text{scattered}}} \tag{2}$$

![Energy level diagram for Raman scattering.](image-url)
\( \lambda_{\text{incident}} \) and \( \lambda_{\text{scattered}} \) are the wavelengths of the incident and Raman scattered photons, respectively.

As can be seen in Figure 2, a typical Raman spectrum is symmetric to the Rayleigh line and the Anti-Stokes lines are lower in intensity than the Stokes shifted lines.

From classical scattering theory, one finds that the intensity \( I \) of scattered light is proportional to the 4th power of the excitation frequency

\[
I \propto v^4
\]

(3)

Exciting a sample with blue light at 400 nm would therefore give a 16 times higher Raman signal than using red light at 800 nm. The problem of using blue (or UV) excitation light, however, is fluorescence. Many samples show fluorescence when they are excited with blue light and Raman emissions are extremely weak compared to fluorescence. If a sample shows significant fluorescence, obtaining a Raman spectrum is usually impossible because the fluorescence background covers the Raman signal. In the red (or even IR) region of the spectrum, fluorescence is usually not a problem, but the excitation intensity must be much higher (\( I \propto v^4 \)). Another problem is that Silicon detectors cannot be used above 1100 nm (band gap energy of Si: 1.12 eV). Other IR detectors (such as InGaAs) show much more thermal and readout noise than silicon and photon counting detectors with low dark count rates are not available yet. In real experiments one must always find a compromise between detection efficiency and excitation power.

Confocal microscopy

Confocal microscopy allows the user to obtain three-dimensional information from the sample. It requires a point source (usually a laser), which is focused onto the sample. The reflected light (Rayleigh, Raman, fluorescence) is collected with the same objective and focused through a pinhole at the front of the detector (Figure 3). This ensures that only light from the image focal plane can reach the detector, which greatly increases image contrast and with the proper selection of pinhole size, slightly increases resolution (max. gain in resolution: factor \( \sqrt{2} \)). As can be seen from Figure 3, light originating from planes other than the focal plane will be out of focus at the pinhole. Therefore, its contribution to the detected signal is strongly reduced. Additionally, by changing the distance between the objective and the sample, the focal plane is moved within the sample thus allowing depth profiling or even 3D imaging (Wilson, 1990).

Pinhole size

The choice of the pinhole size is important because on one hand the signal should be as high as possible, while on the other hand the image should be as confocal as possible (highest depth resolution). To take full advantage of the lateral and depth resolution possible with confocal microscopy, the size of the pinhole should be adjusted and optimized. To obtain the highest lateral resolution, the pinhole size should be below \( v_P = 0.5 \). (The variable \( v \) describes the position in optical coordinates and can be derived from

\[
v = \frac{2\pi}{\lambda} \sqrt{x^2 + y^2} \sin \frac{a}{2}
\]

Here \( \lambda \) is the excitation wavelength, \( x \) and \( y \) the sample coordinates in the focal plane and \( \frac{a}{2} \) half of the aperture angle. \( v_P \) is the radius of the pinhole in optical coordinates when assuming a magnification of 1.) However, at this point the transmission through the pinhole is only 5% of the scattered intensity. In practice, the pinhole size can be up to \( v_P = 4 \) without significantly changing depth resolution and up to \( v_P = 2 \) without significantly changing lateral resolution. It can be shown that if \( v_P > 4 \), the
resolution of at least a conventional microscope remains. This is due to the fact that for a large detector the resolution is always determined by the diameter of the excitation laser spot. Only the depth resolution (and therefore contrast for a thick sample) is lost in this case. In most cases, a pinhole size of $v_P = 2.5$ is a good compromise since good depth resolution is maintained while >75% of the light still reaches the detector (see Figure 4).

For the experiment, the relation

$$M \geq \frac{\pi d_0}{v_P \lambda}$$

should be fulfilled, where $M$ is the magnification, $d_0$ the diameter of the pinhole, and $NA$ the numerical aperture of the objective. The left side of this equation is defined by the objective and the beam path and the right side by the wavelength, the pinhole size itself and $v_P$. If, for example, an objective with a magnification of 100× and a numerical aperture of 0.9 is used at a wavelength of 532 nm the optimum pinhole size would be 50 μm for maximum depth resolution and 10 μm for maximum lateral resolution.

In actual experiments, one usually has to find a compromise between the highest resolution and collection efficiency. This is very important in CRM because Raman is an extremely weak effect. If a very small pinhole is used, the collection efficiency is strongly reduced (Figure 4).

This graphic shows the intensity on the detector as a function of pinhole size, normalized to the total intensity in the image plane. One can see that the collection efficiency is about 75% for maximum depth resolution ($v_P = 2.5$), but only 6% for maximum lateral resolution ($v_P = 0.5$).

Using the appropriate pinhole size, it is therefore always possible to obtain maximum depth resolution.

Resolution

For sample scanning systems, the magnification printed on the objective used is of minor importance. The maximum scan range achievable by the sample scanner determines the maximum image size, independent of the magnification of the objective. The more important property of the objective is the numerical aperture, which together with the excitation wavelength determines the lateral resolution of the objective. The magnification is only important for the choice of the pinhole size.

The maximum resolution of a classical microscope is given by the Rayleigh criterion

$$\Delta x = \frac{0.61 \lambda}{NA}$$

where $\Delta x$ is the smallest distance between two point objects that will appear separated in the image plane, $\lambda$ is the wavelength of the excitation light, and $NA$ is the numerical aperture of the microscope objective. In this case, the image of two point objects will appear just separated (Figure 5).

Confocal Raman microscopy

Instrumentation considerations

When combining confocal microscopy and Raman spectroscopy the main challenge is the low signal intensity. As mentioned earlier, only one in about $10^6$ – $10^7$ photons is frequency shifted by the Raman effect. Thus the number of photons reaching the detector is far less than is the case for confocal or fluorescence microscopy. The two obvious
Efficiency of the spectrometer

Efficiency of the grating

Throughput of the microscope: In order to enhance the Laser power up to 3000 rel.1/cm when used with the 785 nm laser.

> drop to about 30% at 3000 rel.1/cm. The correct grating with a 785 nm laser will cause the efficiency to 3000 rel.1/cm when using a 532 nm laser. Using the same 500 nm will have an absolute efficiency of should be used. As an example: a grating blazed at the correct blazing angle for the excitation wavelength

Some lens-based spectrometers show a transmission at throughput in the spectral range they are designed for. While mirror-based spectrometers only have a transmission of about 30% at this wavelength.

Efficiency of the detector (CCD): Back-illuminated CCD cameras show a quantum efficiency (QE) of >90% over the entire spectral range of interest for 532 nm excitation. Deep depletion back-illuminated CCD cameras, on the other hand, show a QE of >90% for 785 nm excitation. As a comparison, front-illuminated CCD cameras do not exceed 55% quantum efficiency at any wavelength.

Additionally, the dark current of the cameras needs to be minimized, which is achieved through efficient Peltier cooling.

The readout noise is another limiting factor for small signals. As the analog to digital (A/D) converter of all cameras will add at least 5–10 electrons read-out noise to the signal, any signal below approximately 5 electrons will be lost in the noise. Additionally, the faster the A/D converter is operating, the higher the read-out noise will be. Electron-multiplying CCD (EM-CCD) cameras can be used to overcome this problem. With these cameras, the signal is amplified before the A/D conversion, allowing the detection of even single photons and reducing the necessary integration time down to milliseconds.

Principle of operation

Confocal Raman microscopes generally provide a variety of modes of operations. The most common are listed below:

Collection of Raman spectra at selected sample areas (single spectrum): Single Raman spectra can be collected at user-selectable sample areas with integration times ranging from ms to hours. The position of the collected spectrum can normally be fully controlled in 3D. A stable and precise positioning system must be included in the instrument to ensure that the point of interest will remain fixed under the excitation focus. This is very important when spectra with longer integration times for the best quality and signal to noise ratio are to be obtained from extremely small sample volumes. For example, using an oil immersion objective (NA 1.4) with a 532 nm laser and the proper pinhole size allows the sample volume to be as small as ≈230 × 230 × 550 nm.

Collection of time series of Raman spectra at selected sample areas (time spectrum): With this mode, time series of Raman spectra can be obtained to analyze dynamic sample properties. Thousands of spectra can be obtained over time and analyzed with integration times ranging from ms to tens of seconds.

Raman spectral imaging: In the Raman spectral imaging mode, the sample is moved in X and Y and a full Raman spectrum is obtained at every pixel measured. From these data sets images of, e.g., the integrated intensity of various bands can be generated. This is illustrated in the following example.

A drill core section from the Äspö Hard Rock Laboratory, Sweden, was studied using the large area scan mode CRM, aiming to characterize secondary fracture fillings in
a 1.8–1.4 billion years old diorite. The rock sample was obtained from ~450 m below the surface (kindly provided by SKB, Swedish nuclear fuel and waste management). This sample was examined with a 532 nm laser and a 50× air objective (NA 0.55). The scan range was 8000 μm in X and 2000 μm in Y with 800 × 200 points resolution. Each spectrum was integrated for 36 ms. Figure 6 shows the characteristic spectra of calcite, fluorite and quartz found in the sample. Integrating over, for example, the area marked in green in Figure 6d results in a single value for each of the 160,000 spectra. These spectra can be displayed as a color-coded image as shown in Figure 6a for quartz. Here, brighter values indicate a higher integrated intensity of the quartz peak and the distribution of quartz can thus be obtained from this image.

Other spectra of the same scan show the characteristic features of calcite or fluorite (Figure 6d). Using these, the calcite (Figure 6b) and fluorite (Figure 6c) images can be generated by using additional integral filters for the marked regions. Other features of the spectra such as the width of peaks or their position can also be evaluated by applying the corresponding filters.

Further evaluation of the data allows the averaging of similar spectra (for which a cluster analysis is often used) and the subtraction of, for example, pure spectra from mixed spectra to extract the spectra of the various components (spectral de-mixing). These spectra can then be used with the basis analysis, where each of the spectra recorded are fitted with a linear combination of the basis spectra. The result of such an analysis is one image for each basis

Raman Microscopy (Confocal), Figure 6 Spectra (d) and spectral images of quartz (a), calcite (b) and fluorite (c) recorded from the diorite (a) and the fracture fillings (b and c). The data were recorded with a WITec alpha500R confocal Raman microscope.

Raman Microscopy (Confocal), Figure 7 DE-mixed and averaged spectra of the various components in the diorite and the adjacent fracture minerals with the combined image on the left.
spectra which can then be combined to generate a false color image showing the distribution of all components (Figure 7).

The color-coded Raman image and corresponding spectra in Figure 7 allow for the general assignment of mineral phases and their gross distribution over the scanned area. In addition to the mineralogical context information, organic components were identified, spectrally characterized and located, trapped between two generations of fracture fillings (the hydrothermal fluorite and low temperature calcite, Tullborg et al., 2008; Wallin and Petermann, 1999). This information allowed to infer at which point in time a “deep biosphere” (see entry “Terrestrial Deep Biosphere”) was active within these rocks.

Cluster analysis of the data set revealed discrete areas of variation in the mineral phases (Figure 8). This is exemplified by the quartz phase where four distinct regions were identified based on variations in relative peak intensity. Plotting regions of equal intensities of the quartz line at ca. 200 cm\(^{-1}\) shows discrete regions in the sample corresponding to each of the identified spectra.

Quartz was selected as an example to highlight the feasibility of color-coded Raman imaging to locate changes of different mineral phases. These changes can likely be attributed to different levels of crystallinity and crystal orientation. It is noteworthy that the discrete phase colored green only occurs at the interface with the plagioclase minerals, which can be attributed to a secondary phase due to the alteration and phase changes of the plagioclases. Since SiO\(_2\)-phases play an important role in biomineralization, this example highlights the potential benefits confocal Raman imaging may provide in understanding the processes and dynamics involved in these processes.

**Summary**

CRM is a nondestructive analytical technique that merges Raman spectroscopy and confocal microscopy for the visualization of molecular information over a defined sample area. The technique makes use of the Raman effect (Raman, 1928), i.e., the energy shift between exciting and scattered photons which is caused by the excitation (or annihilation) of a molecular vibration. This energy shift is characteristic and therefore a fingerprint for the type and coordination of the molecules involved. By means of CRM, the spatial distribution and association of components in the sample, including organics as well as minerals, can be evaluated from large scale scans in the centimeter range to the finest detail with submicron resolution. This way, CRM may contribute significantly to the understanding of a sample’s chemical composition, and complexity, in geological and geobiological studies.

**Bibliography**


Reduction spheroids are small-scale (diameter: few millimeters to maximum about 20 cm) spheroidal bleached features in red-bed sediments characterized by the absence of hematite and often containing a mineralized core (center, Figure 1). Reduction spheroids result from local chemical reduction of ferric iron and other elements. Host rocks are marine and continental red beds and, more rarely, altered, hematite-stained crystalline rocks. Reduction spheroids have been described from many occurrences in red beds of widely variable age worldwide: Proterozoic of Canada (Tanton, 1948); Cambro-Silurian marine red beds of the NE USA (Beutner and Charles, 1985); Devonian continental red beds of Scotland (Parnell, 1985); Carboniferous continental red beds of New Brunswick and Nova Scotia, Canada (Dyck and McCorkell, 1983); Permian continental red beds of Germany (Eichhoff and Reineck, 1952; Mempel, 1960; Schreiter, 1930), Switzerland (Hofmann, 1990, 1991), Southwest England (Harrison, 1970; Perutz, 1940), and Cretaceous radiolarian cherts of Oman (Hofmann, 1991). Reduction spheroids characteristically consist of a spheroidal volume of rock from which hematite pigment has been dissolved. Central dark cores often display pronounced concentric zonation. The cores are strongly enriched in numerous redox-sensitive elements (dominant V, but also U, Se, As, Ni, Co, Cu, Ag, Au, PGE, and others). The dominant mineral in reduction spheroid cores is the vanadium mica roscoelite KV₂AlSi₃O₁₀(OH)₂. Uraninite is another very common mineral, leading to an often increased radioactivity of reduction spheroids (Figure 2). Arsenides of Ni, Co, and Cu are also quite common. Radiometric dating of roscoelite, uraninite, and Pb-depleted haloes indicates late diagenetic ages (Hofmann, 1990; Hofmann and Frei, 1996). Reduction spheroids often occur in large numbers in red beds and can constitute up to several vol% of the rock. Based on textural evidence, such as fracture-bound occurrences and mass-balance calculations (Hofmann, 1991), reduction spheroids cannot be due to detrital organic-rich particles. Instead, the formation must be due to locally catalyzed redox reactions between a mobile reductant and oxidants. The presence of isotopically light sulfides in some cores (Hofmann, 1991), indicating diagenetic sulfate reduction, as well as the need for a local catalyst, strongly favors a microbiological origin of reduction spheroids. Reductants may be mobile hydrocarbons or molecular hydrogen resulting from the radiolysis of pore water (Hofmann, 1992). The formation of reduction spots is most likely due to chemolithotrophic communities. Kerogen-like organic matter is occasionally present at some localities (Curiale et al., 1983; Parnell, 1985) but is lacking at many other occurrences (Hofmann, 1993).

Conclusion

Reduction spheroids are very common in red-bed sediments, and there are strong indications for an origin due to redox reactions between a mobile reductant and oxidized elements locally catalyzed by microbial activity. Reduction spheroids are thus easily visible features resulting from redox processes catalyzed by a deep biosphere of sediments, and represent a type of biosignature that may also be present in oxidized rocks on Mars. Reduction spheroids contain small-scale
element enrichments that are in many ways analogous in element inventory, geochemistry, and mineralogy to ore deposits such as unconformity-related and roll-type uranium ores.

Bibliography


Cross-references

Biosignatures in Rocks
Chemolithotrophy
Deep Biosphere of Sediments
Hydrogen
Ores, Microbial Precipitation and Oxidation
Radioactivity (Natural)

REEFS

Reefs are laterally confined submarine carbonate structures developed by the growth or activity of sessile benthic aquatic organisms, such as corals, bryozoans, sponges, algae, and microbes (Flügel and Kiessling, 2002). See entry “Carbonate Environments” for further reading and classification.

Bibliography

REMINERALIZATION (OF ORGANIC MATTER)

Degradation of organic matter that results in net conversion of organic carbon to inorganic (oxidized) carbon species such as $\text{CO}_2$ or $\text{HCO}_3^-$. See entries “Carbon (Organic, Cycling)” and “Carbon (Organic, Degradation)” for further reading.

RHODOPHYTA

The Rhodophyta, also referred to as “red algae,” are a distinct eukaryotic lineage of algae with simple plastids (two membranes, derived from primary endosymbiosis). Rhodophytes have a long geological record since the Mesoproterozoic (1.2 Ga). Today, they encompass about 670 largely marine genera with up to 4,500 species predominating the coastal and continental shelf areas of tropical, temperate, and cold-water regions. Red algae are significant as primary producers and providers of structural habitat for other marine organisms. Calcifying (coralline) red algae play an important role in the primary establishment and maintenance of limestone reefs. For further details, see entry “Algae (Eukaryotic).”

Cross-references
Algae (Eukaryotic)
Carbonate Environments
Symbiosis

RNA-WORLD

The term “RNA-World,” first suggested by Walter Gilbert in 1986, describes a popular concept on a hypothetical early stage in the origin of life on Earth. Based on discoveries of enzymatic activities in microbial ribonucleic acids (RNA), a system was suggested where primordial RNA molecules accounted for both, the storage of genetic information and catalytic functions necessary to assemble themselves in a primitive self-replicating system. The RNA molecules evolved in self-replicating patterns, using recombination and mutation to explore new functions and to adapt to new niches (Gilbert, 1986). Such a system would not have required the presence of DNA and/or protein enzymes for biochemical reactions. For further reading, please refer to “Origin of Life.”

Bibliography
SALINE LAKES

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Synonyms
Salt Lakes

Definition
Salt: chloride, sulfate, phosphate, carbonate, bicarbonate salts of primarily sodium, magnesium, calcium, and potassium.
Lake: an enclosed body with or without water entering it from a stream, rain, or snow melt.
Ephemeral lake: a lake formed only following rain or snow melt, which dries up during the rest of the year.
Thalassohaline: salts in a lake derived from the evaporation of seawater with the dominant ions of sodium and chloride as found in the ocean.
Athalassohaline: salts in a lake derived from rocks and geological weathering and dominated by magnesium, calcium, and sulfate.
Alkaliphilic lake: also called soda lake. A lake in which the dominant ions are sodium and bicarbonate and carbonate. This causes the lake to have an alkaline pH.

General description of saline lakes
Saline lakes are a natural worldwide phenomenon. They can be found on all continents and in most countries, and they are frequently recognizable by their name—for example, Salt Lick, Salt Lake, Salt Pond, and Salt Marsh. They range in salinity from just above the salt content of sea water (>3.2% salts) to hypersaline lakes (>20% and up to saturation with respect to salts). Examples of saline lakes include, at the lower end, salt lakes in Antarctica, the Caspian Sea, which borders five countries and has a salinity slightly less than that of seawater, and the Salton Sea in California. At the upper end in salt concentrations are the Dead Sea bordering Israel and Jordan, the soda lakes of North Africa, and Qinghai Lake in China. A general characteristic of saline lakes is that they are terminal lakes, which means that although rivers may flow into them, they have no significant outflow. Therefore, as the water evaporates, the lakes become more salty and in some cases may dry up completely forming salt basins or playas especially in extremely arid environments. In Australia, for example, occasional rains may cause the dry salt basins to become lakes, and these then are called ephemeral salt lakes.

Saline lakes are typically divided into two main types: athalassohaline and thalassohaline. The former refers to lakes which have a different chemical ratio of the main cations from what is found in seawater, while the latter refers to lakes with the cation ratio similar to seawater. Table 1 lists the sodium + potassium ion to calcium + magnesium ion ratios and summarizes the chemical composition of a variety of saline lakes thus showing the wide range of lake types possible.

The data in Table 1 indicate that a subset of the athalassohaline lakes exists. These lakes have a high concentration of carbonate and therefore a high pH. These are the soda lakes or alkaline lakes. They, too, are found worldwide and are described below. No Antarctic lakes are listed in Table 1 because the surface waters are generally only very slightly saline (less than seawater) and it is only with depth, often greater than 10 m, that true saline waters are found. In general, it is the bottom waters that are hypersaline with little turnover to bring the saline waters to the surface. The chemical compositions of Antarctic lakes have been reviewed by Matsubaya et al. (1979).

The reason for the differences in the chemical composition lies in the origins of the various types of lakes. The thalassohaline lakes were generally formed during the
**Saline Lakes, Table 1** Examples of the chemical compositions of saline lakes. Concentrations in g/L

<table>
<thead>
<tr>
<th>Lake name</th>
<th>Location</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Cl⁻</th>
<th>SO₄²⁻</th>
<th>HCO₃⁻</th>
<th>pH</th>
<th>Ratio a</th>
<th>References</th>
</tr>
</thead>
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<tr>
<td>Small lake near Koombekine</td>
<td>Australia</td>
<td>9.36</td>
<td>0.065</td>
<td>0.077</td>
<td>0.38</td>
<td>15.7</td>
<td>0.67</td>
<td>0</td>
<td>3.0</td>
<td>20.6</td>
<td>Geddes et al. (1981)</td>
</tr>
<tr>
<td>Mar Chiquita Lake</td>
<td>Argentina</td>
<td>12.5</td>
<td>0.14</td>
<td>0.35</td>
<td>0.25</td>
<td>16.7</td>
<td>5.1</td>
<td>0.21</td>
<td>8.53</td>
<td>21.1</td>
<td>Martinez (1995)</td>
</tr>
<tr>
<td>Laguna Amarga</td>
<td>Chile</td>
<td>24</td>
<td>0.33</td>
<td>0.002</td>
<td>1.7</td>
<td>8.2</td>
<td>38</td>
<td>4.4</td>
<td>9.4</td>
<td>14.3</td>
<td>Saijo et al. (1995)</td>
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<td>Great Salt Lake – North Arm</td>
<td>Utah, USA</td>
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<td>6.9</td>
<td>0.28</td>
<td>8.5</td>
<td>175</td>
<td>22</td>
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<td>7.9b</td>
<td>12.3</td>
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<td>7.3–8.1b</td>
<td>12.8</td>
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<td>228.6</td>
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<td>0.3</td>
<td>6.3</td>
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<td>30.1</td>
<td>9.8</td>
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<td>ND⁹</td>
<td>ND</td>
<td>86.4</td>
<td>43.4</td>
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<td>ND</td>
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<td>1.47</td>
<td>7.9</td>
<td>ND</td>
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<td>16.9</td>
<td>6.25</td>
<td>8.91</td>
<td>NL</td>
<td>41.9</td>
<td>ND</td>
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<td>Seawater</td>
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<td>11</td>
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<td>0.42</td>
<td>1.32</td>
<td>19.7</td>
<td>2.76</td>
<td>0.145</td>
<td>8.0</td>
<td>6.5</td>
<td>ZoBell (1963)</td>
</tr>
</tbody>
</table>

aCalculation for this paper: (Na⁺ + K⁺)/(Ca²⁺ + Mg²⁺)
bTaylor et al. (1980)
cNot listed
dNot determined
eCombined Na⁺ + K⁺
Pleistocene period (Hammer, 1986) and are the result of seawater intrusion before tectonic activity created the mountains which cut the bodies of seawater off from the ocean. Brine springs are formed from the dissolution of salts deposited during evaporation of the seawater. As surface water seeps into the ground, the water percolates downward and eventually encounters the salt, dissolves it, and forms underground saline lakes. These form the basis of often small salty ponds, springs, licks, and rivers.

Athalassohaline lakes are most generally formed by the weathering of rocks by wind, rain, and freshwater and were formed during the Pleistocene period and later by volcanic and faulting activity (Hammer, 1986). These are also terminal lakes, and they are typically close to saturation with respect to salt. They may also be ephemeral – found only occasionally after the rainy season – or permanent containing water year round.

The soda lakes are considered athalassohaline because they were formed from high carbonate rocks due to an absence of rocks of sedimentary origins. Consequently, they have low concentrations of calcium and magnesium ions and high concentrations of carbonate or carbonate complexes (Jones et al., 1998).

Biology of saline lakes

Despite the differences in chemical composition, all saline lakes have certain features in common. In general, fish are not found in lakes with salinities greater than 4% (40%). Two major reasons for this are egg survival (Gerking and Lee, 1980) and loss of osmoregulation (Morris, 1960). One exception is the European sea bass, Dicentrarchus labrax, which tolerates salinities up to twice that of seawater (Jensen et al., 1998).

Other eukaryotic organisms are found in the higher salinity lakes, specifically algae, brine flies, and brine shrimp. The diversity of the algae depends on the total salt concentration. At salinities lower than around 10–12% salt, a wide variety of diatoms and green algae is found. At salinities greater than 12–15% and up to saturation, the main algae are from the genus Dunaliella. It is also at these higher salinities that the brine fly and brine shrimp are major biomass contributors. These small eukaryotes provide the food sources for the extensive bird populations that are frequently associated with saline lakes. However, other crustaceans such as the mussel shrimp cannot survive in lakes with salinities greater than 18% (Deckker, 1981).

Another group of eukaryotic organisms found throughout the range of saline lakes is the fungi. It is only recently that fungi have been isolated from the higher salinity lakes. The state of our understanding of halotolerant and halophilic fungi was summarized by Frisvad (2005). The presence of protozoans and flagellates in saline lakes has also been reviewed and documented by Hauer and Rogerson (2005) and Cho (2005), respectively.

Just as lower salinities allow for a greater diversity of algae, the same is true for the bacteria. Members of the Domain Bacteria predominate in the salinity range of 3–15% salt (Ventosa et al., 1998). Above this, the members of the archaeal kingdom domain, the salt-loving Halobacteriaceae become dominant though several members of the domain Bacteria grow at these elevated salinities. The predominant Bacteria genera in elevated salinities are Halomonas and Salinibacter. As of August 2007, there were 26 described genera of the haloarchaea and 85 species (Oren et al., 2007), and most, but not all, grow over salinity ranges from approximately 12% to close to saturation. Microbes found in most salt lakes are therefore, salt-loving and prefer a circumneutral pH.

Among those haloarchaea genera, the “Natrono-”genera are primarily found in soda lakes. As mention above, soda lakes have an alkaline pH; therefore, bacteria which grow under those conditions are considered alkaliophiles (alkaline pH-loving). There are many genera of the kingdom Bacteria which also contain genera and species that are alkaliophiles. Thus, all of these bacteria are both salt- and high pH-loving organisms. This distinguishes them from the bacteria described in the previous paragraph.

Halophages are viruses that attack bacteria living in saline lakes. The viruses may incorporate into the bacterial cells or they may cause the lysis of the cells. In either case, halophages, like other bacteriophages, tend to be host-specific and can be found wherever their host organism lives. Dyall-Smith et al. (2005) have recently summarized the state of knowledge about the halophages which are specific for the haloarchaea.

Saline lake sediments

Sediments of saline lakes have been the subject of numerous studies because of the potential for determining stable isotope ratios hence sediment age (Piovano et al., 2004) and using lipid biomarkers (Rontani and Volkman, 2005; Pearson et al., 2007) to indicate the types of biota in the sediments. The exposure history of Dead Sea sediments was determined by examining the various minerals formed within the interstitial waters again improving the understanding of the age and depositional history of saline lake sediments (Yechiel and Ronen, 1997). As the salinity of the overlying water increases, this results in similar increases in the salinity of the interstitial waters within the shallow sediments, and the reverse is also true that decreases in the salinity of the lake due to rainfall or snow melt will decrease the salinity of the pore waters.

These postdepositional changes have also been noted for the alkaline Soda Lake in Washington State in the USA (Mantuan, 1973) and in playa Lake Salines in southeastern Spain (Queralt et al., 1997). Such changes can also affect the biology of the sediments which are anaerobic (low to no oxygen) and are dominated by bacterial mats which can produce sulfides and methane (Miller et al., 1993). These mats are composed of layers of bacteria with the cyanobacteria on the surface and various green and purple sulfur bacteria followed by the sulfate-reducing bacteria that produce the sulfides, and the obligately
anaerobic methane-producing bacteria. More recently, Jioang et al. (2007) have noted, in a core from Lake Chaka on the Tibetan Plateau that the distribution of bacteria within the core is determined by the salinity in the pore waters rather than by the mineralogy, and that the biomass decreased most around 150–200 cm depth and increased slightly at 600 cm with a maximum at 900 cm as determined by phospholipid fatty acid analyses. However, the direct counts remained steady with a slight increase at 900 cm. Most of the organisms identified at these lower depths were anaerobic bacteria, again confirming the importance of oxygen to the types of microorganisms found in any sediment.

Value of saline lakes
Saline lakes are nature preserves and serve as home to extensive permanent and migratory bird populations. Great Salt Lake, Utah, USA, hosts over five million birds annually (and this is true in Africa, Australia, the Caribbean, etc.). They may also act as hatching grounds for flamingoes and other birds at the lower salinities. Thus, saline lakes are important in maintaining the biodiversity of our planet.

A second major value, which is related to the first, is that these environments with their large numbers of microorganisms, brine flies, and brine shrimp provide an important gene pool. Although there are numerous studies on the brine shrimp, very little is known about the brine flies, the algae, or the bacteria found in these lakes. Unknown enzymes, potential antibiotics, drug delivery systems, or mechanisms for adaptation to stress could be present but unknown for lack of research into these areas.

Another value of the lower salinity lakes is their potential for aquaculture. Gamito (1997) successfully showed that the gilthead, Sparus aurata, could be grown in the Ria Formosa because of the high benthic productivity (1997). Undoubtedly, there are other fish species that could be successfully grown in the lower salinity saline lakes.

A major economic value of saline lakes is their salt. The ephemeral or dried salt beds are a source of salt to many groups of people. This is especially true in Africa with the Taureg people, and in Tibet which also has salt caravans, in the Atacama region of Chile, in Australia, and in Bolivia to name just a few of the places where salt is harvested from dry saline lake beds.

Bibliography
measurements of total salts, salinity is now normally expressed as a ratio of the electrical conductivity of the seawater to that of a standard concentration of KCl solution. The concentration of the standard solution is set such that the ratio of the approximate average salinity of the ocean today is equal to 35. The previous concentration value (‰) is thus unnecessary, but it is still widely used to highlight the basic meaning of the term. The relative proportions of the dissolved constituents are approximately constant, so the amount of Cl⁻, the major component, is a proxy for salinity. Salinity history refers to past changes in the amount of dissolved salts in sea water over geologic time.

Significance
Salinity is an important environmental variable affecting chemical balances and osmotic pressures in all living cells. It particularly affects metazoans, because the amount of dissolved oxygen available for respiration depends on oxygen solubility that decreases as salinity increases. Besides its direct effects on cellular biochemistry, salinity governs the temperature of maximum density of sea water and thus the global thermohaline circulation that influences ecosystems. Models of geochemical cycles yield no constraints on past salinity levels, and reliable geochemical proxies, isotopic records, or other possible indicators of ancient oceanic salinity are currently unknown. However, Na⁺ and Cl⁻ constitute about 85% of the salt in the sea, so the geo logic record of halite deposits allows insights into how ocean salinity may have varied with time.

Causes of salinity changes with time
Early ideas that steady riverine input of salt to the oceans causes salinity to increase with time failed to recognize the geologic processes that intermittently cause salt to be removed from the sea and then later released following tectonic uplift. Nor did they correctly assess the probable initial origin of salt in the sea or consider possible small changes in ocean volume over time. Salt in rivers is derived largely from groundwater dissolution of ancient marine salt beds deposited when sea water evaporated under arid conditions in low latitude, geographically restricted arms of the sea. Deposition of these “saline giants” depletes the ocean in salt and thus lowers salinity. Subsequently, these enormous deposits may be tectonically lifted above sea level. Depending upon elevation, descending groundwaters can eventually dissolve the deposit and debouch into streams or lodge as dense, saline brines deeper in the continental subsurface. In addition to the saline giants, carbonate depositional environments in shallow seas that flooded the continents in past times typically contained vast tidal flats and supratidal areas in which sea water intermittently evaporated. Partially evaporated sea water from these environments as well as their ephemeral salts dissolved by falling rains descended into the subsurface and lodged in sedimentary basins even where large saline giants were absent. Most large
sedimentary accumulations on continents thus have highly saline waters in the deep subsurface. The salts in these brines were also in sea water once and their sequestration on continents thus also served to lower salinity of sea water. The presence of salt beds and brines hundreds of millions of years old illustrates the enormous lengths of time salt extracted from the sea can remain isolated.

**Salinity history for the past 540 Ma**

Estimates of the salt inventory in sedimentary rocks have steadily grown as more and more subsurface deposits are discovered. The latest estimate by Hay et al. (2006) confirms that deposition of the saline giants is strikingly episodic (Figure 1). Over half of the salt currently identified was deposited between 100 and 300 million years ago following breakup of the supercontinent Pangea. Presumably, this breakup and subsequent drift of the continental fragments produced the unusual array of geographic, geologic, climatic, and ocean circulation conditions that Sonnenfeld (1984) argued were necessary to produce saline giants. It follows that ocean salinity should have declined during these times of exceptional withdrawal of salts from the sea. The amounts shown in Figure 1 are surely minimal estimates of the amount of salt withdrawn because some significant percentage has been dissolved by groundwaters to become brines in the strata beneath the salt. Additionally, salt depositional basins are rimmed with clastic deposits adjacent to the surrounding highlands and these are conduits to the subsurface for the prodigious amounts of evaporite brine present when the salt was being deposited. Combined geochemical and isotopic studies have shown that deep basin brines are probably mixtures of partially evaporated sea water and brine created by fresh water dissolution of salt (Knauth, 1988).

Salinity should increase during periods when there are no giant salt extractions underway and when older, uplifted deposits are eroding into the sea. This is the current situation, so the old arguments that the oceans are increasing in salinity via riverine input of salt are understandable. There is no direct evidence yielding the amount of salt returned to the sea at any given past time. Instead, it is necessary to use models for continental sediment erosion that are based on an array of assumptions. Hay et al. (2006) estimate that the return of salt to the sea has varied by a factor of three over the past 540 Ma, reaching maxima in the Upper Cambrian (515 Ma), at the Permian/Triassic boundary (245 Ma), and during the Lower Cretaceous (135 Ma).

Using the inventory of currently sequestered salt and the calculated estimate of salt return, a possible salinity history of sea water for the past 540 Ma can be inferred if the volume history of sea water can be estimated. Fresh basalt on the ocean floors becomes hydrated during subsea
weathering and hydrous sedimentary minerals are deposited as the basalt rides tectonic plates to subduction zones. At issue is how much of the hydrated water returns to the surface as it gets progressively heated during subduction. Some models predict a net loss to the mantle over geologic time, so it is not unreasonable to suggest that ocean volumes may have decreased somewhat with time. Combining all these uncertain estimates and models, Hay et al. (2006) have suggested the possible salinity history for the oceans for the past 540 Ma shown in Figure 1. The inferred salinity levels prior to 200 Ma are 50% higher than modern values. Inasmuch as life in the sea is stressed by current salinity values, the idea that Paleozoic life could thrive at such high inferred salinity is likely to be resisted by marine biologists and paleontologists. The amount of brine sequestered during initial deposition and later dissolution remain uncertain and could severely affect such calculated salinity histories. Nevertheless, the provocative Hay et al. (2006) estimates indicate that ocean salinities in the past were likely higher.

**Direct salinity measurements in fossil sea water**

Tiny fluid inclusions in carbonates or other minerals precipitated directly from ancient seawater may be fossil sea water that could be analyzed for salinity. The difficulty is that the initial precipitates typically undergo dissolution/precipitation episodes during subsequent burial and metamorphism. Actual attempts at making this difficult analysis on inferred primary precipitates yield salinities of 31–47% at about 500 Ma (Johnson and Goldstein, 1993). These measurements suggest past salinities not too dissimilar from the modern value, but the large error bars do overlap the Hay et al. (2006) estimates (Figure 1). While reliable ancient salinity values remain elusive, it seems demonstrated that significant variations should have occurred in the past 540 Ma and that salinity was likely higher rather than lower in the past.

**Salinity history of the early ocean**

With regard to deeper time, past salinity can be estimated if it is assumed that the oceans outgassed early in Earth history (Holland, 1978) and that the plate tectonic cycle long ago reached an approximate steady state with regard to hydrosphere cycling through the crust and mantle. Cl\(^-\) does not fit readily into any common silicate mineral and thus should have outgassed as HCl along with water following accretion of the Earth. The earliest hydrosphere would thus contain the entire Cl inventory and been at maximum salinity. The only way this high initial salinity can be reduced is by sequestration of salts and brines in the saline giants deposited on long-live continental cratons. Large continents may have formed shortly after the Earth accreted 4.5 billion years ago (Ga), but recent studies confirm the likelihood that objects in the inner solar system were largely resurfaced by the Late Heavy Bombardment (LHB) at about 3.8–4.0 Ga (Gomes et al., 2005). Continents may have started to reaggregate following the LHB in the interval between 3.8–2.5 Ga, but the kind of long-lived, continental cratons upon which evaporatively deposited salt and brine could be sequestered following its removal from sea water do not appear until about 2.5 Ga (Rogers, 1996). It follows that all the salt and brine currently lodged on the continents was in the Archean sea following the LHB and prior to the development of these stable cratons.

In the absence of a paleosalinity indicator in the rock record, a reasonable assessment of the earliest salinity could be made if the total amount of salt and brine currently lodged on the continents were known. While the inventory of salt in strata younger than 540 Ma is becoming increasingly better quantified (Figure 1), the amount of salt in older strata is yet to be inventoried. Enormous deposits are known in Neoproterozoic strata (540–800 Ma) across the Middle East, through Pakistan, and into Australia. These apparently accumulated in restricted basins that developed following the breakup of supercontinent Rodinia, analogous to those developed following the breakup of Pangea. The geographic extent of these enormous salt deposits exceeds that of the Cenozoic deposits in the Gulf of Mexico, so it might even be the largest accumulation in Earth history. The amount of subsurface brine associated with the saline giants and with innumerable ancient plume carbonate deposits is also unknown. Land (1995) used estimates of the amount and average salinity of pore fluids to suggest that sequestered brines could hold an amount of salt equal to or greater than that of the rock salt. Adding these amounts of possible total salt and brine currently on the continents back into modern ocean water raises salinity by over a factor of 2. Assuming that the Cl inventory and the oceans were outgassed early in Earth history and have not changed their amounts significantly with time, Archean salinities were therefore much higher and could have exceeded 70% (Knauth, 2005).

**Implications**

The geologic history of salt deposition clearly seems to suggest significant past salinity variations with values possibly much higher than current levels. If so, the impact on biology and biologic evolution must have been significant. An assessment of the paleontologic record in relation to the emerging possible salinity history for the past 540 Ma (Figure 1) remains to be done. The inferred very high salinity of the earliest ocean combined with the probable hot climatic conditions of the early Earth may have limited early marine biologic diversity to the appropriate extremophiles. If the great Neoproterozoic salt deposits were really the first saline giants, the prospect arises that high salinity was prevalent throughout the Precambrian. The great salinity decline likely accompanying deposition of the Neoproterozoic salt combined with declining temperatures associated with probable glaciations may have allowed oxygenation of the ocean for the first time. The Cambrian explosion of life in the sea at 540 Ma may have been, in part, a response of respiring organisms evolving...
rapidly in this vast new array of oxygenated marine habitats (Knauth, 2005).

Summary
Salinity is not fixed by any equilibria, feedbacks, or geochemical cycles and appears to have been generally higher in past times. It is apparently a free variable dependent upon the vagaries of intermittent salt (and brine) sequestration on long-lived continental cratons. Ocean salinity declines when the salt and brine accumulate and rises when they are eroded back into the sea. Sequestration seems to have been enhanced following breakup of the two supercontinents in Earth history. These breakups are, in turn, driven by the vagaries of mantle convection. The combination of processes therefore necessary to lower the initial, pre-continent high salinity and to affect the rise and fall of ocean salinity involve a long chain of independent variables. The role of chance and contingency in biologic evolution has long been recognized. The low odds that a rerun of the tape of evolution would yield the same results have been duly noted (Gould, 1990). The same might be said about the evolution of oceanic salinity. Salinity history should therefore probably be added to all those chance occurrences that have likely affected the course of biologic evolution.

Bibliography

Cross-references
Critical Intervals in Earth History
Evaporites
Hypersaline Environments
Pore Waters
Soda Ocean, Hypothesis

SCANNING PROBE MICROSCOPY (INCLUDES ATOMIC FORCE MICROSCOPY)

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Synonyms
Atomic force microscopy; Atomic probe microscopy; Force microscopy; Scanning force microscopy

Definition
Scanning probe microscopy uses a nanoscopic probe that is scanned over a solid surface. The interaction between the probe and the surface may be a mechanical or electromagnetic force. The force signal is enhanced and then composed to a force diagram of the surface. Under ideal conditions, an atomic-scale resolution may be achieved.

Introduction
Since their development during the 1970s and early 1980s, scanning probe microscopes (SPMs) have developed into instruments suitable for the analysis of surface topography (and other surface properties) down to near-atomic resolutions. All instruments belonging to this family contain essential components as depicted in Figure 1. In the scanning process, surface material properties are mapped in all three directions. The essential parts of the system are the sharp scanning probe tip, which moves in the z direction, a piezoelectric scanner that moves the specimen, or, alternatively, the tip, in the x/y directions, and a sensor for tip positioning. Sensor and tip are connected in a feedback loop for the correction of z positioning. The x/y/z positions are used to generate a topographic image of the specimen. The resolution of SPMs is only limited by the size of the probe tip (or the probe–sample interaction volume). Since the tip apex ends up in one single atom and the piezoelectric actuators execute motions down the range of angstroms, resolutions down to atomic scale are possible. In contrast to electron microscopes, the sample can be observed under ambient conditions, which makes the technique potentially attractive for life science, including geobiological research.

The scanning tunneling microscope (STM)
The scanning tunneling microscope (STM) is the ancestor of all SPMs (Binnig et al., 1982). The specimen needs to be conductive or semiconductive. STM is predominantly used in materials sciences and chemistry. Though not ideally suited for biological specimens, the technique has also been used in life sciences (see, e.g., Guckenberger et al., 1988). The instrument provides a surface topography down to atomic resolution.

STMs use a sharpened conducting tip with a bias voltage (in mV to V-range) applied between the tip and the sample. If the distance between the tip and the sample is large, no current flows. When, however, the tip is brought
very close (1 nm), but without physical contact, a small current (pico- to nano-amperes) flows across the gap between the tip and the sample. This current is the result of the overlapping of wave functions between the tip atoms and the surface atoms. Electrons can “tunnel” across the vacuum barrier separating the tip and sample in the presence of the bias voltage. The magnitude of the tunneling current increases exponentially when the tip approaches the specimen surface. This results in high system sensitivity: when the separation between the tip and the specimen changes by 10%, the tunneling current changes by an order of magnitude. This provides, in the end, surface images with lateral atomic resolution.

The image of the tunneling current depicts approximately the topography of the sample. Actually, rather than physical topography, the STM measures a surface of constant electron tunneling probability.

**Atomic force microscopy and related techniques**

Atomic force microscopy (AFM) comprises several derivatives and is applicable for measurement of numerous physical and chemical parameters of sample surfaces. AFM in the strict sense (without a modified probe tip) is used to study the material properties of specimens (stiffness, plasticity, and viscosity; see, e.g., Rotsch et al., 1997). For biological specimens, on the cellular level, the properties of the cytoskeleton and (induced) cytoskeletal changes become visible (see Fung, 1993; Goldmann et al., 1998 for some applications). With the aid of chemically modified tips, AFM is useful for quantification of binding forces between receptors and ligands, protein complexes and cells (Lehenkari and Horton, 1999; Yip, 2001; Allison et al., 2002; Dufrene, 2008). Since AFM is generally suited for solid surfaces, it is especially a versatile tool for the observation of biomineral formation down to nanoscale in situ (see, e.g., Hildebrand et al., 2008).

Image formation in AFM is generally determined by sensing of interatomic forces, making the technique applicable to all solid specimens. Similar to STM, the AFM probes the surface of a sample with a sharp tip, less than 10 nm in diameter. The tip is located at the free end of a 100–500 μm long cantilever. The cantilever is deflected by forces between the tip and the sample surface, and a detector measures the cantilever deflection as the tip is scanned over the sample (or the sample is scanned under the tip). The measured cantilever deflections are used to generate a map of surface topography (Figure 1). Conductors, semiconductors, and insulators may all be scanned. Resolution in the atomic range requires rigid specimens; hence, for most biological samples (except for biominerals), maximum resolution is not achievable.

The topographic data set necessary for image formation can be directly obtained from the measurement of cantilever deflection. When a laser beam is projected onto the cantilever, the position of the reflected beam is shifted, which results in detection of even sub-nm movement at the tip surface. Other methods of signal enhancement are based upon optical interference (e.g., by a Michelson interferometer). A STM tip may also be used to read the cantilever deflection. If the cantilever is made from a piezoresistive material, its deflection can also be detected electrically.
When the cantilever deflection is detected, this can be used to generate the topographic data set directly. This mode (constant height) is often used for producing atomic-scale images of atomically flat surfaces, for which the cantilever deflections and variations in applied force are small. The constant height-mode is also essential for recording real-time images of changing surfaces, which requires high scan speeds. Alternatively, the deflection of the cantilever can be used in a feedback loop that moves the scanner up and down in the $z$ direction, responding to the topography, by keeping the cantilever deflection constant. In this case, the image is generated from the scanner’s motion. With the cantilever deflection held constant, the total force applied to the sample is constant. This mode (constant force) is generally preferred for most applications.

Figure 2a depicts the typical force–distance curve for AFM. As the atoms of the tip and the specimen surface are gradually brought together, they first weakly attract each other by van der Waals forces. This attraction increases until the electron clouds of the atoms are so close together that their electrostatic repulsion progressively weakens the attractive force. The force becomes zero when the distance between the atoms reaches a couple of ångströms, about the length of a chemical bond. In the contact mode, the cantilever is held less than a few ångströms from the sample surface, and the interatomic force between the cantilever and sample is repulsive. For biological (i.e., soft and wet) objects, the contact mode may induce several artifacts. This is avoided by application of modes in which less force is applied to the specimen. However, smaller forces also have to be detected, which in practice reduce the final resolution. In the noncontact mode, the cantilever is held of the order of tens to hundreds of ångströms from the sample surface, and the interatomic force between the cantilever and sample is attractive (largely as a result of long-range van der Waals interactions). In this mode, the cantilever is oscillated with a frequency of 100–400 kHz (the resonance frequency of the cantilever) with an amplitude of a few tens of nanometers. The resonance frequency of the cantilever varies with the force gradient acting on the cantilever. These variations can be used as a measure of changes in the force gradient, which in turn reflects the changes in the tip-to-sample distance, or sample topography. The resonant frequency or vibrational amplitude of the cantilever is monitored and kept constant with the aid of the feedback loop that moves the scanner up and down in the $z$ direction. The system also keeps the average tip-to-sample distance constant. As in contact AFM (in constant-force mode), the motion of the scanner is used to generate the topographic data set. It thus detects changes in the resonant frequency or vibration amplitude as the tip comes near the sample surface. Intermittent-contact (or tapping mode) AFM is similar to noncontact AFM, but the vibrating tip is brought closer to the sample in such a way that the sample is intermittently contacted or “tapped.” As in noncontact AFM, the oscillation amplitude of the tip also changes in response to the distance between tip and sample. Intermittent-contact AFM is also less likely to damage the sample than contact AFM. Though it touches the specimen, lateral forces (friction or drag) between the tip and the sample are minimal. The pulsed force mode (PFM) is used to monitor, besides sample topography, stiffness and adhesion simultaneously (Krotil et al., 1999). In PFM, the probe scans the surface in contact mode. When the cantilever is oscillated with a frequency of about 1 kHz, it is possible to analyze the force output as a function of the distance between the tip and the surface. The force output is depicted in Figure 2b. During an oscillation
cycle the probe tip is attracted by the surface and “snaps” into contact at a certain distance. The tip is then pushed further to the sample, the force signal increases, whereby the slope of the curve depends on the specimen stiffness. When the distance increases, force signal decreases, the tip loses contact (adhesion peak) and oscillates freely until the signal is damped to the baseline. Characteristic points of these curves are taken for processing to generate simultaneous topographic, stiffness (deformation of the specimen under an applied force), and adhesion maps. In the lateral force mode (LFM), lateral deflections (twisting) of the cantilever are measured. These are caused by forces on the cantilever parallel to the plane of the sample surface. LFM studies are used to detect surface friction arising from inhomogeneity in surface material, an artificial enhancement of edges in the image may also be achieved (Overney et al., 1992). In the force modulation mode (FMM), the elastic properties of a specimen are mapped. The cantilever tip is scanned in contact with the sample surface. As in constant-force contact AFM, the feedback loop uses the cantilever deflection signal to maintain a constant force between the tip and the sample and to generate a topography image. When a periodic oscillation is applied to the tip or the sample, the sample surface resists and the cantilever bends according to the elastic properties of the sample surface. The system generates an image, a map of sample stiffness, from the changes in the amplitude of cantilever modulation (Troyon et al., 1997).

Scanning force microscopy is a highly variable technique, because forces between a specimen and an AFM tip may be caused by a multitude of underlying factors. A modified scanning tip may therefore be used to measure a variety of parameters other than the described surface forces. In life sciences, the detection of chemical properties on biological surfaces is of outstanding interest. Some derivatives of AFM, mainly based on a modified scanning tip, are listed below.

Magnetic force microscopy (MFM) is used for measuring the magnetic force between the tip and the sample. For MFM, the tip is coated with a thin ferromagnetic film. The system mostly operates in noncontact mode and detects magnetic domains down to a resolution of approximately 20 nm (de Lozanne, 2006).

Electrostatic force microscopy (EFM) measures the forces originating from an electrical field between the AFM tip and the sample. When a voltage is applied between the tip and the sample (the tip is not in contact with the surface), the cantilever deflects when it scans over charged particles on the sample surface. In this way, EFM maps locally charged domains on the sample surface (Girard, 2001).

Scanning thermal microscopy (SThM) measures the specimen temperature and thermal conductivity of the sample surface. The SThM tip may be made from a thermocouple temperature sensor: a pair of dissimilar metals joined at the tip (platinum/silver Wollaston wires). The temperature difference between the tip and the open end generates a proportional net voltage. In this way, thermal data and surface topography (with the conventional AFM force feedback mechanism) can be measured at the same time. Probes made of Wollaston wires also operate as active heat sources. The probe resistance is proportional to the probe temperature. Changes in the current required to keep the probe at constant temperature produce a thermal conductivity map. When the tip is held at a constant current, the specimen temperature is mapped by changes in the probe resistance (Price et al., 1999).

Photothermal microspectroscopy (PTMS) combines scanning probe microscopy at a submicron spatial resolution with an IR interferometer. IR radiation from a Fourier-transform spectrometer is focused on a specimen. IR absorption causes stretching and bending of molecular bonds. Upon relaxation of these molecular vibrations, evanescent thermal valves are emitted. This signal is detected with a Wollaston wire (SThM) probe. The resulting temperature fluctuations are converted to electrical signals, recorded as a function of time and amplified to produce an “interferogram” which can be transformed back to give a conventional spectrum as in other forms of FTIR spectroscopy. In PTMS, resolutions down to 10 μm are achievable, whereas in conventional FTIR microspectrometric devices, only 100 μm are possible (Grude et al., 2007).

Phase detection microscopy (PDM) is another variation for mapping of surface properties such as elasticity, adhesion, and friction. Unlike FMM (see above), the technique operates in the noncontact mode, which is more suitable for less rigid (biological) samples. The technique monitors the phase lag between the signal causing the cantilever to oscillate and the cantilever oscillation output signal. Changes in the phase lag reflect changes in the mechanical properties of the sample surface. The feedback loop in PDM operates in the usual manner, using changes in the cantilever’s deflection or vibration amplitude to measure sample topography. The phase lag is monitored while the topographic image is being taken, so that images of the topography and material properties can be collected simultaneously.

Scanning near-field acoustic microscopy (SNAM) utilizes an ultrasonic quartz oscillator as resonator and at the same time as a sensor of the damping force exerted by the specimen. Damping increases when the tip approaches the surface. The instruments have been especially developed for scanning of larger areas, with a surface relief in the millimeter range. For low resolution, wide probing tips are used. At resolutions of 100 nm, both hard and soft specimens are well suited for mapping. Higher resolution is obtained with sharp AFM tips (Murdfield et al., 1996).

With chemical force microscopy (CFM) it is possible to obtain modified force curves based on specific interactions between molecules on the specimen surface and a sensor molecule on the probe tip. The tip (normally made from silicon or silicon nitride) is modified by coating a very thin metallic film (normally a 5 nm thick chromium layer followed by a 50 nm thick gold layer) onto the probe, followed by immersion of the probe in a solution of an
organic thiol. The molecules form a monolayer, with the thiol group bound to the gold surface. The distal end of thiol molecule contains an appropriate functional group for interaction with the sample surface. Besides small methyl, carboxyl or amino groups, macromolecules may also be used as specific probes. Coupled enzyme proteins may be used to measure enzyme–substrate interactions. In a similar experimental setup, even whole bacterial cells, coupled to the cantilever, were used to measure distance–force curves between the cell surface and biominerals (Lower et al., 2001; Dufrene, 2008 and references therein).

Near-field scanning optical microscopes (NSOMs) are based upon the AFM operating in the noncontact mode. At the same time, the probe tip generates nonpropagating (evanescent) electromagnetic wave fields to excite a specimen at a small (down to tens of nm) surface spot. Most NSOM setups use an aluminum-coated glass fiber with a sub-light wavelength aperture (25–100 nm in diameter) at the tip of an AFM-like scanning probe (Figure 3). Light, guided through the fiber optics, causes an evanescent wave field that decays exponentially with distance from its origin. Evanescent waves interact with the specimen surface, e.g., by excitation of fluorophores. Fluorescence light emitted from the specimen is scattered from the sample, but only from the evanescent wave field region (at distances from the probe tip of approximately one fifth of the light wavelength). The fluorescence signal is conventionally propagating far-field light. This signal may be collected by a conventional optics below the specimen (e.g., an inverted microscope). Because of the extremely small excitation volume determined by the evanescent wave, together with the high-resolution topographical map generated by the AFM tip, a high-resolution map of the fluorescent targets in the specimen is achieved.

With NSOM, the AFM topographic information is combined with optical properties of a specimen, such as refraction index, reflectivity, transparency, polarization Raman shift, and fluorescence (Gheber et al., 1998; de Lange et al., 2001; Edidin, 2001).

Conclusion

Scanning probe microscopy introduced a purely surface-related technique with atomic resolution into the field of material sciences and also life science. For the first time it was possible to visualize macromolecules directly, under physiological conditions. Even more important is the application potential for mapping all kinds of intermolecular interactions. The fact that just signals from a surface can be collected may be a drawback for the observation and documentation of most biological processes because they are inside cells and other compartments. Most mechanisms in material science, chemistry and biology are, however, surface-related. It is just the question how a surface, which may also be a membrane or the interface of another compartment, can be prepared in a way that it is reachable by an SPM.

Other limitations are the low scanning speed of most modes and the small obtainable image area. Hence, it is straightforward to combine scanning probe microscopy with other imaging techniques. Correlation of low-resolution techniques including fast image acquisition (fluorescence light microscopy or confocal microscopy) with the high-resolution AFM image may be versatile for a multitude of applications, especially concerning the interaction of organisms with solid surfaces (e.g., Arnold et al., 2004; overview in Haupt et al., 2006).

Bibliography


Cross-references
Raman Microscopy (Confocal)

SEDIMENT DIAGENESIS – BIOLOGICALLY CONTROLLED

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Definition
The biological processes that modify unconsolidated sediment after initial deposition and burial.

Overview
Throughout the sediment column, up to the temperature threshold of life, microbial activity plays an integral role in diagenesis. It is widely recognized that through their various chemoheterotrophic pathways, microorganisms are ultimately responsible for the conversion of organic carbon to CO2 and CH4 at temperatures <100°C. Some aerobic bacteria use hydrolytic enzymes to break down complex molecules into simple monomers such as sugars, amino acids, and fatty acids that they can then utilize, while most anaerobes are restricted to simple organic compounds (e.g., acetate, lactate, H2) that are the by-products of fermentation (Lovley and Chapelle, 1995). Typically, the more labile materials (e.g., proteins, carbohydrates) are degraded in near-surface sediments on time scales of days to years, and more refractory materials (e.g., lipids) are broken down deeper in the sediment on time scales of decades, while the most resistant materials (lignins) are transformed only on timescales of thousands to millions of years. The amount of refractory material left after microbial attack will depend on the nature of the primary producers and the degree of processing that has taken place in the river catchment area or oceanic water column. In the end, for example, <1% of the original material buried into marine sediment may ultimately contribute to the sedimentary organic geochemical record (Emerson and Hedges, 1988).

Pore water and mineralogical changes during diagenesis are directly related to bacterial reduction of soluble (O2, NO3−, SO42−, CO32−) or solid-phase (MnO2 and ferric oxyhydroxides) components in the sediment. The terminal electron accepting processes (TEAPs) that occur at any given layer depends on oxidants that are available and, in the situation when multiple electron acceptors are present (as in the uppermost sediment layers), on the free energy yield of the specific reaction. Thus, the decomposition of freshly deposited organic material in sediments proceeds in a continuous sequence of redox reactions, with the most electropositive oxidants being consumed at, or near the surface, and progressively energetically poorer oxidants being consumed at depth until the labile organic fraction is exhausted and the deeper sediments are left with a composition very different from that of the sediments originally deposited (Figure 1). Inorganic by-products of chemoheterotrophy (e.g., HCO3−, Mn2+, Fe2+, NH4+, NO2−, HS−, HPO42− and CH4) seldom accumulate. Physical and macrofaunal processes will cause the net transport of these reduced species from the deeper layers toward the sediment surface, where residing anaerobic and aerobic chemolithoautotrophic bacteria use them as metabolic reactants. Alternatively, their presence in pore waters may trigger important abiotic reactions between the solid and dissolved phases leading to secondary mineral formation, i.e., cementation.

Oxic zone reactions
The sediment–water interface is the site of most intense heterotrophic activity. Aerobic respiration (Reaction 1) is...
the first used metabolic pathway for the degradation of organic matter, not only because it has the greatest energy yield per amount of organic carbon oxidized, but the aerobes are equipped with a full suite of extracellular enzymes capable of degrading complex organic polymers into simpler substrates (e.g., cellulose into glucose).

\[
C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad (1)
\]

\[\Delta G^0 = -2872 \text{ kJ/mol glucose or } -240 \text{ kJ/2e}^-\]

It is estimated that on a global scale, the majority of all particulate organic carbon (POC) deposited is aerobically respired; the percentage varies among different marine environments, with aerobic respiration accounting for >90% of the organic carbon oxidized in deep sea sediments (e.g., Bender and Heggie, 1984), while O₂ is the TEA in only 45% of coastal marine settings and freshwater lakes (e.g., Jones, 1985).

In marine sediment, one of the primary factors controlling the patterns above is the composition of the organic material and the rate at which it is deposited. Plankton-derived organic matter, rich in lipids and cellulose, exhibit significantly higher reactivities than land-derived organic material consisting of vascular plants rich in lignins (Hedges et al., 1988). Thus, in coastal settings, a significant fraction of POC may be relatively difficult to decompose. Furthermore, under the high deposition rates characteristic of coastal settings (1 mm year⁻¹), O₂ diffusion is very shallow and the oxic zone may only extend down a few millimeters in depth, although physical stirring of surface sediments by waves and currents, circulation of overlying water through animal burrows that are connected to the sediment–water interface (known as irrigation), and bioturbation of surface sediments may locally increase the depth to several centimeters (e.g., Hammond et al., 1985). Therefore, with high accumulation rates, freshly deposited organic matter passes through the oxic zone on the orders of 1–10s of years, leaving a significant amount of carbon intact to support fermentative and anaerobic heterotrophic populations (Canfield, 1993). By contrast, the influx rate of POC and nutrients decreases with distance from land and water depth. This results in a progressive diminishment of macrofaunal and planktonic species, as well as the benthic bacterial populations, leading to low organic matter accumulation rates in the deep sea. Coupled with the low sedimentation rates associated with the open ocean (<0.01 mm year⁻¹), the organic fraction will remain exposed to oxygenated waters for thousands of years, leading to almost complete oxidation by O₂ – only the most refractory fractions will remain available for other TEAs in the deeper sediments (Murray and Grundmanis, 1980).
O2 is also consumed by a vast number of chemolithoautotrophic bacteria that metabolize upwardly diffusing reductants (such as HS−, NH4+, Fe(II)). Therefore, as reduced metabolites from anaerobic respiratory pathways diffuse upward, a fraction of the oxygen consumed in the surface layers is diverted away from aerobic respiration, toward reoxidation of the reduced species. As a consequence, the depth of O2 penetration correspondingly decreases. In fact, some diagenesis models show that the amount of O2 partitioned to organic carbon oxidation in coastal sediments can be <1%, with nitrification or hydrogen sulfide oxidation comprising the major O2 sinks (e.g., Blackburn and Blackburn, 1993).

Suboxic zone
Nitrification–denitrification
Nitrogen cycling in aquatic sediments is relatively complex, with nitrate pore water concentrations governed by the balance between nitrification in the oxic zone and denitrification in the suboxic zone. The amount of nitrification is regulated by the concentration of pore water ammonium (NH4+), which largely depends on the C:N ratio of the sedimentary organic carbon being oxidized, and the amount of O2 penetration (Blackburn and Blackburn, 1993). In the presence of O2, ammonium is first oxidized to ammonium-oxidizing bacteria (e.g., Nitrosomonas sp.), and the nitrite is then further oxidized to nitrate by nitrite oxidizers (e.g., Nitrobacter sp.). Collectively, these processes (Reaction 2) cause a subsurface peak of nitrate in the pore water (Mortimer et al., 1999).

\[
\text{NH}_4^+ + 2\text{O}_2 \rightarrow \text{NO}_3^- + \text{H}_2\text{O} + 2\text{H}^+ \\
(2)
\]

The opposing processes in marine N-cycling is denitrification (loss of NO3− to N2) and nitrate ammonification (loss of NO3− to NH4+). Denitrifiers most closely resemble aerobic respirers in that they are capable of completely degrading complex organic matter to carbon dioxide (Reaction 3).

\[
2.5\text{C}_6\text{H}_{12}\text{O}_6 + 12\text{NO}_3^- \rightarrow 6\text{N}_2 + 15\text{CO}_2 + 12\text{OH}^- + 9\text{H}_2\text{O} \\
(3)
\]

\[
\Delta G^0 = -2715 \text{ kJ/mol glucose or } -226 \text{ kJ/2e}^- 
\]

In coastal sediments (and lakes), these bacteria reduce nitrate to negligible levels within 1–10 cm of the sediment–water interface. No nitrate occurs below this depth except where burrows, lined with nitrifying bacteria, irrigate oxygenated water to depths well below the oxic–suboxic boundary (Hansen et al., 1981). In deep sea sediment, the vertical zone of all terminal electron acceptors (TEAs) are expanded, and for nitrate, concentrations may not become negligible until depths of >1 m. Denitrification fluxes range over several orders of magnitude in marine sediments, between values as low as 1 μmol m−2 day−1 in deep sea sediments (Bender and Heggie, 1984) and over 1 mmol m−2 day−1 in some estuarine and coastal waters (e.g., Devol, 1991).

The flux of N2 escaping from sediments is often higher than predicted biological or inorganic denitrification rates based solely on pore water nitrate concentrations. This means that in the sediments, excess N2 is being formed independent of a nitrate intermediate. One way this occurs is through the anammox process, where shunting nitrogen directly from ammonium to N2 (Reaction 4) can promote ammonium deficiencies in sediments where this process plays a key role in nitrogen cycling (Thamdrup and Dalsgaard, 2002).

\[
\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + 2\text{H}_2\text{O} \\
(4)
\]

Ammonium can also react inorganically with MnO2 to form N2 instead of nitrate (Reaction 5). The Mn(II) formed in this reaction is biologically oxidized by Mn(II)-oxidizing bacteria (see below), with O2 as the TEA, generating more reactive MnO2 to continue the oxidation of the soluble ammonium (Luther et al., 1997). Field evidence suggests that this reaction can outcompete the direct oxidation of NH4+ by O2 to NO3− in Mn-rich coastal sediments, and in doing so, potentially account for 90% of N2 formation. In essence, this process short-circuits the traditionally considered nitrification–denitrification process.

\[
2\text{NH}_4^+ + 3\text{MnO}_2 + 4\text{H}^+ \rightarrow 3\text{Mn}^{2+} + \text{N}_2 + 6\text{H}_2\text{O} \\
(5)
\]

Manganese cycling
Detrital mineral phases that survive transport and deposition are for the most part an unreactive component of the bottom sediment. The oxides and hydroxides of manganese and iron are the exceptions. These phases are stable under the oxygenated conditions encountered in the water column, but they quickly become unstable after burial below the oxic layers of sediment. Following denitrification, reduction of Mn(IV) oxides (typically written as MnO2) to dissolved Mn2+ becomes the most energy-efficient bacterial respiratory process (Reaction 6).

\[
\text{CH}_3\text{COO}^- + 4\text{MnO}_2 + 3\text{H}_2\text{O} \\
\rightarrow 4\text{Mn}^{2+} + 2\text{HCO}_3^- + 7\text{OH}^- \\
(6)
\]

\[
\Delta G^0 = -558 \text{ kJ/mol acetate or } -139 \text{ kJ/2e}^- 
\]

In most sediments, the limited amount of manganese (and iron) oxides deposited in sediment, as well as the slower rates of burial of these oxides compared to the downward diffusion of O2, NO3− and SO42−, generally makes their reduction of minor importance (<10%) in terms of the amount of total organic carbon oxidized (Burdige, 1993). There are, however, exceptions to this generalization, i.e., some lakes and marine hydrothermal settings where high rates of metal oxide reduction are driven by a combination of relatively high metal inputs and active macrofaunal activity that mix suboxic sediment with
oxygenated bottom waters, thereby reoxidizing dissolved Mn$^{2+}$ (and Fe$^{2+}$) to MnO$_2$ or Fe(OH)$_3$ (Aller, 1990).

In typical marine sediments, the concentration of Mn$^{2+}$ is negligible at the sediment surface, but at some depth between the NO$_3^-$ maximum and zero NO$_3^-$, it begins to increase toward its maximum values. Despite forming near the base of the nitrate reduction zone, its concentration is never highest at that depth because the Mn(II) that diffuses upward is biologically reoxidized and precipitated as fresh MnO$_2$ at the sediment surface, suggesting that the TEA can be either nitrate or oxygen (Froelich et al., 1979). A characteristic feature of Mn(II) oxidation is the upward convexity of the pore water Mn$^{2+}$ profile and the discrete layer of MnO$_2$ forming at the base of the oxic zone (recall Figure 1). By contrast, downward diffusion of Mn(II), and its reaction with HCO$_3^-$, most commonly results in the formation of manganese carbonates, such as rhodochrosite (MnCO$_3$).

Iron cycling
Below the zone of dissimilatory manganese reduction, and at the depth of complete nitrate removal from pore waters, is where Fe(III) reduction takes place (Reaction 7). Ferrihydrite [Fe(OH)$_3$] is the least crystalline, most reactive, and easily reducible iron oxide phase, with rates of Fe(III) reduction in sediment declining rapidly with depth as these poorly crystalline phases become depleted (Lovley and Phillips, 1986).

\[
\begin{align*}
\text{CH}_3\text{COO}^- + 8\text{Fe(OH)}_3 & \rightarrow 8\text{Fe}^{2+} + 2\text{HCO}_3^- \\
& + 15\text{OH}^- + 5\text{H}_2\text{O} \\
\Delta G^0 & = -337 \text{ kJ/mol acetate or } -84 \text{ kJ/2e}^- 
\end{align*}
\]

Despite the lower reactivity of crystalline phases, in sediment where ferrhydrite has either been depleted or where crystalline iron oxides are inherently more abundant, the latter can serve as significant source of reducible iron that couples the oxidation of buried organic material. According to experimental observations, Fe(III)-reducing microorganisms developed three different strategies to cope with the difficulty of transferring electrons from the cell to the surface of a barely soluble electron acceptor (see Lovley et al., 2004 for review). First, physical contact between the cell surface and ferric iron allows direct delivery of electrons. Second, iron chelators increase the solubility of Fe(III) and hence, alleviate the need for Fe(III) reduction. Third, electron-shuttling compounds transfer electrons from the cell to Fe(III) oxide surface without the necessity of physical contact between cells and mineral. Considering the complexity of natural environments and the wealth of microbial capabilities, it is not surprising that different organisms, as well as single organisms, developed different strategies in order to reduce diverse Fe(III) compounds under varying conditions. For example, some evidence indicates that Geothrix fermentans produce and release both Fe(III) chelators and electron shuttles (Nevin and Lovley, 2002). Furthermore, studies on Geobacter sp. indicate that different cellular compounds are involved in reduction of dissolved Fe(III)-citrate and barely soluble ferrihydrite (e.g., Leang et al., 2005).

The reduction of ferric iron minerals increases the concentration of Fe(II) in suboxic sediment pore waters, with a peak in concentration at the boundary between the Fe (III) and sulfate reduction zones. Some of this ferrous iron may diffuse upward to be reoxidized to ferric hydroxide inorganically by either NO$_3^-$, MnO$_2$, or O$_2$ (e.g., Myers and Nealson, 1988), and it is likely that in some lakes with near-surface sediment, phototrophic Fe(II) oxidation should take place. Some Fe(II) also precipitates as mineral phases. In iron-rich sediments, the high reactivity of Fe$^{2+}$ and the general availability of HCO$_3^-$ in suboxic and anoxic pore waters tend to cause ferrous iron to precipitate quickly as micritic cement in the form of siderite (FeCO$_3$). By contrast, when sulfate reduction rates exceed Fe(III) reduction rates (see below), enough HS$^-$ is produced to react preferentially with any ferrous iron to precipitate iron monosulfide minerals instead (FeS has lower solubility than FeCO$_3$). Therefore, the precise juxtaposition of iron reduction and sulfate reduction in marine sediments controls the formation of siderite and/or pyrite (e.g., Coleman, 1985). The formation of vivianite [Fe$_3$(PO$_4$)$_2$] in many ways resembles that of siderite, namely Fe(II) concentrations must exceed those of HS$^-$.

Anoxic zone
Sulfate reduction
Below the suboxic layers is a highly reducing, oxygen-free zone under which sulfate reduction predominates. In marine sediments, this is the principal process by which simple fermentation products are oxidized (Reaction 8), accounting for approximately 50% of the carbon oxidation in coastal marine sediments (Jørgensen, 1982), but <10% in deep sea and freshwater environments (Bender and Heggie, 1984; Jones, 1985).

\[
\begin{align*}
\text{CH}_3\text{COO}^- + \text{SO}_4^{2-} & \rightarrow \text{HS}^- + 2\text{HCO}_3^- \\
\Delta G^0 & = -49 \text{ kJ/mol acetate or } -12 \text{ kJ/2e}^- 
\end{align*}
\]

Its relative importance in marine environments compared to freshwater environments is based simply on the higher concentrations of dissolved SO$_4^{2-}$ (~28 mM) at the sediment–water interface, some 50 times greater than the combined sum of all other electron acceptors with higher electrode potentials. Moreover, external electron acceptors that yield more energy than SO$_4^{2-}$ typically disappear within the first few centimeters of sediment depth, leaving sulfate as the dominant TEA for most of the sediment column (D’Hondt et al., 2002).
The rates of dissimilatory sulfate reduction are proportional to the quantity and reactivity of organic matter entering the anoxic sediments — the greater the amount of aerobic decomposition, the less labile organic matter remains available for sulfate reduction, and the more refractory the residual material becomes (Westrich and Berner, 1984). As a result of natural variations in the above, sulfate reduction rates in marine sediments can vary by six orders of magnitude, with the highest in rapidly deposited coastal and lagoonal sediments, where it occurs just below the sediment–water interface (>1 mM cm⁻² year⁻¹) and at the lowest levels in deep sea sediments, where rates may be <10⁻³ mM cm⁻² year⁻¹ (Canfield, 1993).

The product of sulfate reduction is hydrogen sulfide, and as mentioned above, can lead to the formation of iron sulfide minerals. The amount and reactivity of the detrital iron phases dictate the amount of pyrite that will form, commonly referred to as the “degree of pyritization.” In slowly accumulating sediments (i.e., the deep sea), little labile organic matter is buried, sulfate reduction rates are slow, less HS⁻ is available for reaction with detrital iron, so less ferric iron is eventually converted to pyrite. Since the saturation state of the pore waters with respect to iron monosulfides controls the pore water concentrations of dissolved iron and sulfide, limited HS⁻ means that pore water Fe(II) will increase in concentration. By contrast, in rapidly accumulating sediments, more labile organics are buried, near-surface sulfate reduction rates are high, HS⁻ is abundant, and more of it is available for reaction with dissolved Fe(II) and solid-phase ferric iron (Berner and Raiswell, 1983). Nonetheless, not all ferric iron phases are pyritized because some ferric iron minerals react only very slowly with dissolved sulfide, and complete pyritization becomes impossible in the time span over which sulfate reduction occurs in the sediment (Canfield et al., 1992).

Only a fraction of the HS⁻ that forms via bacterial sulfate reduction is actually precipitated as pyrite sulfur (Berner, 1982). Instead, some 90% of the HS⁻ is reoxidized to sulfate. Although O₂, NO₃⁻, and Mn(IV)- or Fe(III)-oxides are the ultimate oxidants in sedimentary systems, there exists a dynamic S-subcycle that involves a number of sulfur intermediate products, with thiosulfate appearing to be the most significant (Jørgensen, 1990). In addition, some of the HS⁻ that ultimately escapes the thiosulfate subcycle provides a usable source of reducing power for various chemolithoautotrophs, most of which gain energy from oxidizing sulfide, with O₂ as the TEAP (Reaction 9).

\[
H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+ \tag{9}
\]

**Methanogenesis**

The terminal step in the anaerobic degradation of organic material is methanogenesis (Reactions 10 and 11). As the least energetically favorable process of organic degradation, the reaction product, methane (CH₄), actually stores a major part of the energy available for aerobically respiring species, i.e., methanotrophs.

\[
CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \tag{10}
\]
\[
\Delta G^0 = -31 \text{ kJ/reaction} \]
\[
4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \tag{11}
\]
\[
\Delta G^0 = -34 \text{ kJ/reaction}
\]

On average, methanogenesis is responsible for 5–10 times less organic carbon degradation than sulfate reduction, and it is only important in sediments where a significant amount of relatively fresh organic material is delivered rapidly enough to pass through the sulfate reduction zone that it takes on any prominence (Canfield, 1993). Indeed, methane does not accumulate in sediments until more than 90% of the dissolved sulfate has been reduced (Martens and Berner, 1974). This is unsurprising given that the growth of methanogens is inhibited by SRBs, because the latter have a higher affinity for the oxidizable substrates than do the former (Lovley et al., 1982). In other words, methanogens prosper when subsurface activity of SRBs is high and pore water SO₄²⁻ becomes diminished.

The CH₄ generated by methanogens in the underlying sediment subsequently diffuses upward toward the sulfate-rich sediments, where it serves as an electron donor in the sulfate–methane transition zone (SMTZ) (Reaction 12).

\[
CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O \tag{12}
\]

In some marginal marine sites, it has been estimated that nearly 100% of the downward SO₄²⁻ flux and the upward CH₄ flux can go toward anaerobic CH₄ oxidation. Comparison of these fluxes to open ocean sites even suggests that anaerobic methane oxidation might be the dominant sink for sulfate in marine sediments relative to organic carbon oxidation (D’Hondt et al., 2002). The diffusive flux of CH₄ upward is usually inadequate to completely reduce pore water sulfate, and hence, accumulate above the SMTZ. Yet, it has been observed in some rapidly depositing shallow water sediments that methane saturates pore waters and ultimately may escape the sediment by bubble ebullition, without complete oxidation by sulfate (Martens and Klump, 1984).

**Sediment hydrogen concentrations**

As discussed above, the dominant terminal electron accepting pathways in sediments are generally segregated into distinct zones based on the potential thermodynamic yield of the various metabolic processes. Yet, on a purely thermodynamic basis, reactions yielding less energy should also take place as long as they are energetically favorable. Instead, the segregation can be more accurately explained by competition between different types of
microorganisms for electron donors, such as the fermentation products $\text{H}_2$ and acetate (Lovley and Klug, 1986). Accordingly, microorganisms utilizing electropositive TEAs effectively maintain $\text{H}_2$ concentrations below the capabilities of microorganisms using TEAs that yield less energy, and it is only when the electropositive TEA becomes depleted that the next most electropositive TEA controls the $\text{H}_2$ concentrations in the pore water system (Lovley and Goodwin, 1988). In pore waters, this translates into distinct $\text{H}_2$ concentration gradients for each particular metabolism and a threshold level below which $\text{H}_2$ cannot be further metabolized. When nitrate and manganese reductions are the dominant terminal electron accepting pathways, pore water hydrogen concentrations are extremely low ($<0.05$ nM), with increasing levels through iron reduction (0.2 nM), sulfate reduction (1–1.5 nM), and methanogenesis (7–10 nM). Acetate concentrations follow a similar pattern (Chapelle and Lovley, 1992).

This pattern of $\text{H}_2$ and acetate competition can be ascribed to the physiological capabilities of the microorganisms growing in the sediments. Microorganisms that use amorphous ferric hydroxide as their TEA can metabolize $\text{H}_2$ or acetate to concentrations lower than those that can be utilized by sulfate reducers, and the sulfate reducers can metabolize the same substrates to concentrations below those usable by methanogens (Lovley and Phillips, 1987). These findings are consistent with observations of sediments where sulfate is not reduced, and methane is not produced until the reducible ferric iron minerals have been converted to $\text{Fe(II)}$. Given the pattern above, it is not surprising then that the availability of $\text{NO}_3^-$ or $\text{Mn}$ (IV) oxides diminishes the magnitude of $\text{Fe(III)}$ reduction in suboxic sediment, and under such conditions, some of the $\text{Fe(III)}$ reducers may even switch over to nitrate or manganese reduction when $\text{H}_2$ becomes limiting for them under their normal mode of growth (Lovley and Phillips, 1988; diChristina, 1992).

**Macroscopic biological influence on diagenesis**

On the seafloor, recently deposited sediment is subject to grazing, burrowing, and particle reworking by relatively large animals moving over the surface of the sediment and the more numerous small organisms and microorganisms living within the upper layers of sediment. This influence may extend several decimeters (and even meters) below the sediment–water interface, but their effect is largely concentrated in the uppermost 20 cm. The benthos are typically inhibited when (1) the particulate organic supply is too low; (2) when the sediment is too fine-grained and compacted to provide sufficient space for them to live, (3) when $\text{O}_2$ levels reach values $<0.2$ mg l$^{-1}$, and (4) when strong ebb and current flows quickly redistribute sediment for burrows to become established (e.g., Van Cappellen and Gaillard, 1996).

Burrowing organisms biomechanically and geochemically alter sediments—a process generally referred to as bioturbation—in spatially intricate ways. Biomechanical modification includes the redistribution of grain sizes and the modification of grain size and sediment cohesiveness. It also impacts on the organic properties of the sediment because grazing causes greater disintegration of intact organic remains, leading to higher surface areas for microbial oxidation, while burrowing mixes fresh organic matter with older, more decomposed materials, causing enhanced degradation of the latter. Ingestion of refractory organic carbon may lead to excretion of more labile fecal pellets (Lee, 1992). The style of biomechanical deformation can be variable depending on the intrusive process employed by the burrowing animal. In clastic sediments, the redistribution of sediment grain sizes strongly influences the arrangement of permeability pathways, thereby exerting a control on the flux of pore water near, and within, preserved burrows. Through abrasion and ingestion, carbonate sediments are prone to (biogenic) grain-size reduction. This provides a more reactive medium for diagenetic fluids to react with.

The biochemical modification of sediment is the result of local compositional heterogeneity imposed by biogenic structures. This includes an increase in the surface area at the sediment–water interface, which in turn, increases the rate and amount of chemical exchange between the sediment, the pore water, and the water column. For instance, irrigation of worm burrow tubes with oxygenated seawater results in increased downward and lateral diffusion of $\text{O}_2$ into surrounding sediment and concomitantly the loss of sulfur from the sediment by oxidation of pyrite and enhanced diffusive loss of $\text{HS}^-$ (Berner and Westrich, 1985). Subsurface flushing with oxygenated waters also influences nitrification, $\text{Mn(II)}$- and $\text{Fe(II)}$-oxidation patterns. In the case of iron cycling, as long as ferric iron persists, phosphate remains adsorbed to the oxide surface and is thus unavailable to the pore waters. Meanwhile, the incorporation of concentrated (and typically labile) organic material in the form of mucous or feces not only provides an oxidizable substrate for the microbial communities, but also leads to concentration of metals in, and adjacent to, burrow linings as sorbed cations or as metal oxyhydroxide, sulfide, or phosphate coatings (Over, 1990). These metals may then serve as nucleation sites for authigenic mineral formation, which provides a variable chemical stock for diagenetic fluids to interact with (i.e., dolomitization, cementation).

Collectively, the imposition of burrows at the sediment–water interface perturbs the generally accepted geochemical zonations within (subaqueous) sediments, proffering a complex distribution of geochemical zones (Aller, 1980).

**Summary**

Microorganisms are integral to the chemical and physical changes sediment undergoes during the process of diagenesis. Through various chemoheterotrophic pathways, microorganisms are ultimately responsible for the
conversion of organic carbon to CO$_2$ (or CH$_4$) through the coupled reduction of dissolved (O$_2$, NO$_3^-$, SO$_4^{2-}$, CO$_2$) or Fe(III)/Mn(IV) oxyhydroxides in the sediment. The inorganic by-products of chemoheterotrophy (HCO$_3^-$, Mn$^{2+}$, Fe$^{2+}$, NH$_4^+$, NO$_3^-$, HS$^-$, HPO$_4^{2-}$, CH$_4$) are either transported from deeper sediment toward the seafloor, where residing chemolithoautotrophic bacteria use them as reactants in their metabolism, or their presence in pore waters may trigger secondary mineral precipitation and cement formation. Macrofauna also contribute to diagenetic reactions through their bioturbating activity. This includes the direct effects of burrowing and mixing of fresh sediment with older sediment, grazing and disintegration of intact organic remains, and the excretion of labile fecal pellets. Indirectly, they influence the solid-phase and pore water properties of sediment by increasing the transport of redox-active elements across the sediment–water interface.

Bibliography


Cross-references

- Aerobic Metabolism
- Anaerobic Oxidation of Methane with Sulfate
- Biogeochemical Cycles
- Carbon (Organic, Cycling)
- Carbon (Organic, Degradation)
- Cold Seeps
- Deep Biosphere of Sediments
- Deep Biosphere of the Oceanic Deep Sea
- Fe(III)-Reducing Prokaryotes
- Hydrogen
- Iron Sulfide Formation
- Methane Oxidation (Aerobic)
- Methanogens
- Microbial Degradation
- Pore Waters
- Siderite
- Sulfate-Reducing Bacteria
- Sulfur Cycle

SELENIUM

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Definition

Selenium, element 34, is a metalloid in row VIB of the periodic table. The main oxidation states are selenide (−2), elemental selenium (0), selenite (+4), and selenate (+6). Selenite and selenate are found in the oxic zone, while Se(0) is more abundant in the anoxic zone. Although hydrogen selenide may be produced by microorganisms, it is highly toxic. Se(II) is more often found as organoselenium in the form of selenoproteins. The common methylated species (e.g., dimethylselenide, dimethylselenide) are volatile. Selenium can be a substitute for sulfur and is often found as metal selenide (e.g., Clausthalite, Crookesite). Selenium occurs in eight stable isotopes, the most common of which are Se80 (~50%) and Se78 (~24%), and nine radioactive isotopes.

The microbial oxidation of selenium is a slow process, whereas the dissimilatory reduction of selenite via selenate to Se(0) is rapid (Dowdle and Oremland, 1998). Selenate and selenite may also methylate, and Se(0) can be further reduced to selenide (HSe⁻¹). Se(0) is insoluble, and bacteria form “nanospheres” of roughly uniform diameter (0.2–0.3 μm) (Oremland et al., 2004). These nanospheres can occur inside the cytoplasm or on the outside of the cell wall and may exhibit measurable isotope fractionation (80Se/78Se of ~9.0% to ~14.0%) (Herbel et al., 2000). Selenium may be assimilated via the sulfur assimilation pathway.

Selenium is an essential element primarily needed for the synthesis of selenocysteine and selenomethionine (as reviewed in Stolz et al., 2006). Selenocysteine (Sec, U) is the 21st amino acid (encoded by UGA) and is found in a number of enzymes (e.g., glycine reductase, formate dehydrogenase). Sec requires four specific gene products: SelA, selenocysteine synthase; SelB, a special translation factor; SelC, a Sec-specific tRNA (tRNAsec); and SelD, the selenophosphate synthase. SelB recognizes the alternative coding of UGA for selenocysteine aided by a stem loop structure in the mRNA. The selenocysteine insertion sequence, or SECIS, is found within the 3′ untranslated region of the mRNA in archaea and eukaryotes and immediately downstream of the UGA codon in bacteria.

Over 20 species of selenate respiring prokaryotes are known and include members of the Crenarchaeota, low and high G + C Gram-positive bacteria, Halanaerobacter, and beta-, gamma-, and epsilon-proteobacteria; however, only a few species respire selenite. The respiratory selenate reductase (Ser) from *Thauera selenatis* is a heterotrimer (SerA, SerB, SerC) and belongs to the
DMSO reductase family of molybdenum enzymes. Two different methyltransferases have been identified. Bacterial thiopurine methyltransferase converts selenite and selenocysteine to dimethylselenide and dimethylselenenide. MmtA (the calicheamicin-like methyltransferase) produces dimethylselenide and dimethylselenenide from selenite and selenocysteine (as reviewed in Stolz et al., 2006).

Bibliography


Significance of shales for geobiology

Although still understudied when compared to sandstones and carbonates (Schieber and Zimmerle, 1998), shales nonetheless are geologically important for multiple reasons. They constitute by volume two thirds of the sedimentary rock record and once examined systematically will contribute much to a better understanding of Earth history and the evolution of life. Because overall they accumulate slower than sandstones or carbonates, as much as 90% of geologic time recorded in sedimentary rocks may be preserved in shales.

Shales are an excellent matrix for fossil preservation, and contained fossils are comparatively easy to extract for study. Because their low permeability limits chemical exchange and access by oxygen, metastable skeletal minerals (e.g., aragonite) and even soft tissues may be preserved. It is not by accident that many of the world’s most scientifically significant sites of fossil preservation (Fossil Lagerstätten; Seilacher et al., 1985) are found in shale successions. Notable examples are the Cambrian Burgess Shale (Morris, 1998), the Devonian Hunsrück Slate (Bartels et al., 1998), the Jurassic Posidonia Shale (Kauffmann, 1978), and the Messel oil shale deposit (Wuttke, 1983). Preservation of fossils carries much valuable information, particularly in the case of marine shales. Fossils are useful because taphonomy (the study of organismal decay over time), spatial distribution of fossils, fossil diagenesis, morphological attributes of fossils...
(e.g., reflecting adaptation to substrate conditions), fossil assemblages and comparison to modern counterparts, and paleocommunities (paleoecology) all can provide a wealth of information about the deposition of a shale succession (e.g., Allison, 1988; Brett and Allison, 1998).

Freshly deposited muds, the sediments destined to become shales after compaction, have an enormous internal surface area. Depending on the assumptions about particle geometry and water content, one can readily estimate that a liter of watery mud should have an internal surface area on the order of several hundred square meters (e.g., Fenchel, 1970). That is enough space to provide habitat for trillions \(10^{12}\) of microbial cells (Figure 1).

**Deposition of shales**

The sedimentology of shales has made progress over the past decades (Potter et al., 2005), and sedimentologists are gradually learning to appreciate the utility of the many small scale sedimentary structures observable in shales (e.g., Schieber, 1989, 1990a,b, 1999a; O’Brien and Slatt, 1990). When studied in polished slabs and petrographic thin sections, a wealth of information about depositional conditions and history can be gleaned from most shales (Figure 2).

Laminae are the most typically observed sedimentary feature in shales. They show a large range in thickness and lamination styles (even, discontinuous, lenticular, wrinkled etc.), and these may represent conditions that include quiet settling, sculpting of the sediment surface by bottom currents, and growth of microbial mats respectively (Schieber, 1986, 1999b). Internal lamina features, e.g., grading (a), random clay orientation (b), preferred clay orientation (c), sharp basal contacts (d), and sharp top contacts (e) may be interpreted as indicative of (a) event-sedimentation (e.g., floods, storms, turbidites), (b) flocculation or sediment trapping by microbial mats, (c) settling from dilute suspension, (d) current flow and erosion prior to deposition, and (e) current flow and erosion/working after deposition (Schieber, 1990a,b). Due to somewhat larger grain size, silt laminae are the most readily observed lamina type in shales and may imply

![Shales, Figure 1](image1.png)

**Shales, Figure 1** (a) TEM micrograph of modern mud from the continental slope off northern California at a burial depth of 5 cm. Illustrates the spatial relationships of a colony of bacteria (bc), its extracellular polymeric substances (ep), and the surrounding sediment matrix of clay (c) and organic matter (o). Scale bar represents 1 µm. (b) TEM micrograph of a modern mud from Eckernförde Bay, Germany. Shows the relationships between bacteria (b), their extracellular polymeric substances (ep), and the surrounding sediment matrix of clay (c), and organic matter (o). Scale bar represents 0.5 µm. (From Ransom et al., 1999.)

![Shales, Figure 2](image2.png)

**Shales, Figure 2** Line drawing that summarizes features observed in a mudstone from the late Devonian Sonyea Group in New York. (From Schieber 1999a.) M = mica; Q = quartz; py = pyrite.
somewhat more energetic conditions. Examples are density currents (grading, fading ripples), storm reworking and transport (graded rhythmites), wave winnowing (fine even laminae with scoured bases), and bottom currents (silt layers with sharp bottom and top). Gradual compositional changes between, e.g., clay and silt dominated laminae are another commonly observed feature, and are suggestive of continuous (although slow) deposition, possibly from deltaic sediment plumes and shifting nepheloid flows.

For many years conventional wisdom has held that parallel laminae in shales are the result of settling from slow moving or still suspensions, and the common assumption was that this reflects distal deposition in comparatively deep water with only minor current activity. Recent flume experiments, however, have demonstrated conclusively that muds can be deposited from swift moving currents that are competent enough to move sand in bedload (Schieber et al., 2007). This occurs because muds have a strong tendency to flocculate, regardless of exact particle mineralogy and water composition. The floccules travel in bedload and form migrating ripples (Figure 3) that build up a contiguous mud bed if sediment supply is sufficient. In spite of accumulation via lateral accretion, after compaction these muds have a parallel laminated appearance, just like many shales in the rock record (Figure 4; Schieber et al., 2007). Obviously, it will be difficult from now on to ascribe a quiet, deep water setting to a laminated shale without corroborating evidence (e.g., paleoecology, trace fossils).

A variety of other small scale sedimentary features also occurs in shales, including mudcracks, load casts, flame structures, dewatering structures, graded rhythmites (Reineck and Singh, 1980), cross-lamination, loop structures (Cole and Picard, 1975), bioturbation (Wetzel and Uchmann, 1998), fossil concentrations and lags, all of which carry information about conditions of sedimentation. Among the more subtle sedimentary features are clay-filled mud cracks, brecciation due to desiccation, and sands or conglomerates that consist entirely of shale particles (Schieber, 1985). The latter can, for example, form as a result of soil erosion (pedogenic particles; Nanson et al., 1986; Rust and Nanson, 1989), erosion of cracked mud crusts, and submarine scouring of mud substrates by strong currents.

Biologic agents may produce microbial laminae and protection of mud surfaces from erosion (e.g., Schieber, 1986; O’Brien, 1990), or may manifest themselves as bioturbation and via destruction of primary fabrics (Wetzel and Uchmann, 1998). Infaunal activity that took place early in depositional history, when the muds had a high water content, may be exceedingly subtle and hard to detect after compaction (Schieber, 2003). In many instances of bioturbation sufficient proportions of primary features survive and can still be interpreted in terms of depositional processes. Careful examination of bioturbation features can provide additional information about substrate firmness, event deposits (escape traces), and rates of deposition. Fecal pellets and pelletal fabrics are another by-product of organic activity, and they are probably more abundant in mudstones and shales than commonly recognized (Pryor, 1975; Potter et al., 1980; Cuomo and Rhoads, 1987). Best seen in thin section, pellets typically differ from the shale matrix with respect to texture, color, and organic content. They range in size from 0.2–2 mm, are generally of elliptical outline, and

Shales, Figure 3 Migrating ripples of flocculated kaolinite in seawater, photographed through the bottom of a flume. Width of flume channel is 25 cm, flow direction indicated by red arrow. (From Schieber et al., 2007.) The ribbed “tails” behind the ripples are the eroded remains of foreset laminae.

Shales, Figure 4 (a) Parallel-laminated black shale, New Albany Shale, Devonian, Indiana. Lighter laminae are silt enriched (the steeply inclined and slightly curved lines are saw marks). (b) A sample from the same core interval as seen in (a). In the center, we see inclined (to the left) truncated laminae, forming the outline of a compacted mud-dominated ripple. (c) Tracing of silt laminae visible in (b). Arrow marks an internal erosion surface. This is a fossil equivalent of migrating mud ripples observed in flume experiments. (From Schieber et al., 2007.)
flattened by compaction. If matrix and pellets are similar in composition and composed of particles of similar grain size, pellet identification can be challenging. Pellets of benthic versus planktonic organisms may be differentiated on the basis of composition (Cuomo and Bartholomew, 1994). Schieber, 1994).

Early diagenesis of shales

It is due to the abundance of endosedimentary microbes (Figure 1) that surficial muds are very active sediments from a geochemical perspective. Much of the initially buried organic material is remineralized in these water-rich sediments (80–90% initial water content), prompting the description of surficial muds as “fluidized bed reactors” (Aller, 1998). The microbial breakdown of buried organic matter requires terminal electron acceptors (oxidizing agents) and due to limitations by diffusion, overall abundance, and differences in energy yield for the various oxidation reactions, we observe a systematic exhaustion of available electron acceptors (e.g., oxygen, nitrate, iron (III), manganese (IV), sulfate, carbon dioxide) as we move downwards from the sediment-water interface. For these reasons closely comparable microbial decay profiles (Brett and Allison, 1998; Curtis et al., 2000; Allison, 1988). For others that are not, associated pH shifts can nonetheless be instrumental for their precipitation. For example, early diagenetic silica (quartz) precipitation is not uncommon in shales (Figure 6). In Phanerozoic shales it is derived from the dissolution of biogenic opal (radiolarian, diatoms, sponge spicules) and fills pore spaces that range from tens of microns to as much as a millimeter in size (Figure 7). Quite frequently these pore fills (in situ quartz) are mistaken for detrital quartz (Schieber, 1996; Schieber et al., 2000).

If phosphorus is abundant in the initial mud, such as due to accumulation of phosphatic skeletal decay profiles, phosphatic concretions, ranging in size from less than a mm to more than 100 mm³, may form (Figure 8). An early diagenetic phosphate matrix seems to be an excellent medium for preserving minute details of soft tissues, such as in the Neoproterozoic Doushantuo Formation of China (Shen et al., 2000).

Due to the rapid and systematic expulsion of pore waters and decrease of porosity during compaction, early diagenetic minerals in shales are preferentially preserved. For this reason, shales contain a rich record of geochemical processes that relate closely to the chemistry of the overlying ocean waters and paleoceanographic conditions. When studied systematically, the authigenic component of shales holds the promise to capture a snapshot of seawater and/or porewater chemistry. With the necessary age constraints, this may enable us to develop a much more detailed record of ocean geochemical changes than currently available.

Conclusion

Shales result from the complex interplay of a range of geologic variables and processes, and there are no easy answers and no “one size fits all” models. Although these rocks have an (undeserved) reputation for being drab, uniform, and uninteresting, they repay the investigative challenges they pose by being a rich storehouse of information about the geologic past. Sedimentological study includes recognition and tracing of facies changes, sedimentary features, shale fabrics, erosion surfaces and internal stratigraphy, as well as information that can be derived from interbedded non-shale lithologies. Paleontological studies may provide data on paleo-oxygenation, primary production, substrate conditions, paleocurrents, bathymetry, and paleosalinity (Schieber et al., 1998). Petrographic investigations provide the basic inventory of shale constituents, and may yield clues about provenance, compaction history, chemical conditions, sedimentary processes (via small scale sedimentary structures), and the origin and maturation of organic matter (Taylor et al., 1998). Chemical analysis of major and trace elements can provide information on provenance (Roser and Korsch, 1986), and a range of proxies for paleo-oxygenation (Jones and Manning, 1994). Organic geochemistry can furnish information on the source of organic matter, its dispersal,
and maturation (Engel and Macko, 1993). Certain organic molecules, such as photosynthetic pigments, may survive as "carbon skeletons" (biomarkers), and may identify the original source of diagenetically altered organic matter and potential indicators of water column anoxia (Brassell, 1992).

Fundamentally, shales do not give up their secrets easily. The tools are at hand, however, to extract a wide range of data from them and allow for multiple avenues of inquiry (Schieber et al., 1998). Interpretations that rely on just one perspective (e.g., petrography, fabric study, trace element geochemistry, or organic geochemistry) typically do not match conclusions derived from a different line of inquiry. Shales need to be investigated in a multidisciplinary way and at multiple scales because of the complex interplay of variables that produces them. Conclusions derived from microscopic features must be in agreement with insights coming out of basin scale studies, as well as with findings from all scales in between.

**Shales, Figure 6** SEM photomicrograph (backscatter image) of a pore space in the Devonian New Albany Shale (Indiana, USA), illustrating suboxic to anoxic diagenesis. Light gray mineral an is ankerite (Fe-Mg-Ca-carbonate) that initially grew in the interior of a *Tasmanites* cyst (dark black line), a locally reducing and anoxic microenvironment. Very early growth is indicated by the uncompacted *Tasmanites* wall and the overall circular growth of carbonate (an + mc) cement. Outside of the protected microenvironment of the cyst we see growth of manganan calcite (mc, darker gray), indicating suboxic conditions in the sediment and oxygenated bottom waters. (From Calvert et al., 1996.) The carbonate cement is surrounded by fine crystalline quartz (qu), derived from dissolution of radiolarian opal. (From Schieber et al., 2000.) The image suggests that pore waters were initially alkaline, allowing for precipitation of carbonates and dissolution of biogenic opal, and that this was followed by a lowering of pH that led to precipitation of quartz. This image illustrates the level of insight one can derive from careful petrographic study of early diagenetic cements.

**Shales, Figure 7** Authigenic quartz (chalcedony) in the late Devonian Chattanooga Shale (Kentucky, USA). (a) Photomicrograph in transmitted light. The clear/bright grains are chalcedonic infills of algal cysts (*Tasmanites*). (b) SEM image (backscatter) from the same specimen, showing more clearly the rounded-lobate outlines of the quartz grains and the enclosing cyst walls (black rim). For a detailed discussion of these features see Schieber (1996) and Schieber et al. (2000).

**Shales, Figure 8** SEM photomicrograph (backscatter image) of an example of early diagenetic phosphate precipitation. The image shows what appears to be a colony of phosphate encrusted bacteria (Mid-Proterozoic Bijaigarh Shale, India).
SHEWANELLA

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Classification
Bacteria – Proteobacteria – Gammaproteobacteria – Alteromonadales – Shewanellaceae (Ivanova et al., 2004), fam. nov. – Shewanella (MacDonell and Colwell, 1985), gen. nov.

Definition
Assumed as one of the first metabolic processes of ancient microbial life (prior to oxygen, nitrate, and sulfate respiration), microbial iron reduction is still of particular interest in recent geobiological systems. The efficiency of iron reducing microorganisms in utilizing structural or crystalline solid-phase Fe(III) oxide minerals as electron acceptors with respect to nutrient availability and pH is still matter of discussion; this type of metabolism, however, is an ubiquitous phenomenon, applied by a wide range of species throughout the Archaea and Bacteria (Weber et al., 2006 and references therein). Members of the family Geobacteraceae and the genus Shewanella are among the most significant and common studied organisms with regard to dissimilatory iron reduction and oxidation of organic matter in soils, sediments, and groundwater.

According to the surface complexation model (Dzombak and Morel, 1990), Warren and Ferris (1998) report on an equilibrium between ferric iron sorption and precipitation reactions on bacterial surfaces, emphasizing iron-reducing bacteria like Shewanella as highly reactive key players in pristine and contaminated waters (Pedersen and Albinsson, 1991).

Actually including 50 recognized species (List of prokaryotic names with standing in nomenclature: http://www.bacterio.cict.fr/), the genus Shewanella features Gram-negative, facultatively anaerobic, dissimilatory metal-reducing, rod-shaped organisms. Due to their highly diverse physiology, they inhabit a wide range of reduct-stratified environments varying in nutrient composition, salinity, temperature, redox potential, and barometric pressure (marine and freshwater, surface, ground and deep waters, sediment, mud and soils), but also comprise symbiotic, epibiotic, and pathogenic species (Venkateswaran et al., 1998; Hau and Gralnick, 2007 and references therein). Under anaerobic conditions, most Shewanella species can produce hydrogen sulfide when grown on thiosulfate or polysulfide, and also can grow on lactate, pyruvate, formate, and on amino acids (Nealson and Scott, 2006 and references therein). For respiratory growth, they can use oxygen as well as a variety of different terminal electron acceptors, including nitrate, elemental sulfur, fumarate, dimethyl sulfoxide, and metals like Mn(IV), Fe(III), As(V), Cr(VI), Co(III), Te(VI), and U(VI) (Nealson and Myers, 1992; Haas et al., 2001; Guha et al., 2003; Saltikov et al., 2003). As diffusion across the cell wall is not possible for metals in their solid form, metal-reducing Shewanella species specifically interact with the mineral surface to facilitate the electron transfer process by active cell adhesion in combination with a unique shuttle-system of proteins reaching from the cytoplasm to the outer membrane (Lower, 2001; Korenevsky and Beveridge, 2007; Fredrickson et al., 2008 and references therein).

Near-surface habitats may get limited in oxygen but not in minerals like iron oxyhydroxides (ferrihydrite, goethite, and hematite), in large part consisting of Fe(III) (Uruttia et al., 1998). In case of dissimilatory metal-reducing bacteria like Shewanella, Fe(III) is reductively dissolved during oxidation of carbon substrates, affecting processes like the biogeochemical cycle of iron and phosphorus, the oxidation of natural and anthropogenic carbon sources, and
biocorrosion. The respiratory reduction of metals turns these organisms to key players in groundwater ecology, as they contribute to the mobilization of occasionally harmful metals (e.g., arsenic) from the solid to the soluble phase (Malasam et al., 2008).

Bibliography


Cross-references
Banded Iron Formations
Fe(III)-Reducing Prokaryotes
Microbial Biomineralization
Microbial-Metal Binding

SIDERITE

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Siderite is an iron carbonate mineral (FeCO₃, 48% Fe) belonging to the calcite group (hexagonal crystal system). Magnesium and manganese may substitute for the iron. Siderite crystals are yellow to dark brown in color, rhombohedral in shape, and with curved and striated faces, and also occur in masses. Siderite is commonly found in bedded sedimentary iron ores, including banded iron formations (BIFs, see entry Banded Iron Formations), and in hydrothermal veins. It is also a common diagenetic mineral, forming at shallow burial depths in shales and sandstones where it tends to form concretions. The diagenetic formation of siderite strongly depends on the availability of Fe⁺ ions in pore waters (see entry Pore Waters) and is thus a phenomenon of suboxic to anoxic environments (Berner, 1971). Diagenetic siderite precipitation has been linked to microbially mediated reactions, specifically to the release of ferrous (Fe²⁺) iron due to dissimilatory iron reduction performed by Fe³⁺-reducing prokaryotes (see entry Fe(III)-Reducing Prokaryotes) such as *Shewanella* (Roh et al., 2003; Hansel et al., 2003; see entry *Shewanella*). Microbially assisted siderite precipitation appears to preferentially occur in environments where the rate of bacterial iron reduction exceeds the rate of sulfate reduction performed by sulfate-reducing bacteria (Py et al., 2006; see entry Sulfate-Reducing Bacteria). In sulfidogenic sediments, iron is largely precipitated as iron sulfide rather than as siderite (see entry “Iron Sulfide Formation”).

Bibliography


Cross-references
Banded Iron Formations
Carbonates
Fe(III)-Reducing Prokaryotes
Iron Sulfide Formation
Pore Waters
Shewanella
Sulfate-Reducing Bacteria

SIDEROPHORES

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Definition
Siderophores are low molecular weight iron-specific organic ligands that are exuded by iron-limited organisms as part of a high-affinity iron acquisition strategy.

Introduction
Iron is a nutrient to almost all known organisms. Even though iron is the fourth most abundant element on earth, the acquisition of this nutrient poses a serious challenge to organisms in many natural environments. A particularly iron-depleted system is the photic zone of the marine water column (Kraemer et al., 2005). Here, soluble iron is removed by biological uptake and subsequent sinking of the biomass below the mixed zone. In ocean areas with particularly low iron concentrations relative to other nutrients in the photic zone, the primary productivity is limited by the low bioavailability of iron. This marine iron limitation may affect the global climate by limiting the efficiency of the marine carbon pump. However, even in soils that contain abundant iron-bearing mineral phases, iron acquisition poses a challenge to microbial and plant life. Indeed, the low solubility and dissolution rates of iron-bearing minerals in calcareous soils are responsible for large annual agricultural losses by plant iron limitation (iron chlorosis) (Kraemer et al., 2006).

The ability of organisms to scavenge iron from their surroundings is prerequisite to controlling intracellular iron concentrations within the physiologically required range (homeostasis). An efficient mechanism of iron uptake involves the exudation of siderophores that are designed to bind iron(III) in the extracellular space with a sufficient affinity and specificity to ensure the formation of Fe(III)-siderophore complexes even in the presence of high concentrations of other competing cations and in the appropriate pH range. This high-affinity iron uptake strategy is used by bacteria (including cyanobacteria) in marine, freshwater, and terrestrial environments, by fungi and some plant species. Other iron acquisition mechanisms include the acidification of the environment, the reduction of Fe(III) coupled to Fe(II) uptake, or the secretion of other ligands (e.g., citrate) with lower affinity for iron. High-affinity iron uptake involving siderophores is generally the most efficient system in environments with extremely low iron availability.

Siderophore structure and properties
Siderophores are not defined by their chemical structure, but by their biological function. Several hundreds of distinct and structurally diverse siderophores are known. Siderophores are low molecular mass (<1,500 Dalton) organic ligands, typically chelating iron in hexadentate octahedral coordination, although siderophores with lower denticity are known. Iron is coordinated by hard ligating groups including hydroxamate, catecholate, carboxylate, and hydroxycarboxylate groups (Figure 1). The nature of the ligating groups and the optimization of the overall siderophore structure are responsible for their outstanding affinity for iron. For example, desferrioxamine B (DFO-B), a siderophore produced by the soil bacterium Streptomyces pilosus forms an iron complex with a 1:1 stability constant logK of 32.02 (Martell et al., 2001). The affinity of microbial siderophores for iron binding is much higher than for competing ions that are often found in high concentrations in the extracellular environment. For example, DFO-B does not, to measurable extent, coordinate with calcium in seawater or in pore water of calcareous soils (Kraemer et al., 2005, 2006). Most siderophores excreted by terrestrial organisms are highly water soluble, allowing the diffusion of siderophores toward insoluble iron sources. This is prerequisite to their function as an iron shuttle overcoming the necessity for the organisms to directly contact the iron source (Kraemer et al., 2006). However, amphiphilic siderophores possessing fatty acid tails have been isolated from cultures of distinct genera of marine bacteria (Martinez and Butler, 2007) and pathogenic bacteria. The inhibition of ligand diffusion away from individual bacteria by anchoring of amphiphilic siderophores in the plasma membrane seems to be advantageous in marine systems where bacterial abundances are low.

Graminaceous plant species, including agriculturally important crops, such as barley, wheat, and corn, are the only plant species known to possess a high-affinity iron uptake system. They exude a distinct class of iron-specific ligands with aminocarboxylate and hydroxycarboxylate functional groups, the so-called phytosiderophores or
mugineic acids (Takagi, 1976; Takagi et al., 1984) (Figure 1).

**Uptake of Fe(III)-siderophore complexes**
Gram-negative bacteria bind siderophore complexes by a receptor in the outer membrane followed by shuttling to the cytoplasmic membrane. Gram-positive bacteria have a receptor anchored to the cytoplasmic membrane. The complexes are then delivered to the cytoplasm by a transmembrane protein (ABC-transporter) (Andrews et al., 2003). Similarly, graminaceous plants possess a high-affinity uptake system specific for iron(III)-phytosiderophore complexes (Curie et al., 2001). Some microorganisms are known to be endowed with uptake systems for iron complexes of siderophores that are not produced by the same organism.

The intracellular release of iron from internalized siderophores is promoted by either hydrolysis of the siderophore or reduction of ferric iron to ferrous iron followed by competitive sequestration of the iron (Miethke and Marahiel, 2007). The biosynthesis and exudation of siderophores as well as the expression of their uptake systems are highly regulated as a response to iron limitation (Hantke, 2001).

**Siderophore concentrations in natural environments**
The exudation of siderophores is a costly strategy in terms of cellular nutrient and energy budgets, particularly in systems with low bacterial densities such as oligotrophic marine systems (Völker and Wolf-Gladrow, 1999). Therefore, it is not surprising that observed siderophore concentration in the environment do not exceed iron concentrations required for efficient uptake. Powell et al. (1980) have estimated hydroxamate siderophore concentrations in soil solutions to be between 10 and 100 nM. Essen et al. (2006) found up to 2 and 12 nM of the hydroxamate siderophores ferrichrome and ferricrocin, respectively, in soil solutions of podzolic forest soils. However, the quantitative analysis of siderophores in natural waters remains challenging due to their structural diversity and low concentrations. For example, siderophores have not been detected unambiguously in ocean water even though iron complexes with conditional stability constants comparable to siderophore complexes in the nanomolar and subnanomolar concentration range have been observed by voltammetric techniques (Rue and Bruland, 1995). Römheld (1991) has estimated that phytosiderophore concentrations in the rhizosphere can reach local concentrations of up to 1 mM in the soil solution.

**Sources of iron**
The soluble iron concentrations in oxic soils or surface waters are limited by the solubility of iron oxides, iron hydroxides, or hydrous ferric oxide. In the neutral to slightly alkaline pH range corresponding to the pH of calcareous soil solutions and ocean water, their solubility reaches a minimum below 1 nM (Liu and Millero, 2002). The presence of iron complexing organic ligands can increase the iron solubility. Thus, iron complexes with low molecular weight organic ligands, fulvic and humic acids, as well as iron-bearing mineral phases (including...
primary silicates and pedogenic iron oxides) are the most important iron sources for high-affinity uptake systems (Kraemer, 2004). Prerequisite for iron sequestration from these sources are the superior affinity of siderophores for iron compared to the stability of the iron complexes or minerals that serve as iron source and reasonably fast rates of the associated ligand exchange or dissolution reactions.

Siderophore promoted dissolution of iron oxides and iron-bearing silicates

Siderophores promote iron sequestration from iron oxides (Kraemer et al., 1999) and iron-bearing primary (e.g., hornblende [Liermann et al., 2000]) and secondary silicates [e.g., kaolinite (Rosenberg and Maurice, 2003)]. The effect of siderophores on iron dissolution rests on their ability to increase the solubility of iron and to accelerate dissolution rates. It has been shown that bacterial and plant siderophores promote iron oxide dissolution in the dark by a ligand-controlled dissolution mechanism, where the rate of dissolution is a linear function of the adsorbed siderophore concentration (Kraemer et al., 1999; Reichard et al., 2005). This mechanism is important in high-affinity iron acquisition by graminaceous plants. Considering the low concentrations of microbial siderophores observed in natural systems, their main function seems to be to increase solubility of iron oxides, generating thermodynamic driving force for other dissolution mechanisms (Cheah et al., 2003; Reichard et al., 2007).

Photochemistry of siderophore complexes

Soluble iron complexes of siderophores possessing γ-hydroxy-carboxylate ligating groups photolyze in seawater under irradiation by sunlight, resulting in oxidation of the ligand and formation of transient Fe(II) that is rapidly reoxidized (Barbeau, 2006). Both the Fe(II) and iron bound to the oxidized ligand are bioavailable to phototrophic phytoplankton that cannot take up Fe(III)-siderophore complexes. Moreover, siderophores promote photoreductive dissolution processes that tend to be faster than ligand-promoted dissolution reactions (Borer et al., 2005). These photochemical processes may be of key importance for algal and bacterial iron acquisition in marine systems.

Formation of siderophore complexes with other metals and their uptake

A prerequisite for the efficiency of the high-affinity iron uptake system is the ability of organisms to discriminate against uptake of other (potentially deleterious) metal ions. The selectivity of the uptake system is ensured by the high specificity of siderophores for iron binding relative to most cations in solution and by the specific recognition of iron(III)-siderophore complexes by receptors at the cell surfaces. However, some exceptions to the selectivity of iron binding and uptake are known. Siderophores have a high affinity for metal ions with an equal or higher charge to radius ratio than Fe(III). For example, the siderophore DFO-B forms a Pu(IV) complex with a 1:1 stability constant that is orders of magnitude higher than the corresponding iron complex (Boukhalfa et al., 2007). Moreover, slow uptake of Pu(IV)-siderophore complexes by the high-affinity iron uptake system of Microbacterium flavescent has been observed (John et al., 2001). The formation of plutonium-siderophore complexes and their bacterial uptake illustrate that the selectivity for a metal ion other than iron does not necessarily imply that the metal ion is actively targeted by the high-affinity uptake system for the purpose of nutrition. Also, the siderophore DFO-B has a high selectivity for the trivalent manganese ion (Duckworth and Sposito, 2005). Manganese in this redox state is not commonly observed in the absence of the siderophores, but the shifted redox potential of the complex stabilizes Mn(III) under conditions that are found in natural systems. In order to appreciate the effect that siderophores may have on the geochemistry of metal ions other than iron, it is important to keep in mind the extremely low siderophore concentrations measured in natural systems (vide supra).

Uptake of phytosiderophore complexes of Fe(II), Ni(II), Zn(II), Cu(II), Mn(II), and Cd(II) by maize (Zea mays) has been observed (Schaaf et al., 2004), and it has been controversially discussed if their uptake by the high-affinity iron uptake system is regulated or fortuitous. A high-affinity uptake system also seems to promote the uptake of molybdenum and vanadium by the nitrogen fixing soil bacterium Azotobacter vinelandii (Bellenger et al., 2008). Methanotrophic bacteria use a high-affinity uptake system for copper acquisition involving the exudation of Cu(I) specific organic ligands, the so-called chalcophores. Chalcophores have a distinctly different architecture compared to siderophores involving nitrogen and sulfur as ligands (Kim et al., 2004).

Conclusions

The key role of siderophores in marine and terrestrial biological iron acquisition is well established. However, a thorough and quantitative understanding of their effect on processes involved in, e.g., global carbon cycling or crop production requires a better understanding of how siderophores influence the availability and distribution of iron among prokaryotic and eukaryotic organisms in complex ecosystems. New observations of siderophore photochemistry, their amphiphilic properties and their mineral surface chemistry as well as observations of iron speciation in natural systems are important steps in this direction. Furthermore, recent work has shown that nutrient acquisition involving the exudation of ion-specific ligands is not unique to high-affinity iron acquisition. Discoveries of other similar nutrient acquisition systems may reshape our thinking about the effect of trace metal biogeochemistry on the microbial ecology of oligotrophic natural systems and vice versa. Finally, the unique properties of siderophores and other ion-specific biogenic ligands continue to inspire technical applications in fields such as pollutant remediation or biomedicine.
Bibliography


Cross-references

Gallionella
Leptothrix
Metals, Acquisition by Marine Bacteria
Microbial Biominalization
Microbial-Metal Binding

**SILICA BIOMINERALIZATION, SPONGES**

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**Synonyms**

Biosilification, Silication

**Definition**

Biosilification: The biological formation of opal-like amorphous hydrated silica. This phenomenon occurs on a globally vast scale in a wide variety of organisms, including protists, radiolarian, foraminifera, sponges,
molluscs, brachiopods, copepods, ascidians, diatoms, and higher plants.

**Biomineralization and biosilification**

**Introduction**

The growth in geobiology and biogeochernistry has led to a number of new existing research areas where the distinctions between the biological, chemical, and earth science disciplines melt away (Weiner and Dove, 2003). Of the intriguing topics that are receiving renewed attention, the study of biomineral formation based on organic templates is one of the most fascinating topics today. During the processes of biomineralization, the organic material acts variously as nucleator, cooperative modifier, and matrix or mold for the mineral ions with respect to biominerals formation which are characterized by a hierarchical order (Bäuerlein, 2007).

Biominerals may be deposited within the organism, and within its immediate surroundings or environment, as a result of the metabolism of the living creature (Skinner, 2005). The physiological pathways by which organisms precipitate skeletal minerals and the forms and functions of the skeletons they fashion have been shaped by natural selection through geologic time, and all have constrained continuing evolution in skeleton forming clades (Knoll, 2003). The variety of biomineralizers recently reviewed by Ehrlich et al. (2008a) can best be expressed by the fact that, approximately 128,000 species of molluscs, about 800 species of corals, 15,000 species of sponges including 550 species of glass sponges, 700 species of calcareous green, red and brown algae, more than 300 species of deep-sea benthic foraminifera, and 200,000 diatom species (Mann and Droop, 1996) exist.

Precipitation of silica on organic templates could have been abiotic, in which organic matter merely serves as a template for nucleation, or biogenic, i.e., induced by biological activities or a combination of both (Mukhopadhyay et al., 2004). Silicon, in the form of silicic acid, is a fundamental nutrient for diatoms, silicoflagellates, radiolaria, and many sponges, all of which polymerize it to build skeletons of biogenic silica (Maldonado et al., 2005).

**Sponges**

Sponges (Porifera) are the most simple and ancient multicellular animals on earth and live attached to the seabed or another substratum. Sponges diverged from other animals earlier in evolutionary history than any other known animal group, extant or extinct (Reitner and Mehl, 1996), with the first sponge-related record in earth history found in 1.8 billion year old sediments (Nichols and Wörheide, 2005). The huge diversity with respect to their natural habitat is probably the reason for the estimated number of approximately 15,000 different sponge species (Hooper and van Soest, 2002). To support life, sponges pump huge amounts of seawater (170–72,000 × their own body volume per day) through their bodies (Simpson, 1984) and filtrate it to capture food particles, such as bacteria, micro algae, other unicellular organisms, or dead organic particles. The whole sponge body is designed for efficient filtration of the surrounding seawater, which is essential because of the low nutrient availability at the sea floor.

The phylum Porifera (sponges) is divided into three classes, Hexactinellida and Demospongeidae that comprise a siliceous skeleton and the Calcarea with a calcareous skeletal network (Bergquist, 1978). Significant separation of the Calcarea from the Hexactinellida/Demospongeidae (Silicispongea) clade is also implied by fundamental differences in morphology and development: unlike Silicispongea, calcareans lack morphologically distinct microscleres, and their calcitic spicules are secreted intercellularly within an organic sheath (vs. formation of silica spicules onto an intracellularly secreted axial organic filament in Silicispongea (Botting and Butterfield, 2005)). Sponges bear only a few different cell types of which the sclerocytes produce the spicules, i.e., siliceous structures, which are often needle-shaped. These spicules often form a distinct skeleton, but occasionally they are loosely distributed throughout the sponge body without identifiable order or they are lacking entirely. Sites, type, shape, and arrangement of spicules, and their skeletal arrangements are the fundamentals of current sponge systematics (Erpenbeck et al., 2006; Boury-Esnault and Rutzler, 1997).

Among the siliceous sponges most important are the monophyletic class Hexactinellida, and the polyphyletic “Lithistida,” which includes taxa belonging to different groups within the class Demospongeidae. Both classes are characterized by rigid skeletons of fused spicules.

Among the class Hexactinellida, the order Hexactinosida (subclass Hexasterophora) (Schulze, 1886), known since the late Devonian (Rigby et al., 1981, 2001), developed a rigid and, to a certain extent, firm skeleton that consists of fused hexactins. This fused, three-dimensional, generally right-angled skeletal network stays intact even after the death of the sponge.

**Hexactinellida**

Hexactinellida Schmidt (Porifera) are deep-water marine sponges defined by their production of siliceous spicules of hexactinic, triaxonic (cubic) symmetry, or shapes clearly derived from such forms by reduction of primary rays or terminal branches added to the ends of primary rays. They lack calcareous minerals and sclerified organic spongin as skeletal components. Siliceous spicules may be entirely loose, or partially fused to form a rigid basal and choanosomal framework. Their living tissues are mainly syncytial, with distinctive porous plugs joining differentiated regions of the syncytium to each other or to discrete cellular components. Flagellated-collar units are anucleate. Hexactinellids are viviparous and, from detailed study of a single species, produce distinctive trichimella larvae. Two subclasses are recognized by different microsclere forms – amphidiscs and hexasters.
Hexactinellids include more than 500 described species, 7% of all Porifera, distributed in five orders, 17 families, and 118 genera (Schulze 1886, 1904; Sollas, 1888; Okada, 1928; Reiswig, 1971, 2002; Lévi et al., 1989; Mehl, 1992; Leys, 2003).

The taxonomy of the class Hexactinellida is very incomplete in the sense that probably not even half of its species are known to science. This is obvious from the fact that many of the hexactinellid species collected during recent deep-sea expeditions are new. The spicules of most hexactinellids are larger (Figure 1) and more luxuriously architectured than those in demosponges (Tabachnick, 2002).

Demospongeae

In demosponges (Demospongeae Sollas, 1885) we have much the largest group, with 95% of all extant sponges. They are also the most diverse group. Some are freshwater but predominantly they are marine species living from the intertidal to the deepest seas, with around 15 orders, 88 families, and about 500 valid genera (Hooper and van Soest, 2002). The skeleton is composed of monaxonic or tetraxonic siliceous spicules (never triaxonic) bound together with collagen-like protein sponglin in discrete fibers or loosely aggregated and ubiquitous collagenous filaments forming the ground substance of the intercellular matrix.

Because the morphology of the spicules differs species-specifically, they are used as a major taxonomic character. However, very little is known about the molecular mechanism(s) that determine the position and arrangement of the spicules within the demosponge skeleton (Uriz et al., 2003; Uriz, 2006).

Lithistid demosponges

Like hexactinellid sponges, lithistid sponges also contain siliceous skeletons comprising spicules. These spicules (desmas) interlock, rendering the skeleton rigid and often stony (Pisera, 2000, 2003; Kelly, 2000, 2003). At present little is known about the deposition of silica within this taxonomic order.

Lithistid sponges are commonly found on tropical and temperate seamounts and continental margins down to depths of about 1,000 m (Kelly, 2000). Several Southwest Pacific species, form large cups, bowls, and plates that extend linearly from the sponge margin. Close inspection of the lamellae show irregular or concentric ridges, indicating that silica deposition is not continuous but rather variable in nature (Ellwood et al., 2007) presented detailed records of trace metals and carbon isotopes to understand siliceous spicule formation in the deep-sea lithistid sponge Corallistes undulatus Lévi and Lévi, 1983 (Demospongiae: Corallistidae). X-ray analysis of two longitudinal sections removed from the lamellae of the cup-shaped sponge revealed 144 and 137 light and dark density band-pairs, respectively, within the siliceous skeleton. Although there was some variability in the $^{32}$Si data, the overall age established using these data indicated that the sponge was between 135- and 160-year old.

Forms of the silica deposition in sponges

Spicules, skeletal frameworks and desmas are the main forms of the silica deposition in sponges as shown in Figure 2.

The history of the discoveries related to sponge spicules is well reviewed by Vosmaer and Wijisman (1905). According to these authors, after Schweigger in 1819 had demonstrated that the spicules of sponges in some cases do not consist of calcium carbonate, Grant in 1826 found them to contain silica, and Bowerbank in 1841 showed that, in addition to the silica some organic matter is present. Sollas in 1885 likewise finds that the sponge silica resembles opal. He states in general that the refractive index of sponge silica is that of opal or colloidal silica, and not of quartz. This kind of opal was proposed by Vosmayer and Wijisman to call spicopal, however, the organic matter of the spicule was called by F.E. Schulze spiculine. As to the structure of the spicules, Gray in 1835 had found them in Hyalonema glass sponge to
consist of layers, which became conspicuous by heating. These layers concentrically surround a "central canal," which is filled out, as Kölliker in 1864 has shown, by an organic mass, the axial rod. Claus in 1868 found that the silica which directly surrounds this central rod, is homogenous. He called this homogenous cylinder the axial cylinder. The sponge spicule thus consists of a central organic axis, surrounded by concentric layers of opal, the outermost of which is invested in a spicule sheath of organic matter or rather of organic matter in intimate association with silica (Vosmaer and Wijsman, 1905) (Figure 3).

Using the freshwater sponge *Ephydatia fluviatilis*, Weissenfels and Landschoff (1977) and Weissenfels (1989) demonstrated that the formation of spicules starts in sclerocytes within a specific vesicle. After the production of an axial organic filament, silicon is deposited around it and the whole process of forming a spicule (190 μm in length and 6–8 μm in diameter) is completed after 40 h, at 21°C.

Siliceous sponge spicules have traditionally been separated into two categories termed, according to their size, megascleres and microscleres (Lévi, 1973). They are highly diverse in sponges and the selection pressures responsible are difficult to envisage. There are over 12 basic types of megasclere and 25 types of microsclere reported in Demospongiae, 20 basic types of megasclere, and 24 types of microsclere in...
Hexactinellida, besides a long list of variations of the basic types (Lévi, 1973, 1993; Garrone et al., 1981; Simpson 1984, 1990; De Vos et al., 1991; Bavestrello et al., 1993; Boury-Esnault and Rutzler, 1997; Tabachnick and Reiswig, 2002).

Desmas differ from typical demosponge spicules: first they are joined by articulation; second, they always display irregularity and often complex morphology and sculpture. The most basic difference, perhaps, is in the fact that the crepis or axial filament is usually very short (or even invisible) and extends only a short distance from the spicule center. Growth ceases very rapidly, and an important part of a desma, including secondary branches as well as elements of sculptures, does not depend on the axial filament (Sollas, 1888; Lévi, 1991).

In contrast to the high diversity of spicules, there are relatively few basic types of skeletal framework in demosponges. Six elemental types of skeletons with intermediate forms can be differentiated: hymedesmoid, plumose, axial, radiate, reticulate, and arranged in strength confusion (Boury-Esnault and Rutzler, 1997).

Chemistry of sponge silica
It is established that siliceous sponge spicules of both the Hexactinellida and Demospongiae consist of hydrated amorphous silica (Simpson, 1989; Uriz et al., 2003; Uriz, 2003, 2006). Chemically, they differ only slightly in their $\text{SiO}_2: \text{H}_2\text{O}$ proportions. Desmas bearing demosponges (“Lithistida”) have a ratio of 5:1 whereas Hexactinellida have 4:1 (Hartman, 1981). Holzhüter et al. (2005) reported that spicules of demosponge Suberites domuncula consist of more that 90% silicon and oxygen. They found that at the molecular level silica of sponge spicules has following amorphous structure: $\text{SiO}_4$ tetrahedra are connected to form polyhedra, which are comparable with those found in silica gels. These polyhedra form silica particles up to 3 nm in size which are interconnected forming a dense packing containing pores. However, recently Mugnaioli et al. (2009) reported for the first time crystalline packing containing pores. In subsequent years these molecules were cloned also from other sponges, among them S. domuncula and Lubicmirskia baicalensis (as reviewed in Müller, 2003; Müller et al., 2007). The spicules are produced in special cells, the sclerocytes; that formation starts intracellularly around a silicatein filament and is completed extracellularly by apposition of silicatein onto the growing spicules (Müller et al., 2005, 2006). The silicateins are distinguished from the cathepsins by the replacement of the first amino acid residue in the catalytic triad, cysteine by serine (Shimizu et al., 1998). It has been proposed that serine increases the nucleophilicity during the nucleophilic attack at the silicon atom. The polymerization-promoting activity of the silicateins has been shown to be catalytic and not stoichiometric. It is possible that the controlled punctuated secretion of low mechanisms of silicification in sponges are proposed: enzymatic (silicatein-based) and nonenzymatic, or self-assembling (chitin- and collagen-based).

Silicatein-based silicification
The group of Morse (Shimizu et al., 1998; Cha et al., 1999; Shimizu and Morse, 2000; Weaver and Morse, 2003; Weaver et al., 2003) discovered that organic filament in demosponge Tethya aurantium is composed of a cathepsin L related enzyme, termed silicatein. They cloned two of the proposed three isoforms of silicateins, the α- and β-forms, from this demosponge (Cha et al., 1999). In subsequent years these molecules were cloned also from other sponges, among them S. domuncula and Lubicmirskia baicalensis (as reviewed in Müller, 2003; Müller et al., 2007). The spicules are produced in special cells, the sclerocytes; that formation starts intracellularly around a silicatein filament and is completed extracellularly by apposition of silicatein onto the growing spicules (Müller et al., 2005, 2006). Cathepsin L is an endopeptidase which cleaves peptide bonds with hydrophobic amino acid residues in P2 and P3 positions and occurs in lysosomes as well as extracellularly as secreted enzymes. The silicateins are distinguished from the cathepsins by the replacement of the first amino acid residue in the catalytic triad, cysteine by serine (Shimizu et al., 1998). It has been proposed that serine increases the nucleophilicity during the nucleophilic attack at the silicon atom. The polymerization-promoting activity of the silicateins has been shown to be catalytic and not stoichiometric. It is possible that the controlled punctuated secretion of low
Silica Biomineralization, Sponges, Figure 5 The chemical mechanism proposed by Fairhead et al. (2008) to operate for the polymerization of silicic acid using silicateine molecules. The authors proposed H163 deprotonates Si(OH)₄: the extensive water network could permit proton shuttling. They illustrated one possibility.

concentrations of monomeric silicateins, pulses in the transitory flux of silica-precursor molecules, oscillations in the pH, ionic, or other conditions of the condensation environment, or a combination of these factors may be responsible for the annular patterning and continued deposition of silica in spicule biosynthesis in vivo once the axial filament has become completely covered during the early stages of silica deposition around this proteinaceous core. Alternatively, the continued growth of silica may be a result of dangling bonds at the growth surface of the newly deposited silica (Saito et al., 1995).

There are two possible mechanisms for enzyme catalysis (Fairhead et al., 2008): (1) stabilize at the active site one molecule of deprotonated silicic acid (the nucleophile) which will then react with another molecule of silicic acid; or (2) stabilize a protonated silicic acid (the electrophile) which will then react with another molecule of silicic acid. In proposed mechanism (Figure 5) (Fairhead et al., 2008), the roles of C25S location and flanking mutations are simply to create a sufficiently sized pocket that will allow recognition of the tetrahedral Si(OH)₄ molecule in such a way that H163 location can deprotonate it. The deprotonated Si(OH)₄ protein complex can be thought of as a template for condensation reaction. In this proposal, there is no need to involve a high energy covalent intermediate. The presence of a specific Si(OH)₄ transporter in sponge suggests the true substrate in vivo is indeed silicic acid, not high energy silicon alkoxides. This being so, the simple acid–base activation mechanism proposed by authors seems a good model for the biological process.

According to Müller et al., formation of spicules is a biologically controlled extracellular process (Schröder et al., 2006; Müller et al., 2007). The following model of silicification in demosponges is derived from observations and propositions put forth by Morse’s and Müller’s groups (reviewed in Leys et al., 2007). Sclerocytes secrete axial filaments composed mainly of silicateins but other organic molecules are included. Silicatein acts as a structural template for formation of the highly organized mesoporous axial filament. Silicate is used or is actively taken up into the scleroocyte and complexed with specific proteins to form an as yet unidentified organic-silica substrate. The organo-silica complex is transported across silicalemma to the “silica deposition space.” Silica from organo-silica substrate is polymerized on the outer surface of the mesoporous axial filaments as nanospheres, perhaps at serine and histidine catalytic centre sites, and the complexing protein is released and possibly recycled to the scleroocyte cytoplasm.

Growth of the axial filament continues at spicule tips, providing primary patterning of the spicule, while at the same time transport and deposition of silica continue on lateral spicule surfaces between the tips. Once the first few layers of silica nanospheres encase the axial filament, enzymatic activity can no longer be expressed by silicateins of the axial filament. Continuing deposition of silica on the outer surface of spicules is facilitated and controlled by silicatein at active sites on the silicalemma, resulting in production of specific patterned morphologies of spicules. Several elements of this model remain unverified. Other unknowns include the roles of the minor non-silicatein organic compounds incorporated in axial filaments, whether silicateins associated with the silicalemma are the same forms as those in the axial filaments and how the final high-fidelity submicroscopic external patterning on spicule surfaces is genetically controlled. However, recently Kozhemyako et al. (2009) identified silicatein genes (AcSilA and AcSilB) in nonspicule-forming marine sponge Acanthodendrilla spp. The authors suggest that silicateins could participate also in the functions unrelated to spiculogenesis.

Chitin- and collagen-based silification
Aminopolysaccharide chitin has a nanofibrous structure and the chain of pyranose ring arranges almost parallel to the (100) plane and extends along the fiber axis (Iijima and Moriwaki, 1990). The chitin molecule has C=O, O–H, and N–H groups and oxygen atoms, which have affinity to the calcium, phosphate, carbonate, and hydroxyl ions of corresponding calcium phases. However, the same functional groups possess affinity to silicate ions. Because there is a possibility that such an oriented organic matrix acts as a template, or an ordered structural framework, it was hypothesized the existence of naturally occurring silica-chitin composites (Ehrlich et al., 2007). Moreover, silicon was found associated with glycosaminoglycans bound as an ether or ester-like silicate with C–O–Si or C–O–Si–O–Si–O–C bonds, in amounts of one Si atom/130–280 repeating units of the organic (Schwartz, 1973). The role of chitin in biosilification is still not completely understood. Although chitin is one of the most important biopolymers in nature, knowledge of its interaction with silicon in vivo was absent up to now. Only recently, Ehrlich et al., isolated and identified
chitin from skeletal formations of some marine glass sponges for the first time. The presence of chitin within the framework skeleton of *Farrea occa* (Ehrlich et al., 2007) and *Euplectella aspergillum* (Ehrlich and Worch, 2007a) as well as separate spicules *Rossella fibulata* (Ehrlich et al., 2008c) could also be revealed by gentle NaOH-based desilification technique established by Ehrlich et al. (2006) and Ehrlich and Worch, (2007b). The structure of the chitin extracted from these sponges turned out to be similar to alpha-chitin from invertebrates.

It was suggested that silicate ions and silica oligomers preferentially interact with glycopyranose rings exposed at the chitin surface, presumably by polar and H-bonding interactions (Ehrlich et al., 2008b). On the basis of the obtained results, authors proposed a model for the nanoscale structure of the naturally occurring silica-chitin composite unit, including interaction between poly-N-acetylglucosamine fragment of the chitin nanofibril and silica nanoparticles, which can be seen in Figure 6.

Because chitin could play a crucial role also in biosilification in fungi (Kolb et al., 2004), it was hypothesized (Ehrlich et al., 2007) that chitin molecules are probably part of a very old organic template system involved in a biosilification phenomenon, which was established a long time before the origin of first metazoan (e.g., glass sponges).

Recently, Ehrlich et al. reported for the first time on example of *Hyalonema sieboldi* glass sponge that its spicules are a biocomposite containing a silicificated collagen matrix, and that high collagen content is the origin of the unique mechanical flexibility of the spicules (Ehrlich et al., 2006; Ehrlich and Worch, 2007b). Especially, interest is paid now to newly discovered highly hydroxylated collagen isolated from *H. sieboldi* spicules (Prof. M. Collins personal communication). Glass sponges are largely restricted to deep, cold-water (between 0 and 4°C) habitats (Tabachnick, 1991; Janussen et al., 2004; Dohrmann et al., 2008). Therefore, from the ecological point of view collagen- (as well as chitin-) based silicification which occurs in spicules and other skeletal formations of these sponges is an example of unique cold-water biomineralization. There is no evidence in the literature about silicatein activities at this temperature level. Skeletal biomineralization requires energy and so imposes a metabolic cost on skeleton-forming organisms (Knoll, 2003). The hypothesis is that probably some phosphate moieties and ATPase-based mechanisms are in some way involved in biosilification at temperatures near zero (Ehrlich, 2009).

The procedure of alkali slow etching opens the possibility to observe the forms of collagen fibrils located within silica layers of sponge spicules and their distribution. For example, the results obtained by SEM observations of the desilificated spicular layers of *Monorhophas chuni* provide strong evidence that collagen fibril’s orientation within spicules possesses twisted plywood architecture (Ehrlich et al., 2008b). Typical fibrillar formations were observed within the tubular silica structures of this sponge in all layers starting from the inner axial channel containing axial filament (Figure 7c) up to the outermost surface layer of the spicules as shown in Figure 7b and Figure 7d. The fibrils in each cylinder form individual concentric 2D networks with the curvature of the corresponding silicate layers. These layers of about 1 μm in thickness are connected among each other by protein fibers (Figure 7a), which possess a characteristic nanofibrillar organization. Partially desilificated nanofibrillar organic matrix observed on the surface of silica-based inner layers of the demineralized spicule provides strong evidence that silica nanoparticles of diameter about 35 nm are localized on the surface of corresponding nanofibrils (Ehrlich et al., 2008b). This kind of silica nanodistribution is very similar to the silica distribution on the surface of collagen fibrils in the form of nanopore necklets, firstly observed in the glass sponge *H. sieboldi* (Ehrlich et al., 2006; Ehrlich and Worch, 2007b). It was suggested that the nanomorphology of silica on proteinous structures described above could be determined as an example of biodirected epitaxial nanodistribution of the amorphous silica phase on oriented collagenous fibrillar templates (Ehrlich et al., 2008b). From this point of view, basal spicules of Monorhophas sponges could be also defined as natural plywood-like silica-ceramics organized similarly to the crossed-lamellar layers of seashells. The authors suggested also that the matrix of the *M. chuni* anchoring spicule is silificated fibrillar collagen rather than collagen-containing silica which is the reason for their remarkable mechanical flexibility.

**Biogeological aspects of silica deposition by sponges**

Analysis of the biogeochemical cycle of silicon suggests that the biospheric environmental silicic acid concentration may have varied considerably during the time of life on Earth. Biochemical evolution today is proceeding within environments in which the silicic acid concentration is considerably lower than previous evolutionary periods (Exley, 1998).

Concomitant with the advent of dioxygen, about 2500 million years ago, and its subsequent gradual increase in atmospheric concentration from approximately 1% towards the level of 20% which is characteristic of today, there occurred an increasing number of organisms within which silicic acid was processed to silica. The most important of these, in the terms of their diversity, ubiquity (both freshwater and marine species) and biomass, were the diatoms, which occur however late in earth history (Mesozoic). The diatoms are characterized by a silica frustule which surrounds their cell wall. Interestingly that chitin-based organic networks are an integral part of cell wall biosilica from the diatom *Thalassiosira pseudonana* (Brunner et al., 2009). According to Maldonado et al. (2005), our current understanding of the silicon cycle in the ocean assumes that diatoms dominate not only the uptake of silicic acid, but also the production and
Proposed model (adapted from Ehrlich et al., 2008c) of nanostructural organization of the naturally occurring silica-chitin composite unit (a) isolated from spicules of *Rossella fibulata* glass sponge. Silica nanoparticles tightly surround chitinous nanofibrils (b) Schematic view (c) shows a possible nanodistribution of silica on the surface of chitinous nanofibril. Image (d) represents the hypothetic scheme of interaction between silica and poly-N-acetylglucosamine-fragment of the chitin nanofibril and formation of the corresponding hydrogen bonds.
Silica Biomineralization, Sponges, Figure 7 Proposed model (c) of micro- and nanostructural organisation of the basal spicule of *Monorhaphis chuni* glass sponge with respect to organic matrix. Collagen nets, surrounding the spicules, showed a tight mat of nanofibrils (b, image courtesy C. Eckert). SEM image (a) shows a collagenous fibrillar matrix which could function as glue between concentric layers. Schematic image (c) represents the region of the axial canal and axial filament. The axial canal of *M. chuni* possesses a characteristic quadratic opening and contains oriented bundles of unsilicified collagenous nanofibrils. The base material of the walls of the axial canal and concentric layers distributed above it consists of silicified collagen fibrils with a twisted plywood orientation (d). This kind of fibrillar architecture could be responsible for the remarkable micromechanical properties of the spicule as a biocomposite made by silica and collagen.
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reversing of biogenic silica, and that other organisms with siliceous skeletons, including sponges, radiolarians, and silicoflagellates, play a negligible role. The authors showed that the retention of Si by siliceous sponges in some sublittoral and bathyal environments is substantial and that sponge populations function as Si sinks. Therefore, sponges may affect Si cycling dynamics and Si availability for diatoms, particularly in Si-depleted environments. It was strongly suggested (Maldonado et al., 2005) that the role of sponges in the benthopelagic coupling of the Si cycle is significant. For example, Antarctic giant hexactinellids, such as Rossella nuda and Scolymastra joubini, which may be up to 2 m tall, 1.4 m in diameter, and up to 600 kg wet weight, containing up to 50 kg biogenic silica each (Maldonado et al., 2005). They are extremely slow-growing and seemingly reproduce only at long time intervals (Dayton et al., 1974) and become old, probably more than 1,500 years (Dayton, 1979; Gatti, 2002). While their living tissues represent only a modest biomass, the siliceous spicules of hexactinellids become an important ecological factor. After the death of the sponges, the megascleres do not dissolve but accumulate on the bottom and over large areas to form spicule mats commonly about 50 cm thick but occasionally exceeding 2 m (Koltun, 1968; Dayton et al., 1974, Henrich et al., 1992). The mats structure the fauna living in and on them.

Marine sponges fractionate silicon isotopes during opal biomineralization to a greater degree than any other process on Earth thus far investigated (De La Rocha, 2003). This ability is in keeping with both their extremely negative silicon isotope composition and their inefficient uptake of silicic acid relative to other silicon-bioiomineralizing organisms.

Sponges seem to be important players in numerous geobiological processes (Reitner, 2004). For example, siliceous sponge reefs had a wide distribution in prehistoric times and once constructed the largest reefs known on earth, reaching an acme in the Upper Jurassic when a deep-water reef belt on the northern Tethys shelf existed that was 7,000-km long (Krautter et al., 2001). In present times hexactinellid sponges of the order Hexactinosida have constructed reefs at several localities off the coast of British Columbia, Canada. The reefs occur on subtidal glaciated seafloor areas with a low sedimentation rate and become old, probably more than 1,500 years (Dayton, 1979; Gatti, 2002). While their living tissues represent only a modest biomass, the siliceous spicules of hexactinellids become an important ecological factor. After the death of the sponges, the megascleres do not dissolve but accumulate on the bottom and over large areas to form spicule mats commonly about 50 cm thick but occasionally exceeding 2 m (Koltun, 1968; Dayton et al., 1974, Henrich et al., 1992). The mats structure the fauna living in and on them.

Summary

Sponges are exclusively aquatic, sedentary, filter-feeding invertebrates, producing silica- made skeletons consisting of individualized elements (spicules) of lengths ranging from micrometers to centimeters, which can subsequently fuse or interlock with each other. The high diversity of spicule shapes and sizes in both fossil and living sponges has been repeatedly reported in literature. However, the mechanisms that determined such diversity remained elusive until recently. We are beginning to understand the mechanisms of spicule secretion and formation and the role of spicules and skeletal frameworks in the biogeology.

Molecular techniques and ecological experiments have demonstrated the genetic control of the process and the contribution of environmental factors to the expression of a sponge spicule, respectively. Silicatein proteins govern the enzymatic and structurally controlled synthesis of silica in marine demosponges. However, new biomineralization topics such as the role of chitin and/or collagen as self-assembling and nanofibrillar organized templates in sponge related biosilicification phenomena require further investigation.

Bibliography


Cross-references

Biogeochemical Cycles
Deep Biosphere of the Oceanic Deep Sea
Origins of the Metazoa
Reefs
Sponges (Porifera) and Sponge Microbes

SILICOFLAGELLATES

Silicoflagellates are planktonic, microscopic algae with siliceous skeletons, characterized by their simple geometries and remarkable variability. Taxonomically, they belong to different classes of the Stramenopiles and possess complex plastids derived from secondary endosymbiosis (see entry Symbiosis). Silicoflagellate skeletons may have considerable potential as environmental indicators and are common in fossil deposits, dating back to the middle Cretaceous (ca. 120 Ma), with species diversity reaching a peak in the Miocene (ca. 23–25 Ma). Today, only a single genus Dictyocha with approximately five recognized species exists. For more details, please refer to entry “Algae (Eukaryotic).”

SINTER

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Synonyms
Silica sinter; Siliceous sinter

Definition
Sinter: A sedimentary rock primarily composed of silica that is precipitated from hot waters at the vents of high-temperature (high-enthalpy) hot springs and geysers, and from cooled waters on their surrounding discharge aprons.
Geyserite. A dense, banded or laminated variety of sinter that forms at and near the vents of geysers and some high-temperature springs.

Some banded and laminated types of nonmarine carbonates (calcite or aragonite), including spring travertines and speleothems, have also been termed “sinter” or “calc-sinter.” We suggest that the geological term “sinter” should be restricted to siliceous deposits precipitated from silica-rich waters discharged at hot springs and geysers.

Introduction

Sinters are deposits of silica precipitated by hot waters discharged at the vents of hot springs and geysers. Most sinters are precipitated as noncrystalline opal-A, but they change to quartz during diagenesis. They form almost exclusively in high-enthalpy geothermal fields where the underground reservoir temperatures exceed ~220°C, and so are most common in volcanic and tectonically active regions. Most geysers discharge from vents lined with sinter. Weed (1889) was among the first to discuss the role of microorganisms in the formation of sinter. After his pioneering work, very little scientific study was done on these rocks until Allen (1934) described sinters at Yellowstone. Walter (1976) undertook petrographic study of sinters, including stromatolitic types. Rimstidt and Cole (1983) made one of the first detailed studies of the chemistry of sinter formation. Krumbein and Werner (1983), followed later by Cady and Farmer (1996) and Jones and Renaut (1996), described, illustrated, and interpreted silicified microbes in recent sinters. Since then, interest in these unusual rocks has increased significantly, and more than 140 studies of sinters have been published during the past decade. The possible role of bacteria in silica precipitation has been a focus of much interest. Many filamentous and coccoid microbes preserved in recent sinters resemble the microfossils found in Precambrian quartzose cherts.

Morphology of sinter deposits

Most sinter is gray to white in color, but the addition of trace elements can produce vividly colored deposits such as the orange sinters found around the edge of Champagne Pool, Waiotapu, New Zealand (Jones and Renaut, 2003, Figure 2b). The main types of sinter deposit are mounds, which form around the vents of many geysers, pool-rim dams that precipitate around the margins of hot-spring pools, and various types of terraces and platforms that form downslope from the vent(s) on the spring or geyser discharge apron. Sinter mounds (Figure 1a) develop where aqueous silica precipitates from fluids that overflow the margins of a geyser vent or small-diameter point-sourced spring, or from silica that precipitates from airborne spray. Sinter mounds can be columnar, domal, or broadly conical with slopes that range from nearly vertical to low angled (<10°). Mounds vary from decimeters to meters in height and up to several meters in basal diameter. Many factors control mound morphology including the original shape and dimensions of the vent, the volume and type of discharge (i.e., geyser versus hot spring activity), the original slope, climate (e.g., predominant wind direction), and water chemistry (e.g., silica concentration). Mound morphology can vary with time as the sinter deposits build up or the pattern or volume of discharge changes.

Pool-rim dams (Figure 1b) form where silica precipitates around the edges of hot-spring pools and some (fountain-type) geysers. The discharged water either flows across the lowest parts of the dam or escapes through erosional notches. Nested concentric pool-rim dams, each with different elevations, may form when mean water level in the pool changes because of external (e.g., hydrostatic) controls.

Water discharged from hot springs either flows across the land surface forming a “discharge apron” or it may flow directly into the local (nonthermal) drainage system. Outflow waters that mix with river or lake waters can be diluted below saturation with respect to amorphous silica, so that any sinter deposits are restricted to the vent area. If undiluted, however, the thermal outflow will cool progressively downslope leading to more silica precipitation. Sinter terraces (Figure 1c–f) commonly form where silica-laden flows flow across slopes, precipitating sheets of silica, as they cool and (or) evaporate. Each terrace is composed of a rimstone dam and pool. In scale, they range from miniterraces a few centimeters across, to large terraces tens of meters across, with dams several meters high. Some terrace pools temporarily pond water, but much of the outflow crosses terrace surfaces as largely unconfined sheets a few millimeters deep. Where several active vents lie near each other, their terraces may merge to form extensive flat sinter platforms.

Silica precipitated from hot waters also forms many small features including oncoids and pisoids (geyser eggs), which typically accrete in shallow pools near geyser vents; silica stalactites, which grow below overhanging terraces; and many small-scale ornate forms of geyserite on proximal sinter terraces (Figure 1e and g). Stromatolites develop locally in ponds or spring pools in the outflow system, usually where water temperature is <70°C.

The thickest, most extensive sinter deposits form in neutral to alkaline waters (pH 7–9.5). The sinters that form in acid to neutral waters (pH 2–7) tend to be thin (millimeter to decimeter rather than meter thick). They lack some of the complex ornamentation of sinters forming in more alkaline fluids (Figure 1h), but commonly show spicular and bladed morphologies, and tend to be glassier in appearance than sinters forming in alkaline waters.

Microorganisms and sinter

Petrographic and scanning electron microscope (SEM) studies of sinter commonly reveal abundant silicified remains of microbes including coccolid and filamentous bacteria, eukaryotic algae, diatoms, fungi, and
extracellular polymeric substances (EPS) (Figure 2). Near spring vents and on many terraces where waters have cooled to $<70^\circ$C, microbial mats commonly cover the substrate and can become encrusted and (or) replaced by opaline silica. Bunches of current-aligned, partially silicified, filamentous cyanobacteria (stringers) are present in some channels. If hydrothermal discharge is ephemeral and the terrace surfaces dry out periodically, microbial mats may be absent. However, biofilms, some thinly (millimeter thick) coated by opaline silica, and endoliths may still be present either at or just below the terrace surface, including sites very close to active vents.

Microbial fossils are preserved in most settings from vent to distal discharge apron. In waters $>75^\circ$C, nonphotosynthetic prokaryotes, including heterotrophic and chemolithotrophic bacteria and archaea, are present, although their silicified remains are often difficult to identify. Cyanobacteria and photosynthetic bacteria
Sinter, Figure 2. SEM photomicrographs of silicified biota in sinters from New Zealand (a–j) and Iceland (k, l). (a) Laminae formed of low-porosity opal-A alternating with laminae formed of (sub)erect filamentous microbes. (b) Opal-A spheres that formed in water column, settled to floor, and accumulated in pocket between erect microbial filaments. (c) Silicified microbes encased by opal-A spheres of variable diameters. (d) Large-diameter filamentous microbes (probably *Calothrix*) surrounded by small-diameter filaments (probably *Phormidium*). (e) Large-diameter filamentous microbes (probably *Calothrix*) and associated coccoid microbes (probably *Synechococcus*) encased by opal-A. (f) Dense accumulation of *Synechococcus* held in opal-A matrix. (g) Enlarged view of *Synechococcus* showing specimens in process of binary fusion. (h) Naturally etched surface showing microbes in groundmass formed of amalgamated opal-A spheres; etching has highlighted laminae in spheres showing their growth patterns. (i) Transverse cross section through silicified filamentous microbe – natural etching has highlighted the growth laminae in the opal-A. (j) Silicified pollen grain from a pine tree held in matrix of opal-A spheres. (k) Silicified bryophyte. (l) Transverse cross section through silicified twig showing perfect preservation of cells. Sample locations: a–c, h–j from discharge apron of Waikite Geyser, Whakarewarewa geothermal area, New Zealand; d–g from geyser system at Tokaanu, New Zealand; k and l from discharge apron of Geysir, Haukadalur, Iceland.
commonly inhabit waters from ~65–75°C (e.g., *Synechococcus, Chloroflexus*), with a wider range of cyanobacteria at temperatures <65°C (e.g., *Phormidium, Spirulina, Anaibaena, Oscillatoria, Calothrix*, and others). Eukaryotic algae colonize substrates at temperatures <60°C and diatoms at <45°C. Diatoms, fungi, and *Cyanidium* spp. are often the dominant fossil microbes in sinters precipitated from acidic fluids. Macrophytes, including grasses, wetland species, and trees and their root systems, may also be submerged by hot spring fluids and encrusted or replaced by amorphous silica.

**Mineralogy and petrology**

Most sinter is precipitated as noncrystalline opaline silica (opal-A: SiO₂·nH₂O) that can contain up to ~20 wt.% H₂O; less commonly, chalcedony is a primary mineral. Although sinters precipitated from neutral and alkaline fluids are commonly monomineralic, silica precipitated from acidic fluids is commonly accompanied by kaolinite, ferric oxyhydroxides, alunite, jarosite, pyrite, and gypsum.

Sinters exhibit a wide range of textures and fabrics. Geysersites tend to be laminated, columnar, or stromatolitic, and generally have low primary porosity. Sinters that form distally from the vent are more diverse and include bedded and laminated facies that form on terraces, commonly with in situ silicified microbial mats, fenestral porosity, and intraclasts reworked from silicified mats upslope. Early cementation by opaline silica commonly masks the original fabrics, producing apparently massive sinter, but slight differences in the water content of the precipitated opal-A can help differentiate primary textures and cements (Jones and Renaut, 2004).

During diagenesis, the opal-A transforms to opal-CT, moganite, and ultimately quartz. The opal-A to opal-CT transformation can be rapid (Lynne et al., 2006; Jones and Renaut, 2007) and proceeds mainly by dissolution–reprecipitation reactions.

**Origin of sinter**

Silica solubility increases with increasing temperature, pH, and pressure, and can be influenced by other factors such as the presence of certain ions (e.g., Al, Fe) and salinity. Most silica precipitation at hot springs has been attributed to the cooling of thermal fluids that have equilibrated with respect to quartz, chalcedony, or volcanic glass at depth, and then rise rapidly to the surface, emerging as hot water supersaturated with respect to amorphous silica (White et al., 1956; Rimstidt and Cole, 1983; Fournier, 1985).

Much of the dissolved silica is present in its monomeric form (H₄SiO₄). As saturation increases, silica polymerization occurs through reactions that involve linear, cyclic, and three-dimensional forms of polymeric silica (Iler, 1979). In strongly supersaturated fluids, including some spring pools, nuclei grow by Ostwald ripening into nanospheres and microspheres (10 nm–1 μm) and may form colloidal particle suspensions (sols). Continued growth of spheres can destabilize the sols and fine sediment settles from suspension. If the pH falls or the electrolyte concentrations increase (e.g., by evaporation), suspended particles may flocculate, forming aggregates that eventually sink to the substrate. In both cases, the silica sediment then undergoes cementation to form sinter.

At sites of evaporative concentration, microspheres may coagulate to form gels. In contrast, at relatively low levels of supersaturation, dissolved silica precipitates mainly by heterogeneous nucleation upon existing substrates or suspended particles. Evaporative concentration can induce silica precipitation at hot springs in semiarid and arid regions (e.g., Kenya Rift), although evaporation is usually less effective than cooling in humid environments. However, evaporation is an important mechanism on subaerial wetting including outflow terraces between geyser eruptions and sites receiving airborne spray. Seasonal freezing of geothermal outflow can also induce opal-A precipitation and agglomeration by “cryogelling,” which concentrates silica in residual fluid as ice is formed (Channing and Butler, 2007).

The pH and salinity also affect amorphous silica solubility. Nucleation and polymerization are inhibited in acidic waters because monomeric silica is slow to deprotonate (Fournier, 1985; Mountain et al., 2003), which explains the thin sinter development around acid hot springs. At pH > ~9, silica dissociates into other species, increasing the solubility. A rapid decrease in pH of a highly alkaline, silica-saturated thermal fluid might induce amorphous silica to precipitate on contact with neutral lake waters or groundwaters or if the fluid is acidified by microbes.

The waters that flow from most geysers and many hot springs in the geothermal fields of Iceland, New Zealand, Kamchatka, Chile, and Yellowstone, are strongly supersaturated with respect to amorphous silica so should precipitate silica abiologically upon rapid atmospheric cooling. Physical processes control the gross morphology of most sinter deposits, whereas chemical processes control the silica precipitation mechanisms. There has been much debate about whether or not microbes mediate silica precipitation and silification, particularly in waters where homogeneous nucleation does not occur. Microbes can provide reactive surface ligands that adsorb aqueous silica from solution, and should therefore promote heterogeneous nucleation by reducing activation energy barriers. Three main mechanisms have been proposed: hydrogen bonding, cation bridging by metals (especially Fe), and electrostatic interactions (Phoenix et al., 2003; Lalonde et al., 2005; Konhauser, 2007, pp. 156–160). Although consensus has not yet been attained, most recent field studies (e.g., Jones et al., 2004) and laboratory experiments (e.g., Fein et al., 2002; Yee et al., 2003) suggest that the role of microbes in silica precipitation is largely incidental, especially in strongly supersaturated fluids. At lower levels of supersaturation, they may provide organic
substrates for heterogeneous nucleation. Bacteria (including cyanobacteria), however, control microfabric development in many types of sinter, over a wide range of scales and fluid temperatures (Jones and Renaut, 2003).

**Fossil sinter**

Fossil sinters, also termed paleosinters, are increasingly being recognized in the geological record. They commonly form “caprocks” to some epithermal mineral deposits (e.g., Guido et al., 2002) but are rarely a target of study. Two exceptions are the early Devonian Rhynie deposits (e.g., Guido et al., 2002) but are rarely a target in modern environments and the geological record.

**Conclusions**

By mass, sinters are trivial compared to most sedimentary rocks, but their rarity belies their importance. Hot spring sinters form in one of the few environments where silica precipitation and silification processes can be studied at the Earth’s surface, another environment being highly alkaline lakes. Consequently, they will remain important in studying how microbes and plants are silicified, both in modern environments and the geological record.

**Bibliography**


Bibliography


- Hot Springs and Geysers

- Hydrothermal Environments, Fossil

- Hydrothermal Environments, Terrestrial

- Silica Biomineralization, Sponges
SKELETON

Biologically, a skeleton is a hard or rigid framework that could provide protection and support in many types of organisms. Most of skeletons are mineralized, but some may not be mineralized (e.g., some annelid tubes being agglutinated). They could be subdivided into exoskeletons and endoskeletons, not including hydroëskeletons. Exoskeletons are external hard parts. They may enclose the soft tissues and organs of the body, or may be external sclerites. Endoskeletons are internal hard parts (typical of many vertebrates). Most of endoskeletons are generally surrounded by skin and musculature. See entry “Animal Skeletons, Advent” for further reading.

SMALL SHELLY FOSSILS

Small shelly fossils (SSF, also “Small Shelly Fauna”) is a non-taxonomic term, first used by Matthews and Missarzhevsky in the title of their review paper in 1975, to denote the earliest skeletal fossils, although some of these fossils may not be small and most of them are actually not shells. It has been popularly used in literature somewhat as a catchall term for the early Cambrian skeletal fossils, including spicules, shells, tubes, and diverse disarticulated sclerites. See entries “Animal Skeletons, Advent” and “Critical Intervals in Earth History” for further reading.

SNOWBALL EARTH

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Synonyms
Cryochron; Global glaciation; Hard snowball; Ice-albedo catastrophe; Snowball (in geology); White Earth

Definition
Snowball Earth is a climate in which the oceans and most land areas are permanently covered by glacial ice. As less sunlight is absorbed by ice than by water or land, a critical area of ice will cause surface temperatures to fall below freezing everywhere, resulting in a snowball Earth. This arguably occurred near the beginning and end of the Proterozoic eon. Snowball Earths self-destruct after millions of years due to the buildup of atmospheric carbon dioxide of volcanic and metamorphic origin, which could not be converted into organic matter or limestone. Biological evolution is a postulated cause and consequence of snowball episodes.

History of the concept
Sedimentary deposits of glacial origin were first described from the late Proterozoic (Neoproterozoic) of Scotland in 1871 and from the early Proterozoic (Paleoproterozoic) of Canada in 1907. By 1940, it was apparent that the Neoproterozoic glaciation(s) had been more extreme than those of the Pleistocene and at least equal in severity to the late Paleozoic glaciations of Gondwanaland. In the 1960s, the occurrence of glacial deposits within thick successions of nearly pure carbonate strata suggested that Neoproterozoic ice sheets had entered what were normally the warmest parts of the globe. This was confirmed in the 1980–1990s by reliable paleomagnetic data showing that ice sheets had reached the ocean close to the paleoequator in the Neoproterozoic of Australia and Paleoproterozoic of South Africa (Evans, 2003). Moreover, evidence of marine geochemical anomalies concurrent with glaciation came increasingly to light in the form of sedimentary Fe₂O₃ and MnO₂ ore deposits, large excursions in stable carbon and sulfur isotopic compositions of minerals precipitated from seawater, and distinctive post-glacial “cap” carbonate sediments that rest directly on the terminal glacial deposit at all latitudes (Hoffman and Schrag, 2002). Neoproterozoic glacial deposits are now known from 22 different paleocontinents and microcontinents, and Paleoproterozoic deposits from 6.

The fraction of incoming solar radiation absorbed by ocean water under cloudless skies is 0.9, but for continuous ice it is only 0.4–0.6 and as low as 0.1 if the ice is covered by fresh snow. In the 1950–1960s, calculations by meteorologists of zonal average surface temperatures at different latitudes, assuming radiative energy balance and incorporating crude parameterizations of the radiative effects of clouds, meridional heat transport, and positive feedbacks due to the reflectivity (albedo) of ice, suggested that a lowering of solar luminosity by a few percent would result in a globe-encircling glaciation, or “white” Earth (North, 1990). If the luminosity is incrementally lowered, polar ice caps expand until their edges reach a critical latitude, beyond which ice advances rapidly to the equator without any additional luminosity reduction, or even with increased luminosity. This is due to runaway ice-albedo feedback – more ice reflects more radiation, creating more ice. (The same calculations indicated that a small increase in radiative forcing, due to anthropogenic CO₂ emissions for example, could cause a sudden loss of polar sea ice at a critical pCO₂ threshold.) These sensational findings greatly stimulated the nascent field of climate modeling, but proponents were puzzled how a “white” Earth disaster had been averted given that solar luminosity has increased by 25–30% over geological time (the so-called faint young Sun paradox). They assumed (incorrectly, as it turned out) that such an occurrence would extinguish all life – deep-sea chemoautotrophs and polar extremophiles were then unknown – and would be permanent because of the very large increase (~25%) in radiative forcing required to overwhelm a “white” Earth’s high albedo.
As meteorologists assumed that a “white” Earth never occurred, they did not think to consult geologists, who remained oblivious to their findings.

In 1981, planetary scientists (Walker et al., 1981) proposed that Earth’s climate is modulated within the habitable range by a negative climate feedback resulting from the temperature-dependence of silicate-rock weathering. In the geochemical cycle of carbon, this is the means by which atmospheric CO₂ (dissolved in rainwater) is converted into bicarbonate ion (HCO₃⁻), which dissolved in runoff makes its way to the ocean, where it is removed from circulation by the burial of carbonate sediments and organic matter. The feedback operates on a geological timescale (~10⁶ year) and causes the climate system to slowly seek whatever atmospheric CO₂ level, and hence climate, that is needed to balance the rate of CO₂ removal by silicate weathering with the rate of CO₂ emitted by volcanoes and metamorphic reactions. Walker et al. (1981) suggested that this feedback had prevented a “white” Earth disaster but noted that if one did occur, the consequent reduction of weathering rates would lead to an inexorable increase in atmospheric CO₂ as plate tectonics and consequent reduction of weathering rates would lead to an inexorable increase in atmospheric CO₂ as plate tectonics and its termination occurred before 667 Myr. Interregional correlations are correlated with these based on syndeglacial “cap” carbonates (see below), which are uniquely distinctive in many areas. The onset of the snowball is loosely constrained. It is younger than 654.5 ± 3.8 Ma in South China, based on ion-microprobe U-Pb geochronology (Zhang et al., 2008). The maximum concentration of the youngest snowball occurred in 635 Ma, coincident with the boundary between the Cryogenian and Ediacaran Periods (Knoll et al., 2006b). The age is based on ultra-precise (0.5 Myr uncertainty) U-Pb geochronology of primary zircons from volcanic ash-falls in Namibia and South China (Halverson, 2006). Sections in other regions are correlated with these based on syndeglacial “cap” carbonates (see below), which are uniquely distinctive in many areas. The onset of the snowball is loosely constrained. It is younger than 654.5 ± 3.8 Ma in South China, based on ion-microprobe U-Pb geochronology (Zhang et al., 2008). The maximum duration of this snowball was 23 Myr.

The timing of the older Cryogenian snowball is weakly constrained (Halverson, 2006): its onset is younger than 726 ± 1 Ma in Oman and 725 ± 10 Ma in South China, and its termination occurred before 667 ± 5 Ma in the western USA. In Oman, strata within the glacial succession are no older than 713 ± 1 Ma. Interregional correlation of glacial strata is more uncertain than for the younger Cryogenian glaciation. There is fragmentary evidence for an earlier Cryogenian glaciation of presumed regional scale ~750 Ma in central and southern Africa, while a mid-Ediacaran glaciation of short duration occurred in eastern Newfoundland, Canada, at 582 Ma (Knoll et al., 2006b), with possible correlatives in western and northern Europe, central Asia, Western Australia, and southwestern Brazil.

Paleoproterozoic glacial deposits in the western Transvaal, South Africa, are interfingered with volcanic lavas erupted near sea level at 11 ± 5° paleolatitude (Evans et al., 1997) approximately 2.22 Ga (10⁹ year before present) (Kopp et al., 2005). The duration of the low-latitude glaciation is unknown. Three distinct glaciations occurred between 2.45 and 2.22 Ga in the Huronian succession of Ontario, Canada, and correlative glacial deposits occurred in Wyoming (USA), Quebec (Canada) and possibly Finland (Ojakangas, 1988). The paleolatitudes of these deposits being unknown, they are assumed to represent regional-scale glaciations.
Paleogeographic reconstructions and paleoclimatic indicators in bounding strata

At the time of the Cryogenian snowballs, many continents were in low paleolatitudes and few if any were near the poles (Trindade and Macoun, 2007). This is consistent with cold climates because a preponderance of low-latitude continents maximizes the rate of atmospheric CO₂ consumption by silicate weathering. It was also a time of fragmentation and dispersal of the long-lived (1.0–0.8 Ga) supercontinent Rodinia (Li et al., 2008). Continental fragmentation also enhances silicate weathering by bringing land closer to the source of moisture. Cryogenian paleogeography contrasted with that of the late Paleozoic ice ages, when a supercontinent (Pangea) had just formed and the glaciated lands (Gondwanaland) lay in a polar region. Little is known of early Paleoproterozoic paleogeography, but there are suggestions that they accompanied the fragmentation of land-masses consolidated in the late Archean eon (2.7–2.5 Ga).

Cryogenian glacial deposits are found within thick successions of shallow-water carbonate rocks in Namibia, California, Arctic Alaska, and southern Mongolia (Halverson et al., 2005). This is paradoxical because non-skeletal carbonates form preferentially in the warmest parts of the surface ocean on account of their “reverse” solubility. Throughout Earth history, thick carbonate successions formed only at latitudes less than 35° (Ziegler et al., 1984; Opdyke and Wilkinson, 1990). Their range did not change between warmer and colder climates because their distribution depends on the relative not the absolute temperature. Thus the meridional temperature gradient has never been reversed due to high orbital obliquity (Evans, 2006), as has been proposed to account for low-latitude glaciation (Schmidt and Williams, 1995). If glaciers reached the ocean in the warmest areas of the world, then the colder areas must also have been frozen.

Sedimentology of the glaciogenic deposits

The sedimentology and inferentially the depositional processes of Proterozoic and Phanerozoic glacial deposits are quite similar (Boulton and Dyenoux, 1981; Hambrey and Harland, 1981). Those more interested in the rocks themselves than in their distribution have a hard time believing that Proterozoic ice ages were different in character. They range from terrestrial lodgement, moraine and outwash (sandur) complexes to glaciomarine grounding-line wedges and fans, and proglacial suspension fallout with or without sediment gravity flows and ice-rafted debris (Kellerhals and Matter, 2003).

Shaped and striated clasts, subglacial pavements with grooves and chatter marks, and stratigraphic relations suggestive of ice streams (currents of fast-flowing ice within an ice sheet) all attest to dynamic glacial action (Hoffman, 2005). At first it was thought that dynamic ice sheets were inconsistent with a snowball because the frozen ocean could not resupply them with snow. However, climate models suggest that net sublimation of subtropical sea-ice would supply ice sheets with up to centimeters of ice-equivalent annually, meaning that ice sheets would thicken sufficiently to generate dynamic ice streams within a few 10^5 year, a fraction of the estimated snowballs duration (Donnadieu et al., 2003; Pollard and Kasting, 2004). Accordingly, the evidence for dynamic ice sheets is not incompatible with a frozen ocean.

On the Otavi carbonate platform of Namibia, the younger Cryogenian glaciation left a continuous grounding-line prism situated 500–1,000 m vertically below the rim of the platform (Hoffman, 2005). This is quite unlike any Quaternary ice grounding line in polar seas, which typically switched between the inner and outer shelf. The grounding-line prism in Namibia is directly overlain by a syndeglacial “cap” dolostone (see below) deposited above prevailing wave-base, the only shallow-water deposit at such depth on the slope (Hoffman et al., 2007). This indicates glacioeustatic changes of >500 m, consistent with an average ice-sheet thickness of >1.1 km over all continents. An important implication of this finding is that shelves and platforms were above sea level during most of the glacial interval, and any glaciomarine deposits found there could only have been deposited when the deglaciation was well-advanced. The nature of such deposits has often been cited as inconsistent with snowball conditions (e.g., wave action, phototrophism, ice-rafting, etc.). This is true; by the time they were deposited, the snowball no longer existed.

Associated marine geochemical anomalies

Fe₂O₃- and MnO₂-ore formations

During the Fe-ore exploration boom following World War II, it was learned that the only “banded iron-formations” (BIF) younger than 1.9 Ga are intimately and exclusively associated with Cryogenian glacial deposits (Klein, 2005). Extensive ore-grade deposits of Fe₂O₃ (±MnO₂) were found in SE Australia, SW Brazil, NW Canada, and Namibia. The Australian and Canadian deposits are in the older Cryogenian assemblage, the Brazilian ones are in the younger (Gondwana) Cryogenian assemblage, and the Namibian deposits are in both (?). As only reduced Fe(II) is soluble in seawater, Martin (1964) attributed “this peculiar combination of sediments” to “oxygen deficiency in stagnating bottom waters caused by an ice cover.” Canfield and Raiswell (1999) pointed out that decreased continental runoff during a snowball would lower the influx of sulfate to the ocean, which after bacterial sulfate reduction, removes dissolved Fe(II) from seawater in the form of FeS₂ (pyrite). Lowering the influx of sulfate therefore raises the concentration of Fe(II). The large sea-level fall associated with a snowball also raises the Fe:S flux ratio in mid-ocean ridge hydrothermal vent fluids on account of the drop in hydrostatic pressure (Kump and Seyfried, 2005).

Kirschvink (1992) suggested that the dissolved Fe(II) was oxidized to insoluble Fe(III) at snowball terminations, consistent with the stratigraphic position of the Australian
BIF near the top of the glacial sequence. Elsewhere, BIF occurs as sporadic lenses within glaciomarine sequences and Fe oxidation may have been caused by discharging of oxic subglacial meltwater along the grounding lines of ice shelves.

The 2.22-Ga Makanyene glaciation in South Africa ended with outpourings of mafic lava and the deposition of major Fe- and Mn-ore deposits. The latter are the world’s largest and imply the presence of abundant molecular O$_2$ (not required for Fe oxidation) $\sim$0.22 Gyr after atmospheric O$_2$ first rose above $10^{-6}$ PAL (present atmospheric level) according to mass-independent sulfur isotope fractionation data (Farquhar et al., 2000). The youngest pre-Cryogenian BIFs are limited to collisional (foredeep) basins (1.89 Ga) around the Superior craton, unrelated to glaciation.

**“Cap” carbonates**

“Cap” carbonates are highly continuous, meter- to deca-meter-thick layers of relatively pure carbonate lying sharply without significant hiatus on top of the last glacial deposits of a snowball (Hoffman and Schrag, 2002; Shields, 2005). They typically extend far beyond the actual glacial deposits at the same stratigraphic horizon.

The best developed cap carbonates are those associated with the youngest snowball deglaciation in 635 Ma: they are global in extent and sedimentologically unique in the entire stratigraphic column (Allen and Hoffman, 2005; Shields, 2005; Hoffman et al., 2007). They begin with a layer of pale gray, often pinkish, micro- and macropeloidal dolostone (Ca$_{0.5}$Mg$_{0.5}$CO$_3$). Its global average thickness is $\sim$20 m and it was laid down diachronously, mainly above storm wave-base, during glacioeustatic flooding of continental margins and inland seas (Hoffman et al., 2007). Assuming that strong positive climate feedbacks (e.g., ice albedo, ice elevation, CO$_2$ degassing, gas-hydrate destabilization) drove snowball deglaciation on timescales $<10$ kyr, cap dolostone accumulation rates were $>2$ mm year$^{-1}$.

The dolostone contains anomalous sedimentary features such as giant wave ripples (Allen and Hoffman, 2005) and stromatolite biostromes containing strictly vertical tubes of internal sediment with meniscus-like laminae (Corsetti and Grotzinger, 2005). The top of the dolostone layer in some areas is marked by a continuous crust of primary seafloor BaSO$_4$ (barite) cement in the form of digitate rosettes (pseudostromatolites) with internal growth lamellae (Hoffman and Schrag, 2002). The barite crust and other redox-sensitive geochemical (Hurtgen et al., 2006) and biomolecular (Elie et al., 2007) proxies indicate that an oxic–anoxic interface within the water column intersected the seafloor at this horizon. In subsiding areas, the dolostone is overlain by deeper water shales or limestones, locally with layer- upon-layer of seafloor cements in the form of macroscopic crystal fans originally composed of aragonite, the orthorhombic polymorph of CaCO$_3$ (James et al., 2001). In non-subsiding areas, the dolostone layer ends at a subaerial exposure surface heavily disrupted by products of phreatic and vadose diagenesis (Hoffman and Schrag, 2002; Shields et al., 2007).

The nature and extent of the 635-Ma cap carbonate indicates an ocean in which the mixed layer and thermocline waters were highly oversaturated with respect to (CaMg)CO$_3$ and CaCO$_3$ respectively. Sources of alkalinity responsible for the anomalous saturation state include the weathering of carbonate and silicate rock powder and debris exposed by deglaciation (Higgins and Schrag, 2003; Anderson, 2007), anaerobic oxidation of CH$_4$ released by melting of permafrost in organic-rich shelf sediments glacioeustatically exposed during the snowball (Kennedy et al., 2001; Jiang et al., 2003), and anaerobic respiration at depth of organic matter produced in surface waters newly exposed to sunlight (Font et al., 2006). It was suggested (Kennedy, 1996; Ridgwell et al., 2003) that the 635-Ma cap carbonate can be explained by analogy with the “coral reef hypothesis” of Quaternary glacial–interglacial cycles (Berger, 1982), in which alkalinity builds up in deep waters during glacial stages because of the loss of shelf area, where carbonate is normally sequestered without passage through corrosive deep waters. This mechanism presupposes continued carbonate production in open ocean surface waters during the glaciation, for which evidence is lacking.

The cap carbonate ending the older Cryogenian snowball is different in character but comparable in thickness to the one just described, as well as in its sharp basal contact. Lithologically, it is usually a dark, fetid, limestone or dolostone, locally rhodochrosite (MnCO$_3$) deposited below storm wave-base. Former aragonite seafloor cements are prominent in some areas, notably in eastern Brazil (Vieira et al., 2007). In Namibia, there are hundreds of meters of continuous sublittoral microbialaminites with distinctive roll-up structures (Hoffman et al., 1998a). Missing altogether is the transgressive cap dolostone characteristic of the 635-Ma deglaciation. Carbonate production evidently did not begin until after deglaciation was complete, suggesting a weaker alkalinity flux into the ocean or a lower saturation state when the deglaciation began.

Cap carbonates a few centimeters to decimeters thick occur locally above the mid-Ediacaran (582 Ma) and early Cryogenian (~750 Ma) glacial deposits, believed to represent regional-scale glaciations.

In the Paleoproterozoic, the middle Huronian glacial formation has an impure cap limestone up to 17 m thick, the only carbonate unit in a terrigenous clastic succession up to 12 km thick (Bekker et al., 2005). The low-latitude Makanyene glaciation (~2.22 Ga) in South Africa has a possible cap carbonate (Mooiobrai Formation) known only from subsurface bore holes (Kirschvink et al., 2000).

**Post-glacial isotopic anomalies**

Cap carbonates are typically depleted in $^{13}$C (i.e., $\delta^{13}$C $< 0.0\%$ PDB) and detailed studies of expanded
sections of the 635-Ma cap carbonate in different areas show large negative isotopic excursions (Halverson et al., 2005). Excursions are deviations over time in a positive (higher δ13C) or negative direction, followed by a return to previous values. The excursion begins ~0‰ at the base of the oldest (deepest) cap dolostone, descends through the cap dolostone to a low of ~6‰ in the basal limestones (after a ~1.5‰ step-function across the dolostone–limestone transition), and then rises slowly back to ~0‰. Its stratigraphic thickness depends on the sedimentation rate: on the Otavi platform, Namibia, it is ~400 m thick but only ~50 m thick on the deeper slope sections (formerly the ice grounding-line zone). The origin of the excursion is disputed (e.g., Kennedy et al., 2001; Higgins and Schrag, 2003).

The same cap carbonate hosts negative excursions in δ11B, interpreted as an expression of greatly elevated pCO2 (Kasemann et al., 2005), and δ34S of carbonate-associated sulfate (CAS), interpreted as oxidized sulfidic snowball waters (Hurtgen et al., 2006). The negative δ34S excursion is followed by a positive one with lows and highs of 15 and 45‰, respectively. The positive values (the highest in Earth history) are attributed by a rapid increase in isotopic fractionation between sedimentary sulfate and pyrite, without a compensatory decline in the fractional burial rate of pyrite (Halverson and Hurtgen, 2007).

The older Cryogenian cap carbonate preserves only the rising leg of the post-glacial isotopic excursions, reflecting stratigraphic truncation (non-deposition) during glacioeustatic flooding. Surface waters became strongly enriched (δ13C > 4‰) again soon after carbonate production resumed (Yoshioka et al., 2003). Paired negative and positive δ34S/CAS excursions resemble those above the younger snowball but data are fewer (Hurtgen et al., 2002).

The two known Paleoproterozoic cap carbonates are also depleted in 13C (Kirschvink et al., 2000; Bekker et al., 2005), but lack local isotopic context as they occur in clastic-dominated successions. A major positive δ13C excursion (Lomagundi excursion) appears to begin before age-equivalents of the Makganyene glaciation in North America (Bekker et al., 2006).

Pre-glacial isotopic anomalies
Both Cryogenian snowballs were preceded by long (>10^7 year) intervals of consistent isotopic enrichment (δ13C ≥ 5‰ PDB), followed by Myr-long excursions to much depleted values (δ13C ≤ −5‰), variably truncated by subglacial erosion. There is no accompanying δ34S excursion. The excursions largely predate sea-level falls due to ice-sheet growth. Schrag et al. (2002) suggest that the prolonged isotopic enrichment reflects high fractional organic burial due to rapid sediment burial and expanded suboxic zones on preponderantly low-latitude continental margins. They propose that this situation ultimately led to a slow rise in atmospheric CH4 due to methanogenesis in organic-rich sediments. The rise in CH4 is held responsible for the negative δ13C excursion (Halverson et al., 2002). Silicate weathering feedback would lower pCO2 in response to a slow rise in CH4, causing the climate to become more unstable because of its greater sensitivity to CH4 concentration and the absence of buffering of its concentration by the ocean. Greater stochastic variability in a cold climate increases the risk of exceeding the critical threshold for runaway ice-albedo feedback. Unusual stromatolite and oolite development during the pre-glacial negative δ13C excursions could reflect a rise in pH caused by the proposed lowering of pCO2. The methane substitution model (Schrag et al., 2002) may not be viable, however, because it depends on an unrealistic residence time for CH4 in the Cryogenian atmosphere of >10^9 year. Unless their timing around 1 Myr before the onsets of both Cryogenian snowballs is a coincidence, or merely wrong, the negative δ13C excursions should have some bearing on snowball causation.

Climate modeling
Climate models are used to simulate the conditions under which a snowball could initiate, develop over time, and deglaciate. Ideally, an atmospheric general circulation model (AGCM) would be coupled to a dynamic ocean model, a dynamic ice-sheet model, a dynamic sea-ice model, and an interactive model of the geochemical cycle of carbon. In reality, this is computationally unattainable in the foreseeable future. To begin with, full-blown AGCMs are typically run to equilibrium over ~100 model years, which are ~4 orders of magnitude less than the adjustment time for the geochemical carbon cycle. Therefore, atmospheric CO2 concentrations are either prescribed at various levels, providing “snapshots” under arbitrary conditions, or else a greatly simplified AGCM must be employed.

Snowball climate models must be adjusted for ambient Proterozoic conditions. Solar luminosity was ~7% lower than present in the Cryogenian and 16–20% lower at 2.22 Ga (Crowley and North, 1991). The Earth’s rotation rate was ~10% higher in the Cryogenian (Williams, 2000) and faster by an unknown amount in the Paleoproterozoic. And Proterozoic continents had higher albedos in the absence of forest cover. These factors all tend to lower the global mean surface temperature and would have resulted in compensatory higher levels of atmospheric CO2 (Walker et al., 1981).

Modeling suggests that while snowball initiation is resisted by ocean dynamics (Bendtsen, 2002; Poulsen and Jacob, 2004), it is assisted by atmospheric Hadley convection (Bendtsen, 2002), flowage of ice-shelf ice (Goodman and Pierrickhumbert, 2003), and wind-driven sea ice (Lewis et al., 2007). Both the thickness of tropical sea ice and the CO2 level required for deglaciation are highly sensitive to the prescribed ice albedos (Warren et al., 2002; Pollard and Kasting, 2005; Lewis et al., 2006; Le Hir et al., 2007). The role of clouds is complex
and is a major source of uncertainty in the models (Pierrehumbert, 2005).

**Theories of causation**

In addition to the methane substitution model described above, a number of other astronomical, oceanographic, geodynamic, and biological theories have been proposed to account for low latitude glaciation. Astronomical theories include solar variations (Harland, 1964), large orbital obliquity (Williams, 1975), ice-ring collapses (Sheldon, 1984), impact ejecta (Bendtsen and Bjerrum, 2002; Fawcett and Boslough, 2002), and passage through giant molecular clouds (Pavlov et al., 2005). The large obliquity hypothesis is the most testable and contested of these models (Néron de Surgy and Laskar, 1997; Williams et al., 1998; Pais et al., 1999; Maloof et al., 2002; Levrard and Laskar, 2003). Oceanographic theories include enhanced carbonate burial (Roberts, 1971), intermittent ocean stagnation (Kaufman et al., 1993; Grotzinger and Knoll, 1995), glacioeustatic hypsometry change (Ridgwell et al., 2003), and enhanced organic burial (Kaufman et al., 1997). Geodynamic theories involve continental distribution (Kirschvink, 1992; Schrag et al., 2002), supercontinent breakup (Donnadieu et al., 2004), flood-basalt weathering (Goddéris et al., 2003), and true polar wander (Li et al., 2004). Biological theories include the evolution of biocatalyzed weathering (Carver and Vardavas, 1994), methane destruction (Pavlov et al., 2000, 2003; Catling et al., 2001; Claire et al., 2006), methane substitution (Halverson and Hurtgen, 2007), and the evolution of oxygenic photosynthesis (Kopp et al., 2005). As the ultimate cause of the Quaternary ice ages remains obscure, it is not surprising that the “why” question for Proterozoic snowballs is undecided. Different snowball may have had different causes, but whatever their cause, it was an uncommon occurrence.

**Snowballs and the history of oxygenation**

There is increasing evidence for stepwise increases in environmental oxygenation near the beginning and end of the Proterozoic eon, roughly contemporaneous with the inferred snowballs. This is the empirical motivation for causative theories involving the oxidative destruction of reduced “greenhouse” gases (Pavlov et al., 2000, 2003; Catling et al., 2001; Claire et al., 2006). To make a climatic impact, changes in oxygenation must occur rapidly (<10^6 year), or else silicate weathering feedback will cause compensatory adjustments in pCO2, largely nullifying the effect on radiative forcing.

Mass-independent sulfur isotope fractionation data indicate that atmospheric O2 concentrations remained below 10^-6 PAL (present atmospheric level) until ~2.45 Ga, close to the first Huronian glaciation and ~230 Myr before the Makganyene snowball. The same data suggest the possibility that O2 remained very low (but >10^-6 PAL) during the intervening interval. However, deposition of the succeeding Kalahari MnO2-ore formation required at least transient O2 concentrations >5 orders of magnitude higher than this, possibly caused by a photosynthetic “bloom” and resultant organic burial in the snowball aftermath (Kirschvink et al., 2000) or by H2O2 (hydrogen peroxide) released by the melting of snowball ice (Liang et al., 2006).

There is increasing evidence that the deep oceans remained anoxic, but sulfidic not ferrous, during most of the 1.5 Gyr ice-age gap between the Makganyene and the Cryogenian snowballs (Scott et al., 2008). Mass-dependent S-isotope data imply that seawater sulfate concentrations remained low (<4 mM) between the Cryogenian snowballs (Hurtgen et al., 2002), but rose rapidly to near modern (29 mM) levels in the early Ediacaran (Halverson and Hurtgen, 2007). This rise in marine sulfate, presumably caused by increased atmospheric pO2, appears to follow rather than lead the end-Cryogenian snowball. However, the response time between oxygenation and methane destruction is much shorter than between oxygenation and growth of the marine sulfate reservoir, so an increase in oxygenation to trigger the snowball is not ruled out by these data.

**Implications for evolutionary biology**

**Eukaryotic survival**

The younger Phanerozoic glaciations had little impact on the course of biological evolution as measured by rates of origination or extinction of taxa: only the Hirnantian (latest Orodician) glaciation ~445 Ma was accompanied by major mass extinctions. Snowballs, however, presented qualitatively different challenges to existing biota, if they actually occurred. For millions of years, mean annual surface temperatures at the equator would have resembled those of Antarctica today. Areas where sunlight and liquid water coexisted were severely limited, hot springs and zones of perpetual crack development where mobile sea ice encountered fixed rocky substrates. With permanent ice cover, seawater would be starved of oxygen, becoming anoxic due to sinks for oxygen in the ocean. It would become progressively more acidic due to submarine volcanic CO2 emissions and air–sea gas exchange through cracks at rates lower than the oxygen consumption rate. The geochemical response of the ocean to these afflictions is complex, but seawater pH would probably be lowered by 2.0–2.5 pH units (Le Hir et al., 2008). Deep-sea vent biota would be adversely affected by reduced Eh and pH gradients between hydrothermal fluids and seawater. With low overall productivity, nutrient levels would be high, except for fixed nitrogen due to denitrification. Upon deglaciation, a “bloom” of primary producers would occur, contingent upon nitrate availability. The bloom might be delayed because meltwater injection and surface warming would combine to produce an unusually stable density stratification (Shields, 2005), temporarily suppressing nutrient-upwelling from depth. A 635-Ma
cap carbonate in southwestern Brazil preserves a molecular record of this bloom in the form of an unusual assemblage of biomarkers pointing to a low-diversity eukaryotic flora dominated by red algae and a prokaryotic biota rich in green sulfur bacteria (Elie et al., 2007). The latter imply anoxic waters at the base of the photic zone and the former are consistent with low levels of illumination as exists, for example, in a turbid water column.

No biologist doubts that prokaryotic life, bacteria and archaea, could survive a snowball cryochron and its aftermath. Some do question if eukaryotic algae could survive (but see Vincent et al., 2004). As several extant clades of eukaryotic algae evolved before the Cryogenian, their survival causes some to doubt the existence of snowballs (Knoll, 2003). This is the attraction of more benign but less physically plausible climate states like “slushball” Earth, wherein continents are glaciated at all latitudes (satisfying geological observations) but half of the ocean remains ice-free (Peltier et al., 2004). However, the slushball solution provides no satisfactory explanation for synglacial Fe- and Mn-ore deposits (Klein, 2005), for cap carbonates (Pollard and Kasting, 2005) or for the multimillion-year duration of Cryogenian glaciations inferred from stratigraphic subsidence analysis (Hoffman and Schrag, 2002).

The only fossil record that is sufficiently continuous to bear directly on the evolutionary impact of snowballs is the record of organic-walled, acid-resistant microfossils representing a variety of eukaryotic algae, protists, and possibly fungae (Knoll et al., 2006a). Their Proterozoic record is characterized by extremely low rates of turnover (extinctions and originations) of taxa, roughly 100× slower than in the Ediacaran and Phanerozoic, following the last snowball (Peterson et al., 2005). The sparser macrofossil record is also consistent with slow turnover. Taxonomic diversity drops precipitously during the interval that includes the Cryogenian snowballs (Knoll et al., 2006a), with the inevitable result that it is little studied by palynologists. There have been no systematic studies to determine if the interval of very low diversity actually begins at the first Cryogenian snowball. The microfossil record effectively starts only after the Paleoproterozoic snowball (Knoll, 2003).

**Evolutionary innovation**

Since the mid-twentieth century, geologists have speculated that Neoproterozoic glaciation and its aftermath was somehow responsible for the advent of multicellular animals, the first fossils of which were known to occur not too far above the glacial deposits in many areas (Harland and Rudwick, 1964). (Note this was not the advent of multicellularity *per se*, which occurred earlier in algae (Knoll, 2003).) In the 1990s, with improvements of radiometric dating, this connection appeared less likely: the canonical Cambrian “explosion” in diversity of animal taxa with hard parts did not occur until ~530–520 Ma and the oldest macrofossils arguably attributable to metazoa, the enigmatic Ediacara biota, appear ~580 Ma. This is >100 Myr and 55 Myr, respectively, after the last snowball ended in 635 Ma. In contrast, molecular divergence in modern animals, calibrated by known branching points in the geological record (so-called molecular “clocks”), gave estimates of animals origins much older than the Cryogenian snowballs.

However, recent evidence from microfossils and molecular fossils appears to put the connection back in play once again. In South China, remarkable cysts enclosing metazoan diapause eggs and embryos extend down to strata deposited within ~3 Myr (632 Ma) of the last snowball termination in the same area (Yin et al., 2007). In Oman, molecular fossils (24-isopropylcholestanol) believed to be diagnostic of sponges are found down to and even within correlative glacial deposits (Love et al., 2009). In addition, new molecular “clock” analyses place the origin of eumetazoa more in line with the new fossil evidence (Peterson and Butterfield, 2005), while the molecular signature of animal radiation indicates a geologically instantaneous event (Rokas et al., 2005).

How might the environmental stress of a snowball and its aftermath have contributed to revolutionary biological innovation? One idea involves the dual role of heat-shock protein 90 (HSP90) in suppressing the expression of mutant signal transduction proteins and in promoting the proper folding of stress-damaged proteins (Baker, 2006). Under extreme temperatures, HSP90 is diverted from the former role in favor of the latter, allowing accumulated cryptic mutations to be phenotypically expressed and therefore subject to natural selection. Another possibility is that spatial isolation of biological refugia in a snowball favored the evolution of altruism, a basic requirement for intercellular cooperation, by promoting kin selection and eliminating non-altruistic invaders (Boyle et al., 2007).

The evolutionary impact of the Paleoproterozoic snowball(s) is even more conjectural as there is no detailed fossil evidence for synchrony. Nevertheless, it has been suggested that horizontal gene transfer between taxa at levels higher than at the species level was triggered by environmental catastrophes, and that these gene exchanges were responsible for such macroevolutionary innovations of possible Paleoproterozoic age as thermophilic and photosynthetic bacteria, and the eukaryotic cell (Hartman, 2002). Molecular fossil evidence for an Archean (pre-snowball) origin for oxygenic photosynthesis and for eukaryotes has been presented (Brocks et al., 1999; Summons et al., 1999); however, more recent studies suggest that the “biomarkers” in question are less phylogenetically diagnostic than earlier believed (Fischer et al., 2005; Kopp et al., 2005; Rashby et al., 2007). Finally, the surprising finding that the highest 18S rRNA gene diversity in extant protists and the richest in ancestral lineages occurs in Arctic communities (Stoeck et al., 2007) suggests the possibility that snowballs figured prominently in their evolution.
Conclusion
Important new evidence for extreme atmospheric carbon dioxide concentrations during and after the Marinoan snowball Earth has recently been obtained from mass-independent oxygen isotope ratios in sulfate minerals (Bao et al., 2008, 2009).

Summary
Virtually all continents were covered by large ice sheets during two discrete intervals of the Cryogenian Period (between 725 and 635 Ma). The multimillion-year duration of the glacial intervals, the deposition of syn-glacial Fe₂O₃ and MnO₂ sedimentary ores in different areas and of post-glacial “cap” carbonates at all latitudes support the hypothesis that the world ocean was also ice-covered. These episodes represent a climate state unknown on Earth since 635 Ma, or in the 1.5 Gyr before 725 Ma. Climatic models indicate that the pan-glacial climates, popularly known as snowball Earth, were the result of runaway ice-albedo feedback, and that they were reversed by infra-red radiative forcing due to the accumulation of atmospheric CO₂ of volcanic-metamorphic origin, which could not be removed because of the absence of liquid precipitation when surface temperatures were below freezing everywhere. New microfossil and molecular biomarker evidence opens up the possibility that the origin of multicellular animals was related to these climate shocks. Evidence for glaciation at low paleolatitudes around 2.22 Ga, also associated with sedimentary Fe and Mn ores, suggests the existence of snowball states possibly related to the initial rise of atmospheric O₂.

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SODA LAKES

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Definition

The term “soda lake” designates a class of lakes with waters showing an excess of the total alkalinity (TA ≡ [HCO3−] + 2[CO3²−], i.e., the sum of the charges of the bicarbonate ion plus carbonate ion) over the charges of the alkaline earth ions magnesium and calcium:

[\text{HCO}_3^- + 2\text{CO}_3^{2-} > 2\text{Mg}^{2+} + 2\text{Ca}^{2+}].

When such water is evaporated, the high [CO3²−] concentrations will cause a rise in pH and eventually, Na carbonates precipitate. These are soda (or natron, Na2CO3·10H2O, monoclinic), trona (Na2CO3·NaHCO3·2H2O, monoclinic), and thermonatrite (Na2CO3·H2O, orthorhombic). They are commercially used in the glass-, textile-, paint-, soap- and metal-industry, mined worldwide (Garret, 1992).

Occurrence

Soda lakes occur worldwide, albeit in very specific zones: they seem to be associated with active tectonic and volcanic zones (Figure 1). Most of the famous soda lakes occur in East Africa along the East African rift (e.g., Kraml and Bull, 1998/1999). Here, the succession of soda lakes left deeply buried soda deposits that also serve as the source of natro-carbonatite lavas (Ol Doinyo Lengai Stratovolcano, Tanzania). Another well-known group of alkaline lakes, some fossil such as Searles Lake, occur in the western USA. Less well known are lakes on the Altiplano, or in Australia, Afghanistan, Turkey, and other places around the world. So far no comprehensive list of soda lakes has apparently been compiled.

Setting

However, there seem to be two different types of lakes that acquire soda chemistry: most of the larger lakes are depressions in endorheic regions closed toward exorheic runoff by tectonic movements or by volcanic dams. The ratio between the tributary area and lake surface area is large. The largest soda lake on the Earth is Lake Van (Van Gölü) in eastern Anatolia (e.g., Kemen et al., 1991).

Table 1 gives its morphometric data; it is (because Lake Aral dried up) by volume the third largest closed basin lake on Earth. Lake Van occupies the former headwaters of the Euphrates before it was dammed by an eruption of the Nemrut volcano.

The other class of soda lakes occur in volcanic craters or calderas. They typically do not have any surface tributaries and their ratio between recharge area and lake surface area is small. We have discovered the alkaline nature of several crater lakes, including Satonda Crater,
Indonesia (Kempe and Kazmierczak, 1993), Niuafo’ou Caldera Lake, Tonga (Kazmierczak and Kempe, 2006), and Kauhako Crater Lake, Molokai, Hawaii (unpublished data and Donachie et al., 2004). Other soda lakes occupy the Empakai Crater in Kenya, the crater in Pantelleria, and possibly a score of other craters that have not been investigated as yet. Even Santorini, Greece, must have had an alkaline crater lake for some period prior to the famous Minoan eruption that blew out the stromatolites of that lake.

Chemistry

Soda lakes can reach pH in excess of 10.5 and alkalinites of >150 meq/l. Some of the most alkaline water bodies are Lake Magadi (Eugster, 1986) in Kenya and Mono Lake in California (e.g., Bischoff et al., 1993). Lake Van has a pH of 9.7 and an alkalinity of 151 meq/l (Kempe et al., 1991; Reimer et al., 2009). These are examples of highly alkaline lakes. But there are soda lakes “in the making,” i.e., lakes that obey the basic condition of TA > (Mg + Ca) but have not acquired higher salinities. One of them is the crater lake of the Nemrut Volcano itself that has dammed Lake Van. In Table 2 the water of Lake Van is compared with that of the Nemrut Lake.

The Table lists the main parameters that are used to characterize natural waters, all in all the two lakes are not very different in their respective surface and bottom samples, i.e., they are relatively well mixed. First of all the conductivity gives a rough measure of the amount of total salts dissolved, which is in seawater about 35 g/l, in Lake Van it is about 22 g/l, and in Nemrut Lake only about 440 mg/l, i.e., Nemrut Lake is what would be classified as a “fresh water” lake. But this is only one of the criteria: when calculating the soda lake criterion...
(i.e., TA/(Mg + Ca)) we find 16.65 and 16.85 for Lake Van (0 and 440 m, respectively), 3.11 and 3.18 for Lake Nemrut (10 and 150 m, respectively) and 0.018 for seawater. This ratio tells us that Lake Van is a typical soda lake with >16 times as much alkalinity as alkaline earth ions, but that also Lake Nemrut is a soda lake with >3 times higher alkalinity than alkaline earth ions; whereas seawater has much more Mg and Ca than dissolved carbonates (i.e., >54 times). We also see that the chemical composition of both lakes do not differ much with depth, apart from the pH in Nemrut Crater that drops considerably. Lake Van has a very high pH as expected from its high alkalinity. But Nemrut crater lake has – at the surface – the same slightly alkaline pH as seawater, not identifying it (yet) as a soda lake. When from these data (and temperature not given here) the CO$_2$ pressure of the water (P$_{CO_2}$) is calculated (using PHREEQE; Parkhurst et al., 1990) and compared to the P$_{CO_2}$ of the atmosphere of

### Soda Lakes, Table 2

<table>
<thead>
<tr>
<th>Lake/Depth</th>
<th>pH</th>
<th>P$_{CO_2}$ ppmv</th>
<th>Alkal. meq/l</th>
<th>Cl meq/l</th>
<th>SO$_4$ meq/l</th>
<th>Na meq/l</th>
<th>Mg meq/l</th>
<th>Ca meq/l</th>
<th>Cond μS/cm</th>
<th>SI$_{Cc}$</th>
<th>SI$_{Ara}$</th>
<th>SI$_{Dol}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seawater at surface</td>
<td>8.24</td>
<td>363</td>
<td>2.33</td>
<td>546</td>
<td>56.6</td>
<td>468</td>
<td>106.2</td>
<td>20.6</td>
<td>ca. 48,000</td>
<td>0.59</td>
<td>0.44</td>
<td>1.96</td>
</tr>
<tr>
<td>Van 3 m</td>
<td>9.74</td>
<td>309</td>
<td>151.2</td>
<td>160</td>
<td>48.9</td>
<td>338</td>
<td>8.870</td>
<td>0.210</td>
<td>26,000</td>
<td>1.14</td>
<td>0.99</td>
<td>4.09</td>
</tr>
<tr>
<td>Van 440 m</td>
<td>9.88</td>
<td>257</td>
<td>155.6</td>
<td>166</td>
<td>50.9</td>
<td>348</td>
<td>9.06</td>
<td>0.174</td>
<td>26,700</td>
<td>1.06</td>
<td>0.91</td>
<td>3.81</td>
</tr>
<tr>
<td>Nemrut 10 m</td>
<td>8.27</td>
<td>1,240</td>
<td>4.61</td>
<td>0.43</td>
<td>0.22</td>
<td>3.67</td>
<td>0.52</td>
<td>0.96</td>
<td>456</td>
<td>0.37</td>
<td>0.22</td>
<td>0.43</td>
</tr>
<tr>
<td>Nemrut 150 m</td>
<td>6.89</td>
<td>28,600</td>
<td>4.77</td>
<td>0.45</td>
<td>0.25</td>
<td>3.80</td>
<td>0.53</td>
<td>0.97</td>
<td>–</td>
<td>1.11</td>
<td>–1.27</td>
<td>–2.59</td>
</tr>
<tr>
<td>Same degassed</td>
<td>8.73</td>
<td>380</td>
<td>4.77</td>
<td>0.45</td>
<td>0.25</td>
<td>3.80</td>
<td>0.53</td>
<td>0.97</td>
<td>–</td>
<td>1.30</td>
<td>1.15</td>
<td>2.25</td>
</tr>
</tbody>
</table>

Source: From Kempe and Kazmierczak (2003); data see Reimer (1995); (Reimer et al., 2009)

### Soda Lakes, Table 3

<table>
<thead>
<tr>
<th>Site</th>
<th>Minerals dominating</th>
<th>Saturation index</th>
<th>Kind of CaCO$_3$ precipitate</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Sea 1986</td>
<td>Calcite</td>
<td>0.375–0.904 Cc mean of 122 samples: 0.567</td>
<td>Biomineralization of coccolithophorids</td>
<td>Pegler and Kempe, 1988</td>
</tr>
<tr>
<td>Bahamas banks</td>
<td>Aragonite</td>
<td>0.47–0.6 Ara</td>
<td>Extremely low rates (if any) of aragonite (not the reason of whitings)</td>
<td>Morse et al., 2002</td>
</tr>
<tr>
<td>Satonda crater lake</td>
<td>Aragonite, Calcite</td>
<td>0.98 Cc, 0.84 Ara</td>
<td>Biomineralization by red algae and cyanobacterial sheaths permineralization</td>
<td>Kempe and Kazmierczak, 1993; Kazmierczak et al., 2004</td>
</tr>
<tr>
<td>Lake Van</td>
<td>Aragonite, Calcite</td>
<td>1.14 at 0 m to 1.06 at 440 m Cc, 0.99–0.91 Ara 4.09–3.81 Dol</td>
<td>Permineralization of cyanobacterial sheaths, inorganic chemical gardens, river plume whitening, summer whitings</td>
<td>Kempe et al., 1991; Reimer, 1995; Kazmierczak et al., 2004; Reimer et al., 2008</td>
</tr>
<tr>
<td>Lake Tanganyika</td>
<td>High-Mg Calcite</td>
<td>0.96 Cc</td>
<td>Five zones of massive thrombolites down to 50 m</td>
<td>Kempe and Kazmierczak, 1990 and citations therein</td>
</tr>
<tr>
<td>Andros Island</td>
<td>Low-Mg Calcite</td>
<td>Upon degassing and evaporation 1.14 Cc 1.11 Cc, 0.95 Ara, 3.90 Dol</td>
<td>Inland lakes with weakly calcified cyanobacterial and algal mats</td>
<td>Same as above</td>
</tr>
<tr>
<td>Walker Lake</td>
<td>Low-Mg Calcite</td>
<td>1.11 Cc, 0.95 Ara, 3.90 Dol</td>
<td>Small cabbage-like stromatolites up to 4 cm high, built by filamentous green alga and oscillatory and coccolid cyanobacteriacea.</td>
<td>Same as above; Kempe and Kazmierczak, 1997</td>
</tr>
<tr>
<td>Mono Lake</td>
<td>Aragonite (Ikaite)</td>
<td>1.24 Cc, 1.09 Ara, 3.74 Dol</td>
<td>Large tufa columns at former groundwater outlets</td>
<td>Kempe and Kazmierczak, 1997; Bischoff et al., 1993</td>
</tr>
<tr>
<td>Pyramid Lake</td>
<td>Calcite</td>
<td>1.36 Cc, 1.21 Ara, 3.89 Dol</td>
<td>Huge towers and mounds at former groundwater outlets</td>
<td>Benson, 1994; Arp et al., 1999</td>
</tr>
<tr>
<td>Niuafo’ou, crater lake</td>
<td>Aragonite</td>
<td>0.65 Cc, 0.50 Ara, 2.65 Dol</td>
<td>Head-like stromatolites built by coccolid and filamentous cyanobacteriacea</td>
<td>Kazmierczak and Kempe, 2006</td>
</tr>
<tr>
<td>Kauhako crater lake</td>
<td>Calcite</td>
<td>0.97 Cc, 0.82 Ara</td>
<td>Calcified sheaths of filamentous cyanobacteriacea</td>
<td>Kempe, unpublished</td>
</tr>
</tbody>
</table>

Cc = calcite; Ara = aragonite; Dol = dolomite
380 ppm, then we find that the crater lake has a much higher \( PCO_2 \) than the other samples, indicating that it receives volcanic \( CO_2 \) from subsurface springs, while Lake Van does not.

PHREEQE also allows for calculating the state of saturation of water with respect to minerals. The saturation index (SI) is defined as the logarithm of the ion activity product divided by the solubility constant of a given mineral (here, for calcite as an example):

\[
SI_{\text{calcite}} = \log([Ca^{2+}]_i[CO_3^{2-}]_i/K_{\text{calcite}}).
\]

The SI yields negative values if water is undersaturated, zero if saturated, and positive values if supersaturated. The results (Table 2) show that all samples, with the exception of the deep Nemrut sample, are oversaturated with respect to calcite, aragonite, and dolomite. But when the deep Nemrut sample is numerically degassed, it will also show high carbonate supersaturation (last line in Table 2). The main difference between Lake Van and seawater is that the carbonate supersaturation (which is \( >10 \) times its saturation) of Lake Van allows for in situ precipitation of aragonite, while in seawater, even though supersaturated, no spontaneous precipitation occurs. Rather, the supersaturation of seawater is kept low by the multitude of enzymatically biomineralizing organisms that are generally absent in soda lakes due to the high alkalinity.

**Soda lake microbialites**

Those of the mature soda lakes that reach high supersaturation values (i.e., above \( \approx SI 0.8 \), Kempe and Kazmierczak, 1990) produce cyanobacterial microbialites. Table 3 lists a few of them for which sufficient geochemical data are available to calculate saturation. The water analysis available for Andros Island originally do not show supersaturation, but the permineralizing cyanobacterial mats grow in ephemeral very shallow nonmarine inland lakes that apparently sustain growth only after an extensive evaporative phase. In case of one of the Niuafo’ou caldera lakes (Vai Lahi), the current water composition also does not sustain in situ permineralization, and the stromatolites discovered there stopped growing about 200 years ago. Overall, soda lakes represent the best sites to study modern stromatolites, their structure, microbial ecosystem, and growth rates. Many of them have shown to produce structures strikingly similar to those of Precambrian microbialites.

**Genesis of soda lakes**

These data presented here and in the cited papers show that soda chemistry can arise in various climatic, geologic, and morphologic settings. We find water bodies that are soda lakes in the making that will eventually acquire mature soda chemistry, such as Nemrut Crater Lake, a lake that originated only a few hundred years ago after the last eruption of the Nemrut caldera. Other crater lakes, such as Lake Taupo in New Zealand, will never reach this state, since they have an outlet. Astonishing is also the fact that Lake Kivu and Lake Tanganyika (both with an outlet) have soda lake characteristics (Soda Lake Index for Tanganyika: 1.5) and (at least in Lake Tanganyika) grow microbialites.

It has long been stated that the type of chemistry is dependent on the proportions of the ions in the waters.
delivered to it (Garrels and Mackenzie, 1967). Eugster and Hardie (1978) constructed flow diagrams that show the various possibilities of brine evolution. In case of soda lakes, the criterion is the ratio of TA/(Mg + Ca). From the distributions of the soda lakes along the plate boundaries it appears that this ratio most likely arises in areas with fresh volcanic rocks. Here, biogenic CO₂ and/or (see Lake Nemrut) volcanic CO₂ can react with fresh silicates, mobilizing Na, K, Mg, and Ca, in the necessary proportions. Ongoing weathering within the crater lakes and their hydrothermal system (as in the case of Niuafo’ou), or continuing evaporation in the terminal lakes will then lead to mature soda lake chemistry and to a CaCO₃ supersaturation of around SI = 1 that will sustain microbialite growth. However, this is only a very broad picture. When looking at Satonda and Kauhako crater lakes, we find involvement of a second process that causes the alkalinity to rise. This is sulfate reduction. Both lakes are marine-derived lakes, i.e., are filled originally with sulfate-rich seawater. Microbial disintegration of organic matter must replace the charge of the sulfate ion with that of bicarbonate ions according to

\[ \text{C}_{106}\text{H}_{263}\text{O}_{110}\text{N}_{16}\text{P}_1 + 53\text{SO}_4^{2-} + 14\text{H}_2\text{O} \rightarrow 53\text{H}_2\text{S} + 106\text{HCO}_3^- + \text{HPO}_4^{2-} + 16\text{NH}_4^+ + 14\text{OH}^- \]

This pathway of alkalinity production in anaerobic water bodies was coined “alkalinity pump” (Kempe, 1990; Kempe and Kazmierczak, 1994), supplementing the shift toward a soda chemistry, where sulfate is available.

Summary

Soda lake waters show an excess of the total alkalinity over the charges of the alkaline earth ions magnesium and calcium ([HCO₃⁻] + 2[CO₃²⁻] > 2[Mg²⁺] + 2[Ca²⁺]). They are characterized by high pH values (increasing with evaporation) and, eventually, precipitation of Na carbonates. Mature soda lakes that reach high supersaturation values with respect to calcite, aragonite, and dolomite (above ≈ SI = 0.8) may produce cyanobacterial microbialites. The ubiquitous soda lakes in areas with fresh volcanic rocks and high weathering potential and the microbialites found therein gave rise to the Soda Ocean Hypothesis (see separate entry), stating that the early ocean, by following simple geological rules, should have been highly alkaline, possibly similar to present Lake Van.

Bibliography


Cross-references
Alkalinity
Biofilms
Calcite Precipitation, Microbially Induced Carbonates
Cyanobacteria
Divalent Earth Alkaline Cations in Seawater
Dolomite, Microbial
Microbial Mats
Microbialites, Modern
Soda Ocean Hypothesis
Tufa, Freshwater

SODA OCEAN HYPOTHESIS

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Definition
The “soda ocean hypothesis” (SOH) stands for the concept of an early (i.e., in essence Precambrian) alkaline or even highly “alkaline ocean,” in analogy to the chemistry of the present-day “soda lakes.”

Soda ocean hypothesis (SOH)
The SOH has been advanced in biology (e.g., Snyder and Fox, 1975) for biochemical reasons before it was developed in earth sciences for geochemical reasons (Kempe and Degens, 1985; Kempe et al., 1989; Kempe and Kazmierczak, 1994).

In biology, the SOH rests on the observations that certain reactions considered essential for biogenesis would be favored by alkaline conditions (e.g., Abelson, 1966). One of those is the experimental observation that peptide bonds are more stable in alkaline than in acidic environments (e.g., Dose and Rauchfuss, 1972).

In earth sciences, the SOH rests on elemental mass balances, thermodynamic and kinetic arguments, and the analogy to modern soda lakes. These arguments are in short:

1. A CO₂-rich atmosphere in the presence of water would react vigorously with the highly meta-stable, widespread, fine-grained glassy silicates from impacts and volcanic eruptions as would be present during the period of the terminal cataclysm in the Hadean. Carbonic acid weathering of silicates (“Urey Reaction”) will liberate cations in equivalence to the consumed carbonic acid, producing bicarbonate and carbonate anions.

2. Continental silicate weathering today (aided by high soil PCO₂ due to biological activity; PCO₂ = CO₂ Pressure) consumes annually 0.15 10¹⁴t C (Hartmann et al., 2009); thus today’s volume of atmospheric CO₂ would be consumed in about 5,000 years by the Urey Reaction. This rate suggests that a high PCO₂ atmosphere is not stable on geological timescales.

3. Other primordial acids, such as HCl, have much less mass and therefore the primordial ocean must have had TA (total alkalinity ≡ [HCO₃⁻] + 2 [CO₃²⁻] > [Cl⁻]).

4. Sulfur-based acids were not available in larger quantities since not enough free oxygen was in the system to allow formation of either sulfuric or sulfurous acids.

5. The entire mass of carbon available in crustal compartments (i.e., 65.5 10¹³ gC) as carbonates and organic carbon today could in principle have been dissolved as alkali carbonates in the oceans - as long as H₂O and CO₂ were degassed at similar rates - because this would amount to 48.5 gC/kg H₂O while Na₂CO₃ is soluble at 53 gC/kg H₂O (Kempe and Degens, 1985).

6. Observations of modern soda lakes show that their tributaries have inputs of [Na⁺] + [K⁺] > [Cl⁻] + [SO₄²⁻] and [Ca²⁺] + [Mg²⁺] < [TA] (Garrels and Mackenzie, 1967). When evaporated these waters will become alkaline because Ca and Mg carbonates (and sulfates in modern settings) will reach saturation first and Na and K carbonates will stay in solution causing increasingly higher alkalinity and pH. Soda lakes (i.e., lakes with an excess in Na-carbonates) almost exclusively occur today in or near volcanic regions. The early ocean (as well as possible primordial oceans on other planets such as Mars; Kempe and Kazmierczak, 1997) could have reacted the same way, storing CO₂ as TA as quickly as CO₂ was degassed from the mantle or deposited on Earth by comets.

These arguments are based on simple acid–base reactions but have several derivatives:

1. The atmosphere could not have had a high enough PCO₂ to counterbalance the lower insolation of the “faint early sun”.

2. In alkaline waters SiO₂ can be kept in solution in high concentrations (at least in the primordial seas where
silica-sequestering organisms such as sponges, diatoms, silicoflagellates, and radiolarians were absent.

3. The higher the TA becomes, the lower the concentration of the free Ca-ion will become because of the fact that the IAP (the ion activity product) governing CaCO$_3$ solubility, i.e., IAP = [$Ca^{2+}$][CO$_3^{2-}$] is dominated by the carbonate ion.

4. In the absence of biologically controlled bio-mineralization (as was typical for the entire Precambrian until, at the onset of the Cambrian, enzymatic biocalcification evolved) the ocean must have been of a very high supersaturation, since the spontaneous precipitation of CaCO$_3$-minerals is inhibited in natural settings. Saturation is most commonly expressed as the saturation index (SI), i.e., the quotient between the IAP and the solubility product of the mineral (K$_{\text{Mineral}}$).

\[ SI = \log \frac{\text{IAP}}{K_{\text{Mineral}}} \]

A survey of nonenzymatic carbonate CaCO$_3$-precipitating environments (Kempe and Kazmierczak, 1990; Kazmierczak and Kempe, 2006) shows that these environments have an SI of +0.8 to +1 (i.e., a 10-times supersaturation) while the modern ocean has a much lower supersaturation (North Sea SI$_{\text{calcite}}$ average summer 1986 = 0.567 ± 0.096; Pegler and Kempe, 1988) because it is controlled by enzymatic reactions and not by inorganic precipitation. Dolomite can be supersaturated to SI = 4, i.e., 10,000 times its solubility product in soda lakes (e.g., Kempe and Kazmierczak, 2007). This fact, i.e., the higher carbonate mineral supersaturation of the primordial ocean is today accepted widely (e.g., Grotzinger, 1990; Arp et al., 2001).

Because the soda ocean has vanished and the present day halite ocean prevails, there must have been a transition between the two (Figure 1). This transition must have been driven by a process with a long time constant. This process is thought to be the subduction of seawater with marine crust and sediments in subduction zones and the consecutive formation of continental crust. About 1 km$^3$ of seawater is subducted per year today. Any compound dissolved in the ocean has therefore a half-life of about 1 billion years, depending on the rate of subduction. Thus the soda ocean could in fact have prevailed for several billion years before being gradually diminished. According to the soda ocean model (Kempe and Degens, 1985), Na and K would have been transferred into feldspars of continental granodiorites and the carbon would have been deposited as CaCO$_3$ and kerogen on the continents where they are stored for longer times than deep sea deposits that are lost to subduction within less than 100 million years. The chloride subducted would be recycled as volcanic HCl, in a cycle quicker than continental weathering.

**Consequences for biogenesis**

The alkaline model has also a series of consequence for biogenesis and the evolution of life in the first 3.5 billion years of Earth history.

1. Thermodynamic calculations show that in highly alkaline solutions (up to the saturation of Na$_2$CO$_3$), the free [Ca$^{2+}$] can be as low as 4*10$^{-7}$ mol (Kazmierczak and Kempe, 2004), while, due to ion-pairs, total Ca is significantly higher. In the model calculation shown in Figure 2 seawater (i.e., a solution with high chloride and sulfate) was mixed with the natron-saturated solution based on the dilute water from Nemrut Crater Lake (−10 m), a “soda lake in the making” (see entry “Soda Lakes” Table 3) that leads to a free [Ca$^{2+}$] of 1.5*10$^{-6}$ mol. Very low environmental Ca$^{2+}$ concentrations are favorable for biogenesis because cells have to maintain a [Ca$^{2+}$] $<$ 10$^{-6}$ mol in their cytosol (e.g., Kretsinger, 1983; Carafoli, 1987). Otherwise, the proteins would be denaturalized and lose their ability to function. Thus the formation of life in an acidic and consequentially high [Ca$^{2+}$] environment is even more difficult to understand.

2. The only macroscopic fossils throughout the Precambrian (with the exception of the Ediacara fauna and some problematic biostratigraphies) are microbialites (stromatolites, thrombolites), biosedimentary structures produced by colonies of microorganisms. The majority of researchers accept that they have formed by the permineralization of the EPS of biofilms dominated by cyanobacteria (e.g., Riding, 2000; Altermann et al., 2006). This form of CaCO$_3$-precipitation is nonenzymatic and the cyanobacteria do not instrumentalize these precipitates for functions. The high CaCO$_3$-supersaturation caused by the alkaline marine condition is thus an environment in which these microbialites could have thrived easily. Today soda-lake microbialites are formed by the same processes (e.g., in Lake Van, Kempe et al., 1991; López-Garcia et al., 2005, in Mono Lake, and in Niuafou’ou caldera lakes, Kazmierczak and Kempe, 2006) while today’s
marine stromatolites are apparently growing predominantly by trapping and binding of alloge particles (e.g., Ginsburg, 1991).

3. For three billion years cyanobacteria seem to be among the primary photosynthetic organisms. Most modern cyanobacteria are alkalophiles, suggesting that their evolution proceeded in alkaline environments.

4. The development of eukaryotic organisms in the late Palaeoproterozoic may have also been triggered by the early ocean conditions: The gradual increase in the [Ca²⁺] ambient concentration due to the slow transition between the soda and halite dominated oceans, could have caused the geochemical stress that might have influenced the evolution of a cell nucleus by protecting the genetic material against the disruptive Ca²⁺-influx. Note also that the sulfate concentration would describe an exponential curve between 50 and 100% if subsamples were calculated for that section of the mixing experiment.

5. And even the onset of multicellular organisms may have been triggered by the increasing [Ca²⁺]. Experiments with sponges suggest that adhesion of cells can be triggered by an increase in [Ca²⁺] in the environment (e.g., Kreitinger, 1977). Thus the appearance of faunas in Ediacara times may be the indication that the soda ocean was near its demise.

6. The biomineralization that appears in many different taxa at the turn of the Precambrian/Cambrian is, in view of the SOH, a detoxification reaction to the rapidly rising [Ca²⁺] when the ocean, as TA decreases (e.g., from 10.3 to 2.38 meq/l as in present seawater), goes through a sort of titration endpoint triggering a >10-fold increase in [Ca²⁺] (Kempe and Kazmierczak, 1994). Measurements of Ca in fluid inclusions seem to give this facet of the SOH credibility (Brennan et al., 2004; Petrychenko et al., 2005).

Counterarguments

Some arguments against the SOH were brought forward. These, however, do not really disprove the SOH:

1. Models of early Earth often prescribe a long-lasting high PCO₂-atmosphere to keep the oceans from freezing. However, it may also be argued that the lack of free oxygen in the oceans and atmosphere could have allowed for higher methane concentrations that prevented permanent freezing of Earth.

2. Möller and Bau (1993), Bau and Möller (1994) state that the water of Lake Van has a positive cerium anomaly but Precambrian carbonates (or BIFs) do not. However, these authors compare water with sediments. Measurements showed that Lake Van sediments do not exhibit the same anomaly as the water body (own data, unpublished).

Outlook

The acceptance of the SOH in the literature is mixed. Some authors agree with it in essence (Arp et al., 2001), others accept certain aspects (such as the high CaCO₃-supersaturation; Grotzinger and Kasting, 1993; Riding, 2000; or Mg/Ca ratios; Ries et al., 2008), and some doubt its relevance and follow own modeling lines (e.g., Morse and Mackenzie, 1998). Many papers deal with questions of the rising oxygen and sulfate, redox-reactions or certain sedimentary problems like BIF-formation or microbialite (stromatolite) structures without recurring to the overall chemical evolution of the ocean. Although the majority of researchers tend to ignore the SOH, it found entrance into several textbooks of general geology (Degens, 1989; Einsele, 1992; Bahlburg and Breitkreuz, 1998). Digital modeling appears to be not of much help - apart from stating thermodynamic conditions correctly - since the results depend on the adopted boundary conditions of the Hadean and Archean environment, which are as yet not well defined. For example, when prescribing certain PCO₂ values to avoid freezing of oceans because of the faint early sun, then no highly alkaline ocean can evolve. Future research will have to show which one of the many models explains the past of our planet and its geological and biological evolution best.

Summary

Resting on elemental mass balances, thermodynamic and kinetic arguments, and the analogy to modern soda lakes, the soda ocean hypothesis (SOH) states that the Precambrian ocean was alkaline, with a total alkalinity >Ca + Mg
and a high supersaturation of carbonate minerals (SI ca. 1). The soda ocean was lost slowly by subduction of seawater and replaced by the present halite-dominated ocean toward the end of the Precambrian. This facilitated the development of multicellularity and biomineralization as detoxification reactions of life against the rising Ca-concentrations. The SOH is also consistent with the widespread occurrence of stromatolites and cherts in the Precambrian.

Bibliography
as well as microbiological acidification and release of leading to soil series of different soil types. Root exudates base rock material, climatic conditions, and other factors by humification. The pedogenesis differs with respect to essential for the decomposition of plant litter followed by immobilization. This can be easily seen with root systems or earthworm/mole tunnels, but the metabolic activities of microorganisms by far exceed these visible alterations. Microbes are active players in global cycles (Meir et al., 2006). Agriculture and nutrition in modern systems are coupled to the use of fertilizers and plant protection chemicals which can constitute a threat to the water pathways, if management of agricultural use is not performed according to the laws and provisions on soil and water protection, good agricultural practice, or integrated farming, and monitoring has to be included to ensure best practice. Soil density increases due to the use of heavy machinery which leads to soil degradation in high-yield agricultural systems the world over.

**Cross-references**

Alkalinity
Biofilms
Calcite Precipitation, Microbially Induced
Carbonates
Cyanobacteria
Divalent Earth Alkaline Cations in Seawater
Dolomite, Microbial
Microbial Mats
Microbialites, Modern
Soda Lakes
Tufa, Freshwater

**SOILS**

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**Synonyms**

Pedosphere

**Definition**

*Soil:* refers to the product of mineral weathering and secondary mineral formation, (microbial) mineralization, humus formation, and the resulting element mobilization/immobilization in the upper Earth crust in a pedogenetic process involving chemical, physical, and biological activities.

**Introduction**

Soil is the basis of terrestrial life, particularly for agriculture, forestry, and generally land-use by man (Driessen et al., 2001). It is also the largest terrestrial ecosystem dominated by high numbers of microorganisms and soil-living animals as well as the root systems of plants. The biota within this ecosystem, not only interact but also actively shape their environment (Fiedler et al., 2002). This can be easily seen with root systems or earthworm/mole tunnels, but the metabolic activities of microorganisms by far exceed these visible alterations. Microbes are essential for the decomposition of plant litter followed by humification. The pedogenesis differs with respect to base rock material, climatic conditions, and other factors leading to soil series of different soil types. Root exudates as well as microbiological acidification and release of chelating agents take part in mobilization and immobilization processes of mineral elements, essential or ecotoxicologically relevant alike, and thus are major players in import into food chains, and input into water paths or as volatiles into the atmosphere.

**Soil function**

The soils of the world can be characterized from a more structural or a more functional point of view (FAO-UNESCO, 2002), and the geobiologically important functions involving metabolically active soil dwellers are characterized with reference to microbiology, botany and agriculture, and zoology.

Soil function is essential for plant nutrition. The capacity to solubilize nutrients like P and minerals including K, Na, Mg, Ca, and trace elements, as well as availability of S or N are largely the product of microbial mobilization from minerals, microbial degradation of organic matter, or fixation from air (e.g., for nitrogen). Soil mineralogy largely determines the buffering capacity for minerals adsorbed to different fractions (Jones and Bassington, 1998). The sustainability of microbial activity is connected to water availability for growth, and water storage and availability are also major functions for plant growth. Detrimental processes of soil degeneration like soil acidification, desertification, clay mineral destruction, or decrease of organic contents are global problems mankind experiences which will have a large impact on socioeconomic scales.

In order to assess possible problems with land-use and soil degeneration, soil science has provided a very good reference system to classify and monitor soils based on soil mechanics, soil physics, soil genesis, soil composition, and soil functions reviewed in different contexts below (IUSS Working Group WRB, 2006). As soil can be regarded as a major sink for carbon depending on land-use, this might become more important as climate change discussions are gaining impact. Generally, soils and larger views on landscape should be regarded as eminent players in global cycles (Meir et al., 2006).

**Soil texture**

The soil texture can be divided in the proportion of sand (S: 63 μm–2 mm), silt (U: 2–63 μm), and clay (T: smaller than 2 μm) after removing skeleton particles (larger than 2 mm). While sand soils have more than 70% of sand, clay soil is mainly composed of clay (>50%) with...
only small portions of sand (<20%). Most soils in humid conditions are sandy loam (with at least 1/3 sand, 1/3 silt, and up to 1/3 loam) or sandy silt soils (with at least 1/3 sand, at least 1/3 silt, and lower amounts of loam).

These differences in texture are also reflected in mineral composition of the respective soils. While sand soil contains 80% of quartz, this mineral constitutes only 25% of clay soil. In contrast, 50% clay minerals (mostly illit and vermiculite, as well as smaller portions of montmorillonite and kaolinit) are present in clay soil while sand soil contains only 5% of clay minerals. As clay minerals show the highest buffering potential for water and mineral elements, like alkali and earth alkali elements, soil types with higher clay mineral contents show high sorption capacities. Sandy loam/silt shows the highest fertility because of a harmonized ration between water storage and sorption capacities.

Water-retention potentials are of major importance for soil functioning. While sand soil has a water capacity of only 10 volume %, 2/3 of that water (7 volume %) is bioavailable. Loam has 45 volume % water capacity of which, however, only a small proportion, usually less than 12 volume %, is available to plant roots. Sand soil has the highest aeration with 30 volume % of gas-filled pores. The function of mineralic, climatic, and biological conditions over time leads to formation of soils defined by different horizons. These can extend over cm up to 100 m and more, depending on the conditions experienced.

Soil type
Soil types are distinguished depending on the development of soil (pedogenesis) over space and time. The formation of a soil type generally takes a long time, upward of 1000 years. The soil type also is dependent on the climatic conditions under which the pedogenesis has taken place. A typical example of this is the formation of laterite soils in tropical areas. Such soil types show strong coloration, with iron-rich red soil types dominating in most areas. This is due to mobilization and wash-out of elements released from minerals and retaining of insoluble iron(II)oxides.

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Horizons and soil profiles
A visual inspection of a soil profile already leads to immediate distinction of several layers in a typical soil. In an autochthonous soil formation, the lowest typical layer above groundwater is the C horizon, the base rock on which the soil develops. It presents the basis for the elemental composition of the soil developing on its surface.

The B horizon (subsoil) is the next layer, clearly dominated by weathering products. Immigration of clay and sesquioxides (Fe and Al-oxihydroxides) may be found to dominate such B horizons which is specified in Bv (weathering dominated), Bs (loam accumulation), and Bk (sesquioxides) subclassification.

The uppermost layer is the A horizon (topsoil) which is rich in humus resulting from degradation of leaf litter and often shows a darker color due to organic contents enriched in this horizon. The uppermost layer of humus also is specifically termed Ah horizon. In the A horizon, microbial populations are largest and metabolic activities of soil microorganisms can be shown easily by soil respiration analyses. The A horizon also is the site of dominant plant root penetration which again stabilizes and supports the soil structure as well as the microorganisms present. Again, this horizon can be subdivided into layers Ah or A1 (uppermost layer rich in humus), Ap (altered upper layer due to ploughing) and Ae or A2 (subsequent layer influenced by wash-out processes). A layer of litter above the topsoil horizon is formed by decaying plant material. Depending on soil type, not all horizons have to be present.

Soil biology
The animals found in soil, or edaphone, are earthworms, nematodes, insects, specifically collemboles, and moles, to name the most prominent. Their function in soil is especially to mix the soil, to draw leaves under the soil surface which accelerates decay because of higher humidity and better accessibility for microbes, and to form passages for water interflow allowing rain to enter a soil more easily.

Plant roots stabilize soil and protect from wind and water erosion. Decaying plant roots leave passages also allowing for preferential water flow. And living roots contain special tissues for aeration and therefore improve oxygen availability for microorganisms in soil. At the same time, up to 30% of plant net photosynthesis products can be excreted by the roots in the root exudates. These consist mainly of organic acids, like acetate, oxalate, malate, and citrate. These are able to feed microbes in the rhizosphere and also chelate iron and supply the plant with iron. On the other hand, these organic acids, as well as sugars and amino acids form root exudates, provide nutrition for soil microorganisms which multiply in the vicinity of roots. This constitutes the rhizosphere, the specific area found around a plant root (Watt et al., 2006). More directly associated with the plant root are the rhizoplague bacteria which colonize the surface of the plant root. Symbiotic root associations include the nitrogen-fixing rhizobial symbioses with leguminaceous plants, and the mycorrhizal mutual symbioses between fungi and plant roots. It has been stated that microbial populations structure the plant biodiversity seen above-ground by such soil-derived associations (van der Heijden et al., 2007).
The soil surface, if no plants are present, is often covered by a crust consisting of a biofilm with cyanobacteria, fungi, bacteria, and lichens. This crust will lead to covering of bare soils which will then be prone to succession with mosses and plants.

**Soil microbiology**

Soils are the major place of element turnover and biogeochemical cycling in terrestrial ecosystems (Crawford et al., 2005). The degradation of plant litter, decay of leaves, carcasses, feces, and wood rotting are maintained by soil microorganisms with subsequent processes of humification. In addition, denitrification, nitrogen fixation, and element mobilization are performed by the metabolic activities of soil microorganisms. The formation of humus and the binding of elements with humic and fulvic acids drive soil development, and its function for the soil ecosystem cannot be overestimated.

Populations of soil microorganisms contain eukaryotes, bacteria, and archaea. Depending on the metabolic functions prevailing, the soil generated at the site will be dominated by formation of humic and fulvic acids, like in moors, by high mobilization of kations, and the formation of dissolved organic matter (DOC) in luvisols and podzols (in woodlands) or tschernozem (in agricultural land and grasslands). To have an idea of the scale, in 1 hectare soil it has been calculated that 8 tons of microorganisms are present, while the edophone makes only for 2 tons of biomass. With the smaller size of microbes, this makes for very high numbers of soil microorganisms consisting of eukaryotic, bacterial, and archaeal cells.

The eukaryotic population is dominated by fungi with minor populations of ciliates, flagellates, algae including oomycetes and amoebae including myxomycetes. Of special importance are the mycorrhizal fungi forming a mutual symbiosis with plant roots thereby influencing not only plant physiology, but also soil as the hyphae extending into soil can add to soil structure and texture (Rillig and Mummey, 2006).

The archaea found in soil include especially the anaerobic methanogens which can lead to methane exhalation. However, methane oxidation performed by other groups of related archaea, already sequester a large portion of methane in soils. Methane exhalation is more often seen from swamps and flooded areas.

The major taxa of soil bacteria are the Gram-negative proteobacteria, the cytophaga, and Gram-positive bacilli and actinobacteria. Microbial interactions are a driving force for the community structure (Wardle, 2006). Per gram dry weight of soil, usually $10^7–10^9$ bacteria are found. However, the bacterial population so far mainly had been analyzed by plating techniques, which will allow only those to be found that are growing under the applied laboratory conditions. Estimates of direct counts have indicated higher numbers with only 1–10% of bacteria being cultivable. Modern techniques relying not on cultivation but on DNA extraction from soil with subsequent amplification and cloning of 16S rDNA sequences give a better insight in microbial soil populations. New taxa occur by these analyses and the phylogenetic associations observed from the obtained 16S rDNA sequences show that entire branches in the phylogenetic tree of bacteria can be described. It will remain a task to now find possibilities to cultivate these organisms in order to evaluate their physiological properties. Only such new investigations will allow to assess the role of new classes of bacteria in soil function and specific pathways for microbial degradation and soil formation will be uncovered as such investigations and modeling making use of the results will progress (O’Donnell et al., 2007).

**Humification**

Soil organic matter is the basis for humification (Kögel-Knabner et al., 1990). The characteristics of soil organic matter have been analyzed in some detail (Schaumann, 2006). Humus (Stevenson, 1994) provides essential functions in soils. The formation of humic acids and fulvic acids is essential for $A$ horizon development, and especially the $A_h$ horizon of tschernozem is rich in humus. Both humic acids (brown and grey) and shorter-lived fulvic acids have been described as recalcitrant matter with turnover times of thousands of years. These figures are re-evaluated now as it becomes clear that microbial metabolism uses these recalcitrant substrates and incorporates the carbon moieties into their cell mass. However, it is clear that the humic and fulvic acids constitute a major portion of soil matter and that their specific function in water retention and mineral element adsorption contributes a great deal to soil fertility.

Humus constituents also are important for (temporal) fixation of minerals and organic exudates. Their release will be controlled by the microbiota prevalent in the environment (Watteau and Berthelin, 1994).

**Summary**

Soil is the basis of terrestrial life and both evolves from biological activities and in turn allows and shapes terrestrial ecosystems. Soil types can be distinguished with different compositions, functionalities, and fertilities for production in agronomy and forestry, or water quality. The development of a soil type, such as tschernozem, rendzina, and podzol (and in tropic conditions, latosol) is dependent on base rock, climate, physical and chemical weathering, and formation of humic substances which takes thousands of years. The pedogenesis, in addition, is a result of root functions and plant litter degradation. The resulting soil can be characterized in vertical direction by soil horizons, which are distinguished from the $A$ horizon with high humus contents, over a $B$ horizon with lower humus contents, to the $C$ horizon mainly consisting of base rock material. Soil series attempts to combine soil types and soil structure to reveal parameters for soil function.
Microorganisms exhibiting a high degree of phenotypic and genetic similarity; a pragmatic approach to demarcating “microbial species” using molecular biology tools has been 97% 16S rRNA sequence identity or 70% genome identity based on DNA-DNA hybridization; thus, microbial species are often defined by operational characteristics (operational taxonomic unit). The microbial species concept is still a matter of controversy. See entry “Microbial Communities, Structure, and Function” for further reading.

**SPECIES (MICROBIAL)**

Microorganisms exhibiting a high degree of phenotypic and genetic similarity; a pragmatic approach to demarcating “microbial species” using molecular biology tools has been 97% 16S rRNA sequence identity or 70% genome identity based on DNA-DNA hybridization; thus, microbial species are often defined by operational characteristics (operational taxonomic unit). The microbial species concept is still a matter of controversy. See entry “Microbial Communities, Structure, and Function” for further reading.

**SPELEOTHEMS**

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**Synonyms**
Cave deposits; Cave sinters

**Definition**
The word “speleothem” is from Greek and means “cave deposit.” According to its origin, this term can be used for any cave deposits, but it is used mostly for secondary mineral deposits formed (precipitated) in caves. In this entry, speleothem is understood as constructively formed, new cave material. Materials originated by destructive phenomena, such as erosion, weathering, etching, biocorrosion, or bioerosion are not subjects of this entry.

**Biogenic and abiogenic speleothems**
Most of the speleothems originate inorganically by precipitation of various minerals from solutions. The minerals are dissolved by undersaturated fluids and precipitate when the solutions become supersaturated, e.g., by evaporation, outgassing, or pH changes. Mineralogical compositions of speleothems vary depending on the environment in which the caves are formed. The most common speleothem-forming mineral is calcite (as most of the caves are formed of limestones), forming well-known sinter forms, such as dripstones (stalactites, stalagmites) and flowstones. Locally, mostly in hydrothermally influenced cavern systems, speleothems may be formed by its rhombic modification — aragonite. Gypsum and other sulfate minerals commonly form speleothems in gypsum karst but are widespread also in other types of caves. Speleothems formed in silicate rocks (e.g., sandstones, quartzites, granites, etc., Figure 1) are relatively rare, composed mostly of various forms of silica (from opal-A to microquartz depending on its recrystallization). Other speleothems can be represented by ferroan and manganese oxides and hydroxides forming cave flowstones.

**Cross-references**
Bacterial Clay Autigenesis
Biogeochemical Cycles
Carbon Cycle
Denitrification
Microbial Degradation
Microbial-Metal Binding
Mycorrhizae
Nitrogen Fixation
Siderophores
Overall inventory of cave minerals is very large (for a summary see Hill and Forti, 1986).

The formation of some speleothems is influenced by organisms. The organisms can be passively covered (encrusted) by speleothems or they can directly biologically mediate their formation. In some cases it is not easy to distinguish between the active and passive role of the organisms. Biological mediation of mineral precipitation usually involves biomineralization (biomineralization (general aspects); microbial biomineralization; silica biomineralization (sponges)) and/or organomineralization. Biomineralization means direct precipitation of mineral skeleton (either internal or external) or encrustation by the organism itself (syn vivo). Organomineralization in which the precipitation of minerals is mediated by organic matters produced by the organisms and by changing the ambient conditions (e.g., pH, enrichment or depletion of the solution by metabolism, etc.). They can occur either syn vivo (when the organisms are alive – they influence the ambient environment actively) or postmortem (after the organisms are dead – the ambient environment is influenced by the decay products of the organisms).

**Organisms which mediate speleothem formation**

The speleothem-forming organisms are mostly microorganisms (Archaea, Bacteria including Cyanobacteria, and Algae); more evolved multicellular organisms are...
mostly represented by Fungi, mosses and lichens. Also roots of higher plants, penetrating to caves can form speleothems. All the mentioned organisms form speleothems from the autochthonous cave material (mostly taken from solutions). Excrements of animals (e.g., bats) can turn to well-known cave deposit – guano. Unlike the previously mentioned speleothems, guano and its minerals (rich group of phosphates, nitrates, and organic minerals) are completely allochthonous in caves, introduced originally from outside.

Microbes

Microbes inhabiting caves are mostly chemolithoautotrophic or chemoheterotrophic, i.e., their nutrition is not dependent on sunlight (for review see Konhauser, 2007). The exception is the so-called lampenflora, represented by phototropic cyanobacteria and other algae which live on the lightened places in show caves. Phototrophic microorganisms normally inhabit the entrance areas and the number of microorganisms declines rapidly toward the twilight areas. Unicellular algae – diatoms – were found to contribute to the formation of speleothems, which occurred close to the entrance (Kashima et al., 1987; Kashima and Ogawa, 1995). Some cyanobacteria, although originally phototropic, can adapt themselves to darkness and change their life mode to heterotrophic. For instance, the genera Fisherella and Calothrix were found to be able to change their mode of life to slow heterotrophic in complete darkness (Whitton, 1987). Some cyanobacteria were found to inhabit dark cave environment e.g., Geitleria calcarea and Scytonema julianum (Friedman, 1955; Bourrelly and Depuy, 1973) and contribute to speleothem formation. Nostoc is also easily adaptable to a heterotrophic mode of life, and is a common symbiont (endobiont) in lichens and higher plants. In addition to photosynthesis, Nostoc is able to perform nitrogen fixation (symbiosis of higher plants with Nostoc provides them with nitrate). Nostoc has also been reported from caves in Israel, growing in various zones, from well illuminated to complete darkness (Vinogradova et al., 1998). Moreover, Nostoc is also supposed to form large opal stromatolites in the Venezuelan sandstone caves (Figure 1; Aubrecht et al., 2008).

The most common in cave environments are chemolithoautotrophic and chemoheterotrophic microorganisms. Similar to other microorganisms, they use CO₂ as their carbon source but their source of energy comes from different chemical reactions. Chemoheterotrophs utilize preformed organic compounds, whereas chemolithoautotrophs utilize mostly inorganic compounds (Konhauser, 2007). These organisms and processes they utilize are numerous but only some of them are important speleothem-forming factors. Sulfur-oxidizing bacteria, such as Beggiatoa or Thiothrix, are responsible for deposition of elemental sulfur and sulfates in caves. They are particularly important in the caves with sulfur-rich waters, e.g., Parker Cave, Kentucky (Barton et al., 2001). Bacteria also mediate formation of iron oxide and manganese oxide flowstones (Gradziński et al., 1995). Finally, they may also mediate formation of the carbonate speleothems, which are the most widespread speleothems in the world (Taborośi, 2006).

Fungi

Fungi are ubiquitous in all caves, being mostly chemoheterotrophs. They not only inhabit the caves but they actively contribute to the formation of some carbonate speleothems, such as a particular, soft form of sinter – moonmilk (Gradziński and Szulc, 1997; Gradziński et al., 1997).

Mosses

Mosses (often together with lichens) occur mostly in the near-entrance areas and are mostly passively encrusted by minerals (mostly calcite), thus forming moss tufas (Taborośi, 2006).

Plant roots

In shallow-placed galleries, plant roots sometimes penetrate from the surface. They are often mineralized, forming the so called rooticles (Taborośi, 2006). It is not yet known whether they represent passive encrustations, or the roots actively mediate the precipitation. This is also true for other plant (and animal) remnants which are locally transported to the caves from the surface.

Marine organisms forming speleothems

Marine organisms represent a special chapter in the genesis of organic-mediated speleothems. Marine water, bringing nutrition to submarine caves, enables many organisms to live in this cryptic environment. Sessile organisms overgrowing the cave walls easily turn into speleothems. Very common are cryptic stromatolites (Reitner, 1993) formed by nonphotosynthetic organisms or serpulid buildups (Taborośi, 2006; Schlägl et al., 2008) formed by these filter feeders in submarine caves.

Methods of recognition of the speleothem-forming microorganisms

Cave-dwelling microorganisms, including those which contribute to speleothem formation, are considered to be extremophiles. That means they are organisms living in and adapted to environments which are not prolific for many organisms and which lack many aspects necessary for life; in caves, it is mainly the lack of sunlight and generally oligotrophic character of the environment. Adaptation of these organisms to the cave environment makes them uneasy to be determined by classical microbiological methods (Barton, 2006). For instance, for the complete identification of cyanobacteria, about 37 characteristics are needed, e.g., cell morphology, ultrastructure, morphology of colonies or filaments, genetic characteristics (DNA), cultivation conditions, and life conditions (Castenholz, 2001). Cultivation was so far the most
important tool in microbiology. This tool was developed mostly for medical purposes and was optimized for the research of microbes inhabiting animal bodies. However, only a small portion of free-living microbes is cultivable from environmental samples, including caves. Therefore, new culture-independent in situ methods have been developed for the detection of microbial groups in natural microbial assemblages, based on the molecular analysis of their DNA (Barton, 2006). Among these methods, one of the most important is in situ hybridization (FISH). Biomarker analysis may also be a suitable tool for the analysis of speleothems, in which only organic matter remained after dead, decayed organisms.

**Conclusion**

Speleothems are secondary mineral deposits formed in caves. They can form either inorganically or originate biologically. The inorganic formation pathway is based on physical precipitation from solutions after they become supersaturated. Biologically mediated precipitation is influenced by organic matter produced either by living or dead organisms and does not necessarily involve supersaturated solutions. This kind of precipitation acts via biomineralization or organomineralization, and may result in various sorts of minerals, such as calcite, elementary sulfur, sulfates, iron and manganese oxides, etc. The main biological protagonists forming speleothems are microbes. They are mostly chemolithoautotrophic and chemoheterotrophic microorganisms, or phototropic organisms that changed their life modes and adapted themselves to the cave environment. Normally, the phototrophs are active only in near-entrance pars of caves. Other organisms forming speleothems are mosses, lichens, higher plants, or animals. These are however, mostly passively encrusted by minerals; their potential for active biological mediation to form speleothems is still the matter of research.

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SPONGES (PORIFERA) AND SPONGE MICROBES

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Synonyms
Sponge-associated bacteria; Sponge-associated microbes; Sponge-associated microbota; Sponge bacteria; Sponge–microbe systems; Sponge symbionts

Definition
Sponges are sessile multicellular aquatic animals (Lat. Porifera = bearing pores) whose bodies have a typical body plan which allows water to pass through the animals. Sponges are characterized by a skeleton made of calcareous, siliceous spicules or horny fibers.

Sponge microbes: Bacteria and archaea that live in close and permanent association with sponges, usually in the mesohyl between sponge cells.

Introduction
Sponges are sedentary benthic organisms found in shallow waters on tropical coral reefs to the Arctic and the deep sea. Some species, however, are restricted to freshwater ecosystems. Sponges are characterized by a typical body plan built around a system of water canals and chambers (Figure 1a). Within the chambers, flagellated cells called choanocytes produce a water current that enters the sponge body through surface pores (ostia) and leaves through larger openings called oscula. Hence, sponges are filter feeders and incorporate particles from the water as energy and nutrient source. An external cell layer (pinacoderm) encloses the sponge mesohyl, a glycosidic matrix containing several cell types which perform a variety of functions. Sponge cells show a low degree of specialization and a high degree of independence so that the sponge body in some respect resembles a protozoan colony. However, sponges are, without any doubt, placed as true members of the Metazoa (Ax, 1995; Müller, 1998a, b).

Sponges have been grouped into three classes: Calcarea, Hexactinellida, and Demospongiae. Calcarea are characterized by the presence of calcareous spicules. The upgrade of this class to the phylum level has recently been proposed by Borchiellini et al. (2001). Hexactinellida are characterized by siliceous spicules of hexactine structure and syncytial tissue organization.

Demospongiae, the most numerous and diverse class, is a nonmonophyletic group (Boury-Esnault, 2006). They generally have a mineral skeleton made of siliceous spicules, but several lineages, like the common bath sponge, have no mineral skeleton but a network of fibers instead. Modern sponge phylogeny is now based on a combination of molecular methods and sponge morphological features, but sponge spicules are still important features for species determination and identification of sponges in the fossil record.

Sponges form one of the deepest radiations of the Metazoa and can be regarded as one of the oldest animal phylum still alive. The spicule record for sponges starts in the late Proterozoic (Reitner and Wörheide, 2002), while chemofossil records even indicate the presence of sponges – or their direct ancestors – already in the early Proterozoic: specific C30 steranes (24-isopropylcholestenes), which are unambiguous biomarkers for demosponges, were found in 1,800 Ma old stromatolites (McCaiffrey et al., 1994; Moldowan et al., 1994).

Choanoflagellates, a monophyletic group of protists which can show a colonial lifestyle, are the closest sister group of the Metazoa (Medina et al., 2001) and show a morphological resemblance to the flagellated choanoocytes of sponges. It is, therefore, widely agreed that the “urmetazoa,” the last common ancestors of all Metazoa, would have many traits found in sponges (see review in Gaidos et al., 2007).

Numerous sponge species host vast amounts of microbes in their mesohyl matrix (Figure 1b and c) or sometimes in specialized cells, while others contain only few or no microorganisms at all. The former have been termed bacteriosponges (Reiswig, 1981) or high-microbial-abundance sponges (Hentschel et al., 2003) while the latter are referred to as “low-microbial-abundance sponges.” Bacterial population densities in bacteriosponges may reach 10^8–10^10 microbes per gram of sponge wet weight, while those in low-microbial-abundance sponges are within the range of natural seawater.

The first studies exploring the microbial community of sponges were based on electron microscopy and isolation of microbes and yielded invaluable information about the microorganism morphotypes and their specific location within the sponge (e.g., Wilkinson, 1978). Microbes were found to exist extra- and intra-cellularly (e.g., Vacelet and Donadey, 1977) and even inside the nucleus of sponge cells (Vacelet, 1970). More recently, the advent of molecular techniques have enabled a detailed phylogenetic description of the microorganisms associated with sponges (Hentschel et al., 2006), whereas environmental genomics have given insights into their metabolic and physiological properties (Grozdanov and Hentschel, 2007). Phylogenetically complex, yet highly sponge-specific microbial communities were identified in numerous species over large geographical ranges (Hentschel et al., 2002). Sequences representing 16 bacterial phyla and both major archaeal lineages have been recovered to date (see...
review by Taylor et al., 2007; Hentschel et al., 2003, 2006). One of these phyla, the Poribacteria, represents a new bacterial phylum, which is related to the Planctomycetales and has, so far, only been found in sponges (Fieseler et al., 2004, 2006). The other microbial phyla found in sponges are also found in other environments; however, many sponge microbes seem to form, within these phyla, monophyletic, sponge-specific 16S rRNA sequence clusters, which are absent in seawater (Taylor et al., 2007; Hentschel et al., 2003, 2006).

These recent review articles provide an excellent summary of our current knowledge about the diversity and phylogenetic affiliation of sponge microbes, and this topic will therefore not be addressed here in more detail. This article, in contrast, aims to focus on the geobiological aspects of the sponge–microbe system: the question of a long-standing relationship between sponges and microbes; the consequences of fluctuating oxygen concentrations in sponge tissue for the sponge–microbe system; and the nature and possible function of microbial processes within sponges.

**Sponge microbes: secret passengers through evolutionary history?**

Did sponges and their associated microbes have an ancient partnership which remained unchanged since the origin of sponges (Precambrian), or is it a more recent but stable association which pertains to date? In an extensive review of this topic, Taylor et al. (2007) propose three scenarios which may explain the occurrence of sponge-specific

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*Sponges (Porifera) and Sponge Microbes, Figure 1* The sponge–microbe system (a) schematic representation of a sponge with microbes in the mesohyl (b) fluorescence micrograph of a DAPI stained tissue section of the sponge *G. barretti*, CC = choanocyte chamber, Ca = canal, Ce = sponge cells. Large DAPI signals represent nuclei of sponge cells, while small signals represent sponge microbes. Scale bar = 20 μm (c) transmission electron microscope image of the sponge *G. barretti* showing sponge cells (lower right corner) and numerous, morphologically different sponge microbes. Scale bar = 5 μm.
microbes. Firstly, it can be the product of a symbiosis of ancient origin perpetuated through the vertical (i.e., from adult to embryo) transmission of sponge-specific microorganisms. Secondly, the association can be the product of environmental acquisition through specific (immune system recognition, presence of favorable substrate, or bacterial protective capsule) or unspecific enrichment (rare sea-water microbes get trapped, accumulated and become thus detectable in the sponge). Finally, a combination of vertical and environmental transmissions is also possible.

The strongest evidence for a long-standing, close symbiotic relationship between sponges and some microorganisms comes from the demonstration of coevolution by comparative phylogenetic studies of sponges and their associated microbes. Erpenbeck et al. (2002) used a mitochondrial marker, cytochrome oxidase subunit 1 (CO1) and showed that the derived phylogenetic trees of four out of six symbionts were largely congruent with a tree containing sequences from the corresponding host sponges. This suggests that cospeciation had occurred (Erpenbeck et al., 2002). A subsequent study indicated a high degree of host specificity between the filamentous cyanobacterium Oscillatoria spongeliae and various dictyoceratid sponges (Thacker and Starnes, 2003). When comparing the phylogeny of 23 geographically, morphologically, and environmentally distant sponge species (based on their 28S rDNA gene sequences) and their associated archaea (based on 16S rDNA), a genus-specific association of these sponges with Crenarchaeota of the Marine Group I was apparent (Holmes and Blanch, 2007). These results suggest close coevolution and thus, a long-term symbiosis between these archaea and their sponge hosts. Coevolution requires that the host and symbiont maintain close association over evolutionary time. Vertical transmission, the mechanism through which coevolution could occur, has been shown for numerous sponge species belonging to all three classes of Porifera (Ereskovsky et al., 2005; Sharp et al., 2007; Schmitt et al., 2007; and references therein), including in species with highly varied reproductive strategies.

A pitfall for these comparative phylogenetic studies is that the phylogeny of sponges themselves is not yet fully resolved. This is particularly true for the Demosponges where phylogeny from the subclass to the family level is still in a state of flux (Boury-Esnault, 2006). Accordingly, our understanding of symbiont evolution in sponges will continue to develop only in parallel with improvement of our knowledge about host phylogeny. A recently initiated CO1 sequencing project for taxonomically diverse sponges (www.spongebarcoding.org) may provide data to achieve this goal in the future.

Further support for a possible ancient origin of the sponge–microbe system comes from biomarker studies. Various fatty acids of probable microbial origin occur in a wide range of sponges, irrespective of host phylogeny or geographic location (Thiel et al., 1999, 2002). The apparent absence of some of these biomarkers from marine sediments and seawater led to the suggestion that the fatty acids and their microbial producers have been present in the sponges since ancient times.

The final type of evidence for an ancient and close association between sponges and microorganisms comes from the fossil record. Reef mounds constructed by siliceous sponges and cyanobacterial mats in the early Cambrian show that sponges and microbes closely coexisted hundreds of millions of years ago (Brunton and Dixon, 1994). Whether these microbes actually lived within the sponge tissue, as modern sponge microbes do, remains, at this stage of our knowledge, unclear.

In other words, Precambrian microorganism acquisition which started as a mere hypothesis is gaining support through studies of phylogeny, biomarkers, and fossil record. Thus, there is increasing evidence for a long-standing coevolution between sponges and certain microbial lineages, which may even date back to the roots of sponge evolution in the anaerobic, microbe-dominated world of the Proterozoic. There is evidence that a close association between sponges and bacteria may even have provided some of the genetic source material for Metazoa evolution (Lakshminarayan et al., 2004).

The alternative scenario, environmental acquisition, can, however, not be ruled out for certain sponge microbes or, in some cases, may be the more likely explanation. In this scenario, it is assumed that sponge-specific microbes are in fact also present in seawater but that our techniques fail to detect them because of their rarity. In the sponge, specific enrichment of those rare microorganisms could occur as a result of a selection through the sponge immune system (Müller and Müller, 2003) or because those microbes which possess a capsule are protected from sponge cells phagocytosis and would increase in number within the sponge body, thus becoming detectable. Unspecific enrichment is also possible and could happen through the ability of sponges to filter large amounts of water through their body. The likelihood of rare microbes being detected by our current techniques is higher in a sponge than in seawater as they might accumulate and be present in high enough numbers to be found.

The third scenario proposed by Taylor et al. (2007) is a combination of both vertical and horizontal symbiont transmissions (parental and environmental acquisition, respectively) which seems the most likely scenario to explain microbial diversity that we find in sponges today (Hentschel et al., 2002).

**An anaerobic world in sponges**

Until recently, sponge metabolism was viewed as being based on aerobic respiration, similarly to all Metazoa. Oxygen is usually supplied in excess to the sponge body through the water current created by the choanocytes (flagellated cells) (Reiswig, 1974). The remarkable ability of sponges to pump large amounts of water through their bodies has led to the assumption that permanent oxygen saturation exists within the sponge body. However, the
presence of anaerobic microbes in some sponge species hinted toward the presence of spatial or temporal anoxic niches (e.g., Webster et al., 2001; Hoffmann et al., 2005a; Schläppy et al., 2010a).

Gatti et al. (2002) and Schönberg et al. (2004) were the first to use microsensors to investigate the oxygen distribution in the tissue of living sponges at a macroscale. Gatti et al. (2002) used oxygen micro-optodes on sponges’ primorphs (multicellular aggregates composed of previously dissociated single sponge cells) and described oxygen deficiency in *Suberites domuncula*. Clark-type oxygen microelectrodes have since been used to explore the fine-scale oxygen dynamics in several sponge species from the Mediterranean and the North Atlantic. Anoxic regions have been detected in the cold-water sponge *Geodia barretti* (Hoffmann et al., 2005a) and in its explants (Hoffmann et al., 2005b), as well as in the Mediterranean sponges *Dysidea avara* (Schläppy et al., 2007) and *Chondrosia reniformis* (Figure 2) kept in aquaria or cultivation tanks with recirculating seawater.

Similarly, fluctuating oxygen concentrations leading to anoxia lasting up to 1 h were also found in *Dysidea avara* in the field when measured with an oxygen microelectrode applied by SCUBA diving (Schläppy et al., 2010a). A positive correlation between pumping activity and oxygen content was described in the tissue and in the exhaled water of *Dysidea avara* (Schläppy et al., 2007) and *Aplysina aerophoba* (Hoffmann et al., 2008). When the sponge ventilated, its body was oxygen saturated. Figure 2a shows a typical profile of a pumping sponge, with oxygen saturated water above the sponge surface and near-saturated water within the sponge tissue (compare also Hoffmann et al., 2007; Schläppy et al., 2007).

Oxygen concentrations decreased dramatically when pumping activity ceased. After 15 min without ventilation, the entire sponge body was anoxic with the exception of a 1 mm surface layer where oxygen penetrated due to molecular diffusion over the sponge surface (Hoffmann et al., 2008).

Oxygen profiles are typically diffusive over the surface and into the tissue of a nonpumping sponge (Figure 2b) (compare also Hoffmann et al., 2005b, 2008; Schläppy et al., 2007, 2010a). In situ, many sponge species reduce or even stop their pumping activity for several hours at irregular intervals (Reiswig, 1971; Vogel, 1977; Pile et al., 1997). It remains unclear whether the interruption of ventilation is caused by external events such as, for example, a high sediment load in the water (Gerodette and Flechsig, 1979; Leys et al., 1999), decrease in salinity (Fell et al., 1989), or by an intrinsic rhythm specific to a species (see Reiswig, 1971). In nonpumping sponges, molecular diffusion across a diffusive boundary layer at the sponge surface is the only source of oxygen.

The consequences of these findings are twofold. Firstly, all sponge cells and microbes which lay as deeper as 1 mm below the sponge surface need to be able to survive without direct and regular access to oxygen. Since a lack of ventilation causes tissue anoxia, the parts of the sponge body which are not affected by surface diffusion must be alternately exposed to oxic and anoxic conditions. Secondly, by modulating the presence or absence and the magnitude of water flow through their bodies sponges have the possibility of actively switching between aerobic and anaerobic metabolism. It follows that sponge-associated microorganisms must be able to tolerate both situations and their metabolism may be activated or inactivated depending on the oxygen concentration in the sponge. This means that anaerobic microbial processes, such as sulfate reduction, for example, may take place (Hoffmann et al., 2005a; but see Schläppy et al., 2010b).

Another possible consequence of varying oxygen concentrations in sponge tissue is reported by Müller et al. (2004a, b). Using *Suberites domuncula* and its alphaproteobacterial symbiont (SB2) as a model system, they showed that the expression of genes which were essential for metabolic interaction of microbe and host was maximal under aerated conditions. Coupled with the observed loss of SB2 cells from the sponge surface under low-oxygen conditions, it was concluded that the oxygen level is responsible for regulating the bacterial fauna in *S. domuncula*. Whether this type of mechanism is equally important in other sponge–microbe systems remains to be investigated.

Tissue anoxia in sponges may act as a controlling factor toward the number of anaerobic sponge-associated microbes and act as a regulator of the nature and extent of their metabolic activity. Alternatively, it may act as a mechanism through which the sponge can kill undesirable seawater microbes. Regardless of whether anoxia in sponges is intentional or inevitable, many sponges can survive long periods of tissue anoxia and either tolerate or even foster anaerobic microbes and corresponding anaerobic microbial activity within their bodies. A close association with a diverse microbial community including (facultative) anaerobic or microaerophilic microorganisms may have been particularly advantageous at a time when fluctuating oxygen conditions were present on the Earth.

**Interactions between sponges and sponge microbes**

While our knowledge about phylogeny and metabolic capacities of the sponge microbes continuously increases, the role that microbes play for the sponge host is still a matter of debate. Microbes undoubtedly benefit from the nutrient-rich and protected environment of the sponge mesohyl, but the benefit to the sponge host remains unclear.

Sponge microbes may consume carbon sources which are not accessible to the sponge and may be subsequently ingested by the sponge cells. This process is commonly known as “microbial farming” and is well described (Wilkinson and Garrone, 1980; Ilan and Abelson, 1995; Vacelet et al., 1996 and own observation). Autotrophic
symbionts transform inorganic carbon (DIC) into organic carbon, which may then be transferred to the host. Energy transfer between symbiont and sponge is best described for phototrophic symbionts (e.g., cyanobacteria: Wilkinson, 1983). Chemolithotrophic processes, such as nitrification, are similar in principle. The energy which is needed for carbon fixation, however, is gained by a chemical process, the aerobic oxidation of ammonium to nitrite first and then of nitrite to nitrate. The presence of nitrifying microbes as well as the transformation of ammonium to nitrite and nitrate have been demonstrated in many sponge species (e.g., Diaz and Ward, 1997; Diaz et al., 2004; Bayer et al., 2007; Schläppy et al., 2010b). In addition to carbon fixation, these microbes efficiently remove the sponge waste product ammonium and the toxic nitrite. Heterotrophic microbes consume simple compounds (dissolved organic carbon, DOC), while sponge cells prefer small particles such as pelagic bacteria or phytoplankton (Willenz, 1980; Pile et al., 1996; Witte et al., 1997; Ribes et al., 1999). Some sponges, however,
have been identified as important DOC sinks (Yahel et al., 2003; de Goeij et al., 2008). Interestingly, these sponges host high amount of associated bacteria. This leads to the conclusion that DOC is consumed by the sponge microbes, which in turn may be consumed by the sponge cells. A diverse community of associated microorganisms with high metabolic diversity is able to metabolize a wide array of simple molecules and to transfer them into biomass as a food resource for the sponge.

The second possibility for a mutual interaction between sponges and microbes lies within the production of secondary metabolites. Sponges produce a large variety of bioactive compounds (Blunt et al., 2003; Paul and Puglisi, 2004), which act as defenses against surface fouling, infection, or predation. Sponge microbes, which are obviously not harmed by the antimicrobial metabolites, are in fact often the actual producers of these compounds (Hildebrand et al., 2004; Piel, 2004, 2006). In addition to protecting the sponge, secondary metabolites act as a controlling factor in the host–microbe interaction.

At least some groups of sponge microbes are clearly beneficial to the sponge host, while others may be commensals.

**Summary**

Sponges are amongst the most ancient Metazoans on the Earth and are distributed over a wide range of aquatic ecosystems. Phylogenetically complex, yet highly sponge-specific microbial communities live in close association with numerous sponge species. A combination of both vertical and horizontal symbiont transmissions (parental and environmental acquisition, respectively) seems the most likely scenario to explain microbial diversity that we find in sponges today. Tissue anoxia is a feature found in several sponges species and is related to sponge pumping activity. Sponge microbes with autotrophic, heterotrophic, aerobic, or anaerobic metabolisms may contribute to the nutrition of the host. Other interactions between sponges and sponge microbes involve the synthesis of secondary metabolites. Due to modulation of internal oxygen concentration and the production of secondary metabolites, sponges are able to control their microbial communities. While our knowledge about the phylogeny of sponge microbes is increasing rapidly, many questions concerning the activity of sponge microbes and their interaction with the host sponge still remain unanswered. Future challenges will involve linking microbial phylogeny to microbial function on a macroscale within the sponge tissue, and to examine the complex interactions within the sponge–microbe system from the origin of Metazoan life until today.

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STROMATACTIS

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Definition
Stromatactis is defined as a mass of spar (with partial substitution of internal sediment) which has smooth base, digestive roof, occurs in swarms and has reticulate distribution (Bathurst, 1982). Neuweiler et al. (2001) distinguished the terms “stromatactis,” “inhibited stromatactis,” and “aborted stromatactis” (Figure 1). Stromatactis originated when early internal sedimentation occurred in the open space of the cavity network followed by centripetal cementation by early marine cements in residual cavity space. Aborted stromatactis resulted from internal sediment filling of the entire or near-entire original cavity network. Inhibited stromatactis refers to incipient marine cementation of the stromatactic cavity followed by entire or near-entire internal sediment filling.

Stromatactis often (but not always) occur in mudmounds (see entry Mud Mounds). Spar bodies similar to stromatactis but not fitting to all the points of this definition are termed in literature as “stromatocoid” or “stromatactis-like” bodies. Similar to stromatactis but smaller are fenestral pores occurring in intertidal limestones. Similar to limestones with stromatactis structures are “zebra limestones” in which the spar bodies are flat, straight, elongated, and the individual spar layers are not always interconnected.

Evolution of the term stromatactis
The term stromatactis was first used by Dupont (1881, 1882). Until present, there is still no agreement in the way of its origin. There are two main groups of opinions about the origin of stromatactis: abiogenic and biogenic. Most researchers agree that stromatactis originally represented cavities which were later filled with internal sediment and sparry calcite. The suggested abiogenic origins for stromatactis included internal erosion and reworking of small cavities (e.g., Kukal, 1971; Wallace, 1987; Bridges and Chapman, 1988; Matyszkiwicz, 1993, 1997), dewatering or escape of fluids (Heckel, 1972; Desbordes and Maurin, 1974; Bernet-Rolland et al., 1981), slumps (Schwarzacher, 1961), and freshwater karstification (Dunham, 1969). Most recent ideas involve frozen clathrate hydrates in the calcareous mud, after which the stromatactis cavities remained (Krause, 2001) or the cavities are interpreted as a result of sedimentation of stirred polydisperse sediment (Hladil, 2005; Hladil et al., 2006; Hladil et al., 2007). Some researchers supposed that stromatactis did not represent cavities and originated either by neomorphism or recrystallization of the calcareous mud (Black, 1952; Orme and Brown, 1963; Ross et al., 1975) or by dynamic metamorphism (Logan and Semeniuk, 1976). Among the opinions
favoring biogenic origin, the most widespread interpretation is that they are cavities which remained after decomposition of an unknown soft-bodied organism or by neomorphism of carbonate-secreting organism. The suggested organisms include stromatoporoids (Dupont, 1881, 1882; Lowenstam, 1950; Carozzi and Zadnik, 1959), bryozoans (Textoris and Carozzi, 1964), algae (Philcox, 1963; Textoris, 1966; Coron and Textoris, 1974), stromatolites (Cross and Klosterman, 1981), microbial colonies (Tsien, 1985), and burrowing activity of crustaceans (Shinn, 1968). The organisms that are most frequently considered to be involved in stromatactis formation are sponges. The sponge theory was first suggested by Bourque and Gignac (1983), followed by Bourque and Boulvain (1993), Neuweiler et al. (2001), and Delecat and Reitner (2005). Aubrecht et al. (2009) provided evidence that sponges were indeed involved in stromatactis formation within Middle Jurassic mud-mound deposits. Some authors suggested an opinion that a combination of several processes played role in the onset of stromatactis, such as microbial binding of the sediment and excavating of the unbound mud (Bathurst, 1982; Pratt, 1982) or a succession of sponges and microbial colonies (Flajs and Hüssner, 1993; Flajs et al., 1996).

**Stratigraphic distribution of stromatactis**

Stromatactis cavities are time-dependent structures (Bosence and Bridges, 1995; Flajs and Hüssner, 1993; Neuweiler et al., 2001). The age of stromatactis cavities ranges from the Neoproterozoic (Pratt, 1995) and Cambrian (James and Gravestock, 1990), through a maximum in Devonian and Carboniferous, up to the late Jurassic (Matyszkiewicz, 1993, 1997; Jansa et al., 1989;
Pratt, 1995; Aubrechet et al., 2002). Some younger occurrences of problematic stromatactis-like cavities from the Aptian–Albian of northern Spain were reported by Pascal and Przybyla (1989), Pratt (1995), and Neuweiler et al. (1999). Even younger stromatactis-like cavities occur in Lower Albian mud-mounds of the Pyrenees (Canoerot, 2001), in Turonian mud-mounds of Tunisia (Camoin and Maurin, 1988), in late Cretaceous methane-seep mounds of Tepee Buttes, USA (Kauffman et al., 1996), and in Holocene lithoherms found in the Straits of Florida (Neumann et al., 1977).

Neuweiler et al. (2001) ascribed the stratigraphic dependance and rare occurrences of stromatactis in Meso–Cenozoic sediments to the taphonomy of younger sponge taxa which was different from the Paleozoic. The post-Paleozoic taxa were prone to more rapid decay resulting in common cavity collapse and sediment filling (inhibited and aborted stromatactis).

Conclusions
Stromatactis is a mass of spar with smooth base, digitate roof, occurring in swarms, and having reticulate distribution. Various forms of stromatactis were distinguished: stromatactis, inhibited stromatactis, and aborted stromatactis, where the latter two refer to stromatactis cavities partly or completely filled by internal sediment.

There is still no agreement on how stromatactis originated. There are two main groups of opinions: abiogenic and biogenic. The theories on an abiogenic origin involved internal erosion and reworking of small cavities, dewatering or escape of fluids, slumps, dynamic metamorphism, fresh-water karstification, neomorphism, or recrystallization of the calcareous mud, frozen clathrate hydrates after which the stromatactis cavities remained, or the cavities were interpreted as a result of sedimentation of stirred polydisperse sediment. The biogenicity theories preferred the opinion that the stromatactis cavities remained after decomposition of an unknown soft-bodied organism including various hypothetical representatives, such as stromatoporoids, bryozoans, algae, stromatolites, microbial colonies, and burrowing activity of crustaceans. The most favorable are sponges for which direct evidence was found.

Stromatactis is time-dependent. Its age ranges from the Neoproterozoic and Cambrian, through a maximum in Devonian and Carboniferous, up to the late Jurassic. Some but problematic younger occurrences were found in Cretaceous and Quaternary (lithoherms in the Florida Straits). The stratigraphic dependance and rare occurrences of stromatactis in Meso–Cenozoic sediments are considered to result from the taphonomy of younger, post-Paleozoic sponge taxa.

Bibliography


SUBSURFACE FILAMENTOUS FABRICS

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Synonyms

Definition
Subsurface filamentous fabrics (SFF, Figures 1–5) result from the encrustation of microbial filaments with minerals in subsurface geological environments, followed by oxidation of organic matter (Hofmann, 2007; Hofmann and Farmer, 2000; Hofmann et al., 2008). Encrustations of fibrous minerals may yield similar fabrics, but a biogenic origin is indicated by evidence for high flexibility, and a low variability of the smallest diameter of the filamentous forms, typically 1–3 μm. SFF textures vary from delicate mineral webs to massive rocks containing enclosed filaments, depending on the degree of mineral precipitation after initial filament encrustation in voids. SFF are most commonly found in voids in volcanic rocks and in the oxidation zone of ore deposits rich in reduced elements (S, Fe), but also occur in paleokarst environments (Baele, 1998; Feldmann et al., 1997; Kretzschmar, 1982), Mississippi Valley-type base metal deposits (Hofmann et al., 2008), cavities in fossils (Weitschat, 1986), mineral veins (Hofmann, 1989; Reitner, 2004; Trewin and Knoll, 1999), and cavities in impact rocks (Hofmann et al., 2008). Many of these occurrences show low-temperature hydrothermal influences. Historically, SFF have aroused the interest of researchers because of their often conspicuous nature. Examples of long-known forms are particularly the so-called moss agates, for which various interpretations have been proposed.

Subsurface Filamentous Fabrics, Figure 1
Characteristic example of pseudostalactitic SFF. Encrustation of mat-like, vertically draped fabric by goethite. From the oxidation zone of a sulfide deposit, Kintore open cut, Broken Hill, New South Wales, Australia. Scale in cm.

Subsurface Filamentous Fabrics, Figure 2
SEM image of goethite-encrusted microbial filaments from the oxidation zone (in contact with glacial till) of a pyrite-rich small orebody, Lengenbach, Binntal, Switzerland. Image width: 2.9 mm.

Subsurface Filamentous Fabrics, Figure 3
Image of filamentous forms enclosed within chalcedony in a fracture within a lithophysa in Tertiary rhyolite, Priddy Ranch, Oregon, USA. Image width: 4 mm.
been brought forward (Bowerbank, 1842; Daubenton, 1782; Razumovsky, 1835). Similarities do exist with some types of dendrites (present in some moss agates) and speleothems. Many examples of “stalactitic” minerals (e.g., goethite, chalcedony, chrysocolla) were formed around filamentous centers with diameters in the order of microns, much too small for true speleothems the size of which is governed by the diameter of a water droplet (Hill and Forti, 1997). Features discriminating SFF from similar fabrics are (Hofmann et al., 2008) (a) median width of filamentous substrate of <5 μm with narrow, near-normal distribution; (b) evidence of bending (e.g., U-loops) and aggregation to mat-like (biofilms), stromatolite-like, or rope-like fabrics; (c) filamentous cores show a high degree of irregular bending (>0.5°/μm); (d) filamentous cores show many direction changes (>10/mm);

Subsurface Filamentous Fabrics, Figure 4 Filaments encrusted by iron hydroxides, associated to numerous folded mats, and enclosed by chalcedony. Host rocks are basaltic volcanics of Jurassic/Cretaceous age. This assemblage is known as moss agate. Image width: 18 mm. Arts Bogd Uul, Mongolia.

Subsurface Filamentous Fabrics, Figure 5 Filaments encrusted by iron hydroxides, enclosed in a single quartz crystal. Paleokarst within Devonian limestones (mineralization probably of Tertiary age), Hohenlimburg, Germany. Image width: 2.8 mm.
Conclusions

SFF represent a common, but often overlooked, type of biosignature from subsurface environments. SFF often display macroscopically visible fabrics that are easily recognizable and may be regarded as subsurface microbialites and the most conspicuous expression of the deep biosphere, representing in some ways an analog to stromatolites from surface environments. The very common occurrence in a wide variety of different rock types demonstrates that SFF are a common expression of subsurface life, wherever filamentous forms generate a large amount of biomass in relatively short time, allowing mineralization before organic filaments are destroyed. Organisms involved were chemolithotrophs (based on oxidation of Fe, S), but may also include heterotrophs living on chemolithotrophic primary producers. The formation in subsurface environments implies that no photosynthesizing organisms are involved. SFF-like features represent a target in the search for past life on Mars (Hofmann, 2007), as they might potentially be found in nearly all rock types.

Cross-references

Biofilms and Fossilization
Biosignatures in Rocks
Chemolithotrophy
Deep Biosphere of the Oceanic Deep Sea
Fe(II)-Oxidizing Prokaryotes
Gallionella
Hydrothermal Environments, Marine
Hydrothermal Environments, Terrestrial
Karst Ecosystems
Microbialites, Stromatolites, and Thrombolites

Bibliography

Physiological groups

The most important substrates utilized by SRP are fermentation products as H₂, alcohols, and organic acids like acetate, propionate, and butyrate. While most sulfate reducers have a limited spectrum of substrates that support growth, new isolates have been obtained that metabolize a wide variety of compounds including hexadecane, toluene, and several types of substituted aromatics (Hansen, 1994). Physiologically, sulfate reducers can be separated in two groups: Representatives of the first group generally grow fast and oxidize organic substrates incompletely to acetate, while members of the second group, grow more slowly, but oxidize their substrates completely to CO₂. An important electron donor oxidized with sulfate as terminal electron acceptor is methane. However, so far, it has not been clarified whether sulfate reducers can oxidize methane directly or if they receive reducing equivalents in another form from an archaean partner.

Pathway of sulfate reduction

Sulfate reduction is a rather complicated process (Figure 1). First, the double-charged anion has to be taken up by specific transport systems. Then ATP is hydrolyzed to activate sulfate to adenosine phosphosulfate (APS). This is now reduced to (bi-)sulfite, which is reduced to sulfide by sulfite reductase. APS reductase and sulfite reductase are the key enzymes. The genes coding for them (apr and dsr) are often used in molecular studies to analyze phylogenetic or community structures of sulfate-reducing SRP.

Reduction of sulfate with H₂ has a ΔG° of −151 kJ/per mol of sulfate, which is about 1/6 of the value possible with oxygen as electron acceptor. Accordingly, there is not more than 1 ATP conserved per sulfate reduced.

Alternative electron acceptors

Most sulfate reducers can utilize other sulfur compounds than sulfate, often even with a better growth yield. Sulfate is an intermediate of sulfate reduction, but does not require an energy-dependent activation. Thiosulfate can be transformed to sulfide and sulfate in a single-step catalyzed thiosulfate reductase. Some sulfate reducers can also reduce elemental sulfur. Several sulfate reducers can utilize partially reduced compounds in the absence of an electron donor: Thiosulfate, sulfate, and elemental sulfur can be disproportionated to sulfate and sulfide, and the resulting (low amount of) free energy can be used for energy conservation (Bak and Cypionka, 1987).

Furthermore, some SRP can reduce nitrate or nitrite to ammonia, some can reduce metal ions as Fe³⁺, Mn⁴⁺ or even U⁶⁺ (Lovley, 1993), some can reduce CO₂ to acetate (homoacetogenesis), and many can grow by fermentation of simple organic molecules like pyruvate.

Several SRB have been found to reduce molecular oxygen at high rates. The process is coupled to ATP conservation. However, it appears to be a defense mechanism. Sustainable growth of sulfate reducers with O₂ as electron acceptor has never been observed (Cypionka, 2000).
Summary

Sulfate-reducing prokaryotes reduce sulfate to sulfide in an anaerobic respiration process. Sulfate reduction is ancient and found in Bacteria and Archaea. The main electron donors used by sulfate reducers are fermentation products. Sulfate reduction yields little energy and proceeds via several steps. Sulfate reducers are anaerobes, although some of them are able to reduce oxygen not coupled to growth. Alternatively, other sulfur compounds, nitrate or nitrite, and some oxidized metal ions can be used by some strains.

Bibliography


Cross-references

Acetogens
Anaerobic Oxidation of Methane with Sulfate
Archaea
Bacteria
Biogeochemical Cycles
Chemolithotrophy
Deep Biosphere of Sediments
Fe(III)-Reducing Prokaryotes
Sulfur Cycle
Sulfur Isotopes
SULFIDE MINERAL OXIDATION

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Synonyms
Sulfide-mineral weathering; Sulfide-ore oxidation

Definition
Sulfide mineral. A metal-sulfide compound, such as pyrite (FeS2), which forms at high temperature (>50°C) in well-crystallized veins or masses and at low temperatures (<50°C) in poorly crystalline and fine-grained particles. Oxidation. The chemical process of reacting with oxygen. More generally, the chemical process of removing electrons from an atom or group of atoms.

Introduction
Metal-sulfide minerals are valuable as ores for metals that have a wide variety of uses from jewelry to components in vehicles and electronic equipment. They are found primarily in hydrothermal mineral deposits that occur in numerous geologic environments. The most common sulfide mineral is pyrite; other important sulfide ore minerals include chalcopyrite (copper ore), molybdenite (molybdenum ore), sphalerite (zinc ore), galena (lead ore), and cinabar (mercury ore). When these minerals are exposed to the Earth’s surface, either through natural processes or as a result of mining activities, they react with oxygen in air in the presence of water to form drainage that is frequently acidic and detrimental to the environment because of the high metal concentrations. The general term for this water is acid-rock drainage. When it originates from mining activities, it is called acid-mine drainage (Nordstrom and Alpers, 1999). Acid-rock drainage is produced by numerous chemical and microbiological processes within a complex hydrogeological environment. Extensive research has been employed to understand these processes, so that mine-site remediation can be more cost-effective and long-lasting. Weathering of sulfide minerals leads to natural enrichment in copper, gold, and silver in a process known as supergene enrichment. Copper dissolves in the infiltrating water and precipitates when it reaches unweathered sulfides downgradient, enriching the original sulfides. Gold and silver are highly insoluble and are enriched in the residual iron oxide cap or gossan that forms above the unweathered ore deposit. Sulfide-mineral oxidation from both mining and natural weathering is a major source of both dissolved sulfate and dissolved metals in natural waters.

Metals extraction of primary ores and of waste piles from previous mining activities is also accomplished by hydrometallurgical techniques that have optimized sulfide-mineral oxidation by increasing the temperature of leaching, adding catalytic reagents, and bioleaching by adding microorganisms that are specially suited for increasing the rate of sulfide-mineral oxidation.

Chemolithotrophy
Acid-mine drainage has been known since the early days of mining by ancient civilizations and was documented by early Greek and Roman writers (Nordstrom, 2009). Oxidation of sulfide minerals by microorganisms, however, has a more recent history. Winogradsky (1888) recognized that microbes could actually derive metabolic energy from the oxidation of inorganic compounds. These microbes gain energy from the oxidation (or reduction) of inorganic compounds and acquire their carbon needs from the carbon dioxide in the air (autotrophy). Hence, many of them are chemolithioautotrophs or chemoautotrophs for short. During the first quarter of the twentieth century, it was suspected that microbes affected the oxidation of sulfide minerals, but it was not until Rudolfs and Helbronner (1922) demonstrated that microbes could oxidize zinc sulfide, and Colmer and Hinkle (1947) isolated Thiobacillus ferrooxidans, now Acidithiobacillus ferrooxidans, from acid-mine drainage that microbes were recognized to be important catalysts in the production of acid-mine drainage from sulfide-mineral oxidation.

Biogeochemistry
The overall reaction of a metal sulfide like pyrite with air and water can be represented by the equation:

\[
\text{FeS}_2(s) + \frac{3}{2}\text{O}_2(g) + \text{H}_2\text{O}(l) \rightarrow \text{Fe}^{2+}(aq) + 2\text{SO}_4^{2-}(aq) + 2\text{H}^+(aq)
\] (1)

Pyrite + oxygen + water → acid ferrous sulfate solution.

The acidity originates from the protons needed to balance out some of the loss of electrons from iron and sulfur oxidation. The dissolved ferrous iron further oxidizes to ferric iron:

\[
\text{Fe}^{2+}(aq) + \frac{3}{4}\text{O}_2(g) + \text{H}^+(aq) \rightarrow \text{Fe}^{3+}(aq) + \frac{1}{2}\text{H}_2\text{O}(l)
\] (2)

Dissolved ferric iron easily hydrolyzes and precipitates as a colloidal phase, usually a mixture of fine-grained minerals, represented by either ferric hydroxide or commonly called hydrous ferric oxide (HFO):

\[
\text{Fe}^{3+}(aq) + 3\text{H}_2\text{O}(l) \rightarrow \text{Fe(OH)}_3(s) \downarrow + 3\text{H}^+(aq)
\] (3)

Dissolved ferric iron is the oxidant that directly attacks the pyrite surface so that the more correct way to write the reaction would be:

\[
\text{FeS}_2(s) + 14\text{Fe}^{3+}(aq) + 8\text{H}_2\text{O}(l) \rightarrow 15\text{Fe}^{2+}(aq) + 2\text{SO}_4^{2-}(aq) + 16\text{H}^+(aq)
\] (4)

Because dissolved ferrous iron oxidizes (Equation 2) much more slowly than the oxidation of pyrite by dissolved ferric iron (Equation 4), the oxidation of pyrite
would become negligible were it not for chemolithotrophic microorganisms. Many types of bacteria and archaea have been found to gain energy from the oxidation of ferrous iron and reduced sulfur in acidic environments (Norris, 1990; Nordstrom and Southam, 1997; Bond et al., 2000; Ehrlich, 2002). Some bacteria, such as *Acidithiobacillus ferrooxidans*, are capable of oxidizing both ferrous iron and reduced sulfur compounds (hydrogen sulfide, elemental sulfur, thiosulfate, and tetrathionate). Other bacteria, such as *Leptospirillum ferrooxidans*, are only able to oxidize ferrous iron and some bacteria, such as *Acidithiobacillus thiooxidans*, are only capable of oxidizing reduced sulfur compounds. Iron-oxidizing archaea include *Acidianus brierleyi*, *Sulfolobus acidocaldarius* (Nordstrom and Southam, 1997), *Ferroplasma acidiphilum* (Golyshina et al., 2000), and *Ferroplasma acidarmanus* (Edwards et al., 2000). Although *Acidithiobacillus ferrooxidans* has been the subject of more studies, *Leptospirillum ferrooxidans* seems to be the more common species in acid-mine drainage (Rawlings et al., 1999; Bond et al., 2000).

The geochemistry of sulfide-mineral oxidation and its effect on surface-water quality can vary substantially depending on the composition and grain size of the sulfide minerals. Common sulfide minerals are usually either disulfides like pyrite (FeS$_2$) and molybdenite (MoS$_2$) or monosulfides like sphalerite (ZnS) and galena (PbS). Molybdenite is hydrophobic and rather insoluble so that it oxidizes and weathered very slowly whereas sphalerite is more soluble and readily reacts with acid water to produce hydrogen-sulfide gas. The grain size of these minerals can greatly affect their oxidation and dissolution rate. The smaller the grain size, the faster they react. Hence, fine-grained crystals of pyrite can react more quickly than large crystals of sphalerite even though sphalerite would dissolve more quickly if it were the same grain size as pyrite. Marcasite has the same composition as pyrite but it reacts more rapidly because it has a different crystal structure that is more amenable to oxidative weathering.

As acid-mine drainage mixes with surface waters, the dissolved ferrous iron oxidizes and precipitates to hydrous ferric oxide (HFO) which is an excellent sorbent for trace metals. Consequently, some of the dissolved copper, lead, zinc, arsenic, and other metals will be sorbed onto colloidal particles of HFO and decrease the potential toxicity, one of several processes known as natural attenuation. This process is enhanced by the growth of masses of bacteria and algae which also sorb some of the metals, removing them from solution and decreasing the concentration transported further downstream.

Environmental consequences of sulfide mineral oxidation

Acid-mine drainage from sulfide-mineral oxidation usually discharges into streams, rivers, and lakes (Figure 1). The pH values of acid-mine drainage range from 1 to 4 although values as low as $-3.5$ have been measured (Nordstrom et al., 2000). The high concentrations of metals such as copper, chromium, zinc, lead, cadmium,
mercury, manganese, iron, and aluminum found in acid-mine drainage is too toxic for invertebrates, amphibians, fish, and other forms of aquatic or terrestrial life. Only bacteria, algae, and fungi seem to survive when the pH is about 3 or less and the concentration of metals is a few hundred parts per billion or higher. Occasionally cyanide is also released into surface waters from gold processing with harmful effects. Releases of acid-mine drainage have caused the loss of enormous quantities of fish as well as harming livestock, mammalian wildlife, and crops. Two of the largest reported spills destroyed temporarily the biological habitat of two separate river systems. One spill was from the Aznalcollar impoundment in southern Spain in April, 1998, discharging 6 million cubic meters of acid water and pyritic fines into the Guadalariver and ruining thousands of hectares of farmland. The other spill occurred in January, 2000, releasing a hundred thousand cubic meters of cyanide and heavy metals from a 4 km impoundment at the Aurul gold extraction plant near Baia Mare in northwestern Romania. The cyanide-rich water moved down to the Lapus River, then to the Tisza River, and finally to the Danube River all the way to the Black Sea. Smaller spills from the same region occurred again over the next 2 months. Approximately, 1,200 t of fish were killed from this spill, bird life was affected, thousands of fishermen were out of work, and water supplies for several towns and rural communities were badly contaminated.

Treatment technology

Innumerable ideas have been suggested for alleviating the harmful effects of sulfide-mineral oxidation and acid-mine drainage production. These suggestions include passive wetlands treatment, bioreactors, electrochemical treatment, exchange resins, reverse osmosis, and the use of various biosolids. However, the most commonly used, safe, and cost-effective treatments today are lime–limestone neutralization and precipitation of the metal-rich sludge in settling ponds combined with water-management techniques. An important engineering aspect is the construction of containment structures for hazardous materials that can withstand intense rainstorms and floods and that will prevent releases into surface-water and groundwater supplies.

Conclusion

Sulfide-mineral oxidation is a complex hydrobiogeochemical process involving the oxidation of metal-sulfide minerals, catalyzed by numerous bacterial and archaeal microbial species, and producing acid-rock drainage. Mining activities enhance the rate of this process, causing the production of acidic metal-rich drainage that make receiving streams, lakes, and rivers unfit for general usage and injurious to aquatic and terrestrial biota. Major spills of mine wastes into receiving waters have severely damaged valuable aquatic resources, farmland, community water supplies, and the economic stability of those who depend on farming and fishing. Much effort has gone into the remediation of old inactive mines and current mining activities to improve water quality for beneficial uses but the scale and complexity of the problem continues to challenge engineers and regulators.

Bibliography


Cross-references

Acid Rock Drainage
Heavy Metals
Ores, Microbial Precipitation and Oxidation
Pyrite Oxidation

SULFIDE MINERAL OXIDATION
SULFUR CYCLE

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Synonyms
Biogeochemical sulfur cycle; Sulfate reduction and sulfide oxidation; Transformation of sulfur compounds

Definition
Sulfur. Chemical element that is one of the constituents of the Earth’s compartments and living organisms. While the average sulfur content of the whole Earth is about 2%, the crust contains only about 0.07% sulfur (Brimblecombe, 2003).

Sulfur cycle. Biogeochemical system of biotic and abiotic transformations of inorganic and organic sulfur-bearing components, in and between, the lithosphere, hydrosphere, atmosphere, and biosphere. Sulfur initially enters the biogeochemical cycle via volcanic activity and continental erosion. Today, the modern sulfur cycle is influenced by human activity. The Earth sulfur reservoir is assumed to have been essentially constant through time (Garrels and Lerman, 1984).

Assimilative sulfate reduction. Sulfate (SO$_4^{2-}$) is reduced to organic R-SH groups by plants, algae, and fungi.

Dissimilative sulfur and sulfate reduction. Hydrogen sulfide (H$_2$S) is produced from elemental sulfur (S$^0$) or dissolved sulfate by sulfur- and sulfate-reducing microorganisms, respectively. For sulfate reduction coupled to the oxidation of marine organic matter or methane, the overall reactions are:

$$(\text{CH}_2\text{O})_{106}(\text{NH}_3)_{16}(\text{H}_3\text{PO}_4) + 53\text{SO}_4^{2-} + 14\text{H}^+ 
\rightarrow 106\text{HCO}_3^- + 16\text{NH}_4^+ + \text{HPO}_4^{2-} + 53\text{H}_2\text{S}$$

CH$_4$ + SO$_4^{2-}$ → HCO$_3^-$ + HS$^-$ + H$_2$O

Desulfurisation. Hydrogen sulfide is produced from sulfur-containing organic molecules.

Oxidation of hydrogen sulfide. Production of sulfur intermediates, elemental sulfur, thiosulfate (S$_2$O$_3^{2-}$), sulfate (SO$_4^{2-}$), and/or sulfate by bacterial or abiotic oxidation (Jørgensen and Nelson, 2004). Possible reactions are:

$12\text{H}_2\text{S} + 6\text{CO}_2 \rightarrow \text{C}_6\text{H}_12\text{O}_6 + 12\text{S}^0 + 6\text{H}_2\text{O}$

$\text{HS}^- + 2\text{FeOOH} + 5\text{H}^+ \rightarrow 2\text{Fe}^{2+} + \text{S}^0 + 4\text{H}_2\text{O}$

Oxidation of sulfur. Sulfate is generated by the oxidation of elemental sulfur. A possible reaction is (gradient bacterium Thioploca):

$4\text{S}^0 + 3\text{NO}_3^- + 7\text{H}_2\text{O} \rightarrow 4\text{SO}_4^{2-} + 3\text{NH}_4^+ + 2\text{H}^+$

Disproportionation of sulfur intermediates. Sulfate and hydrogen sulfide are produced by the low-temperature bacterial metabolism of sulfur intermediates. The stoichiometries of the reactions are (Finster, 2008):

$\text{S}_2\text{O}_3^{2-} + \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + \text{HS}^- + \text{H}^+$

$4\text{SO}_4^{2-} + \text{H}^+ \rightarrow 3\text{SO}_4^{2-} + \text{HS}^-$

$4\text{S}^0 + 4\text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 3\text{HS}^- + 5\text{H}^+$

In the presence of oxy(hydrox)ides of iron(III) or manganese (IV) the following stoichiometries are obtained (Böttcher and Thamdrup, 2001; Finster, 2008):

$3\text{S}^0 + \text{FeOOH} \rightarrow \text{SO}_4^{2-} + 2\text{FeS} + 2\text{H}^+$

$\text{S}^0 + 3\text{MnO}_2 + 4\text{H}^+ \rightarrow \text{SO}_4^{2-} + 3\text{Mn}^{2+} + 2\text{H}_2\text{O}$

Hydrolysis of elemental sulfur. Sulfate and hydrogen sulfide can be produced by the abiotic hydrolysis of elemental sulfur at high temperatures (Smith, 2000), according to:

$4\text{S}^0 + 4\text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 3\text{HS}^- + 5\text{H}^+$

Formation of iron disulfide (pyrite). The low-temperature formation of iron disulfide typically takes place via the polysulfide (S$^*$) or hydrogen sulfide pathway by the reaction with the precursor iron monosulfide (Rickard and Luther III, 2007):

$\text{FeS} + \text{S}^* \rightarrow \text{FeS}_2$

$\text{FeS} + \text{H}_2\text{S} \rightarrow \text{FeS}_2 + \text{H}_2$

The degree of pyritization (DOP) indicates the relative amount of reactive sedimentary iron that has already been reacted to pyrite.

The global sulfur cycle

The global reduced and oxidized reservoirs of sulfur, carbon, oxygen, and selected metals are coupled on different scales via redox-driven chemical, biological, and geological processes (Figures 1–4). Parts of this cycling, such as the reaction between sulfuric acid (that later became popular in industrialized times as “acid rain”) and carbonate rocks, were already described in the nineteenth century by Johann Wolfgang von Goethe (Figure 1) in his famous novel “Elective affinities” (German: “Wahlverwandtschaften”; published in 1809).

On Earth, pure sulfur occurs in elemental form as for example in volcanic regions, and the quantitative use of sulfur-bearing compounds by man has been used as a proxy for economic wealth (Brimblecombe, 2003). Iron sulfide sulfur occurs in almost all types of rocks and sediments and is even found in extraterrestrial meteorites. In sediments that are not under the influence of hydrothermal activity, the occurrence of sulfide sulfur is indicative for the activity of sulfate-reducing bacteria.
Sulfur Cycle, Figure 1 Coupling of the sulfur and carbon cycles according to Johann Wolfgang von Goethe. (Translation from Niles, 1872.)

Sulfur Cycle, Figure 2 Mass balance model of coupling between the young and old global carbon and sulfur reservoirs. (Redrawn from Berner, 1987, as a modification of the model by Garrels and Lerman, 1984.)

Sulfur Cycle, Figure 3

(e.g., Jørgensen and Kasten, 2006). Whereas sulfur is assimilated by living organisms where it is bond to, for instance, amino acids, most of the organic sulfur found in sediments is derived from early diagenetic reaction of organic matter with reduced and intermediate sulfur species (Sinninghe Damsté and de Leeuw, 1990). The decomposition of organic matter in the presence of sulfate is catalyzed by microorganisms that gain energy from the dissimilatory reduction of sulfate (e.g., Desulfovibrio and Desulfobacter; Rabus et al., 2001). Microbial oxidation of sulfide (e.g., by green and purple sulfur bacteria; gradient bacteria as Beggiatoa or Thioploca; Jørgensen and Nelson, 2004), disproportionation (e.g., Desulfovibrio, Desulfocapsa, Finster, 2008), or further oxidation of sulfur intermediates (e.g., Thiobacillus) can take place aerobically or anaerobically. In the aquatic low-temperature environment, dissolved and metal-bond sulfide may also be abiotically oxidized, e.g., by manganese dioxide (Jørgensen and Nelson, 2004). The lowest oxidation state of sulfur is $\text{-II}$ (e.g., in hydrogen sulfide). In its highest oxidation state $\text{+VI}$ (e.g., in sulfate), sulfur is bond to oxygen and found as (biogenic or abiotic) barite or evaporitic sulfate minerals (e.g., gypsum or anhydrite).

Sulfur in the Atmosphere

The first atmospheric sulfur components on Earth (mainly $\text{SO}_2$ and $\text{H}_2\text{S}$) were derived from volcanic activity and may have led to the first acid rain on Earth (Farquhar et al., 2000). Volcanoes still contribute to the atmospheric sulfur budget, besides emissions from the oceans (e.g., dimethyl sulfide (DMS), carbon disulfide), coastal and wetland areas. Most of the different components temporarily end as sulfate. The anthropogenic combustion of sulfur-bearing fossil fuels in the past century not only produced $\text{CO}_2$, but also led to the formation of sulfuric acid (“acid rain”), which partly led to increased rock weathering that again linked the carbon and sulfur cycles (Figure 1). In the ongoing discussion of dealing with climate change, the observations from the eruption of Mount Pinatubo are taken as an event-type base for geoengineering concepts to decrease the global surface temperature by the artificial release of sulfur components into the stratosphere (Crutzen, 2006).

Sulfur in surface and groundwaters, and freshwater sediments

Rainwater sulfate, sulfur dioxide, and organic sulfur enter the water-unsaturated soil zone, where further
transformations take place. Geological contributions from soil and aquifer minerals vary strongly and may include gypsum dissolution and metal sulfide oxidation. Near-surface oxidation of pyrite from coal-mining activities leads today to the anthropically induced phenomenon of “acid mine drainage” (Holmer and Storkholm, 2001). Sulfur cycling in freshwater lake sediments associated with organic matter diagenesis is controlled by a number of factors, including temperature, light conditions, the availability of sulfate, organic matter, electron acceptors and nutrients, and macrofaunal abundance (Holmer and Storkholm, 2001). Groundwater may play an important role in influencing sulfur cycling, for instance, via the supply of evaporite-derived sulfate (Swiss Lago di Cadagno, as an example) and rock weathering (Böttcher, 1999). Sulfur cycling via organic sulfur compounds (Figure 4) plays an important role in freshwater sediments and soils (Mitchell et al., 1992; Holmer and Storkholm, 2001). Finally, sulfate may reach the ocean where further processing takes place (Figure 4).

**Sulfur Cycle, Figure 3** Major reservoirs and burdens of sulfur in Tg. (Modified after Brimblecombe, 2003.)

**Sulfur Cycle, Figure 4** Simplified sedimentary sulfur cycle and its link to selected metals (neglecting, e.g., transformation of polysulfides, thionates, thiols, and methane). OSC: Organic sulfur compounds.

**Sulfur Cycle, Figure 5** Frambooidal iron disulfide (pyrite) as indicator for the activity of sulfate-reducing bacteria in sediments. (Example from marine intertidal North Sea sediments; Böttcher and Bahlo, unpublished scanning electron microscope picture, 2008.)
Sulfur in seawater and marine sediments

With a residence time of at least 8 million years, dissolved sulfate in seawater is well-mixed and reflects changes in the fluxes between and sizes of the major reservoirs (Figures 2 and 6). Whereas at mid-oceanic ridges, chemical sulfate reduction occurs in heated seawater that circulates through the Earth’s crust, the low temperature dissimilatory sulfate reduction is linked to the enzymatic activity of sulfate-reducing bacteria which requires the abundance of metabolizable dissolved organic carbon compounds or methane (Jørgensen and Kasten, 2006). The anaerobic oxidation of methane associated with microbial reduction of sulfate is an important trap to prevent methane from escaping into the water column (Jørgensen and Kasten, 2006). In continental margin sediments, microbial sulfate reduction accounts for up to more than 50% of the organic matter oxidation (Skyring, 1987; Jørgensen and Kasten, 2006). Sulfate reduction may also take place in anoxic water columns (e.g., Black Sea). Most of the biogenic sulfide is reoxidized at the benthic or pelagic redoxclines by microbial or chemical processes (Jørgensen and Nelson, 2004) leading to the metastable formation of intermediate sulfur species, or finally sulfate. The sulfur intermediates can be further oxidized, reduced, or disproportionated (Figure 4). Only a small portion of sulfide is finally buried as iron sulfides (essentially pyrite; Figure 5) or organic sulfur (Figures 2 and 4).

A number of external factors may directly or indirectly influence the rates of microbial sulfate reduction and sulfide burial efficiency, on different scales that can be reflected by the contents of sedimentary sulfur and the “reservoir” effect reflected in overall isotope discrimination (Figure 7).

Geochemical tracers for sulfur cycling: Stable sulfur and oxygen isotopes

Fractionation of stable sulfur (in dissolved and solid phase sulfurs) and oxygen isotopes (in sulfate and sulfate-bearing minerals) takes place via biological and abiotic transformations and leads to isotope signatures that can be used to identify sources and biological, chemical, and physical processes involved (Canfield and Raiswell, 1999; Böttcher, 2010). Oxygen isotope fractionation can be used...
to trace sulfate sources in aerobic environments (Clark and Fritz, 1997). The composition of dissolved sulfate and sedimentary diagenetic barite after modification by microbially sulfide, on the other hand, is finally governed by intra-cellular oxygen isotope exchange toward equilibration with surrounding water (Wortmann et al., 2007). The fossil sedimentary isotope record of pyrite (Figure 6) and anhydrite/barite/carbonate-bond sulfur, reflects the coupled changes in the atmospheric composition, the biogeochemistry of the sedimentary sulfur cycle and the fluxes between the different sulfur-bearing reservoirs (Figures 1–2, 4 and 6) (e.g., Berner, 1987; Canfield and Teske, 1996; Canfield and Raiswell, 1999; Canfield, 2004).

Conclusion

The sulfur cycle is of general importance due to its involvement in the anaerobic mineralization of organic matter, and therefore, tight coupling to the carbon cycle. Its geochemical signature in sediments provides hints for identification of key processes associated with the evolution of life and the atmosphere on Earth. Some of the many open questions pertaining to the (bio)geochemistry and geomicrobiology of sulfur are (1) the importance of microbial versus chemical sulfide oxidation, (2) the quantitative importance of metastable intermediate and reduced sulfur species in the low-temperature sulfur cycle (Rickard and Morse, 2005; Finster, 2008), (3) the quantitative role of sulfur cycling for the deep biosphere (Jørgensen and D’Hondt, 2006), and (4) the role of metal sulfides in extreme environments on the pre-biotic Earth for the later development of the precursors of life (Rickard and Luther III, 2007).

Bibliography


Cross-references

Anaerobic Transformation Processes, Microbiology
Biogeochemical Cycles
Sulfur Isotopes

**SULFUR ISOTOPES**

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**Synonyms**

Radioactive sulfur isotopes; Stable sulfur isotopes

**Definition**

*Sulfur*. A chemical element that is one of the constituents of living organisms and of the Earth.

*Isotopes*. Isotopes are different nuclear forms of the same element. For a given element, a constant number of protons but different numbers of neutrons in the nucleus correspond to different isotopes. Sulfur of standard atomic mass 32.065 u has 18 isotopes, most of which are not stable and undergo radioactive decay.

*Stable sulfur isotopes*. Stable isotopes are isotopes that do not decay within an experimentally observable time frame. Stable isotopes have essentially the same chemical characteristics and, therefore, their behavior is chemically almost identical. The different masses lead to isotope fractionation in chemical, physical, and especially biological reactions. Four stable isotopes occur in nature: $^{32}$S (95.02%), $^{33}$S (0.75%), $^{34}$S (4.21%), and $^{36}$S (0.02%) (Hoefs, 2008). Typically, the ratio of the two most abundant sulfur isotopes is measured and presented in the conventional δ notation as opposed to the Vienna-Canyon-Diablo-Troilite (V-CDT) standard ($\delta^{34}$S values per mil versus the V-CDT standard). In aerobic surface environments, the sulfur isotopic composition of sulfate is often used for source identification (e.g., anthropogenic versus geogenic sulfate in rain, river, and ground waters (e.g., Clark and Fritz, 1997)). Only recently has the fractionation of the minor isotopes $^{33}$S and $^{36}$S also been introduced into sulfur isotope biogeochemistry (e.g., Farquhar et al., 2003).

*Radioactive sulfur isotopes*. Sulfur has 14 unstable isotopes. Radioactive sulfur with mass 35 is formed in the atmosphere from cosmic ray spallation of argon with mass 40 and has a half-life of 87 days. Therefore, it is used in hydrological studies of biologically less active surface environments. $^{35}$S-labeled sulfur compounds are used in experiments to determine microbial and chemical sulfur turnover, upon microbial dissimilatory sulfate reduction, for instance (e.g., Kallmeyer et al., 2004). The other radioactive sulfur isotopes are all short-lived.

**Fractionation of stable sulfur isotopes**

Sulfur isotopes are fractionated upon microbial and abiotic sulfur transformations and the isotope signal may be preserved in sedimetary sulfide- and sulfate-bearing solids, organic matter, and to a minor extent, elemental sulfur (e.g., Canfield, 2001; Amend et al., 2004). The majority of geochemical and microbial studies on sulfur isotopes have so far focused on the fractionation of $^{32}$S to $^{34}$S. The most important isotope discrimination step occurs during dissimilatory reduction of dissolved sulfate within the bacterial cell. The overall change in the valence state of sulfur by pure cultures leads to hydrogen sulfide that is enriched in the lighter isotope by up to about 50 per mil when compared to sulfate (Figure 1). The magnitude of isotope discrimination depends on conditions such as cellular sulfate reduction rate, substrate and sulfate concentrations, and the organisms (e.g., Kaplan and Rittenberg, 1964; Chambers and Trudinger, 1979; Boliger et al., 2001; Canfield, 2001; Amend et al., 2004). A maximum fractionation upon sulfate reduction of up to about 70 per mil is obtained from pore water modeling in natural environments (e.g., Wortmann et al., 2001), which is close to the value predicted for thermodynamic equilibrium conditions. Further partial or complete oxidation of hydrogen sulfide is associated with only small isotope discrimination, but may lead to the formation (of metastable) sulfur intermediates. The bacterial disproportionation of these compounds (e.g., elemental sulfur, thiosulfate, sulfate) yields hydrogen sulfide that is further enriched in the lighter isotope compared with the original sulfide (Figure 1).

This may contribute to the magnitude of isotope partitioning found in shallow natural environments and the fossil record (Canfield, 2001).
Conclusions

Stable sulfur isotope partitioning preserved in the sedimentary record may serve as a proxy for microbial sulfur cycling (e.g., Amend et al., 2004; Canfield, 2001). One of the many open questions within the biogeochemistry of stable sulfur isotopes regards the conditions leading to the sulfur isotope extremes in sediments, which differ from experimental results with modern bacterial cultures. New insights are expected to derive from experiments carried out under boundary conditions that more closely approach natural environments, the consideration of new cultures, and the application of multi-isotope approaches including analysis of the minor sulfur isotopes (e.g., Farquhar et al., 2003) and sulfur-bond oxygen (e.g., Brunner et al., 2005).

Bibliography


SYMBIOSIS

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Definition
A close, prolonged physical and/or metabolic association between two or more distinct organisms.

Introduction
Most living organisms interact intimately with one or more symbiotic partners that are often vital for their health and survival. Symbiotic organisms are important ecosystem engineers (e.g., corals); they significantly impact biogeochemical cycles (e.g., legumes), and they contribute substantially to geological processes such as rock weathering (e.g., lichens and mycorrhizal fungi). Moreover, symbioses have played a crucial role in the origin and diversification of eukaryotic life.

Important terms and concepts
Symbioses are categorized as being mutualisms, where both organisms involved benefit, commensalisms, where one organism benefits and the other remains unaffected, or parasitisms, where one organism benefits but the other is harmed. When metabolite exchange occurs between symbiotic organisms, it is called syntrophy. Symbiotic interactions among microorganisms can drive otherwise thermodynamically unfavorable reactions forward due to the removal of metabolic end products.

In symbiosis terminology, the smaller symbiotic partner (often a microbe) is referred to as the “symbiont,” whereas the larger partner is called the “host.” In ectosymbioses, the symbiont is located on the external surface of the body of its host, whereas in endosymbioses, the symbiont lives inside the body of its host, either within its cells or in extracellular spaces. Symbioses vary from being obligate, where the interaction is critical for the survival of at least one organism involved, or facultative, where the organisms involved can also survive independently of each other. The method by which the host acquires its symbionts is called the mode of transmission. In vertical transmission, symbionts are inherited from the previous generation, as they are passed from the parent to the egg or embryo. In horizontal transmission, symbionts are acquired anew in each generation either from co-occurring hosts or from the environment.

History
Symbiosis is derived from the Greek words, syn (together) and bio (life). German biologist Anton de Bary is usually credited with coining the term in the mid-nineteenth century, though an equally important contribution was made by his contemporary, Albert Bernhard Frank (who instead used the term symbiosis) (Sapp, 2004). De Bary introduced the term to discuss lichens, which he discovered were composites of algae and fungi. Frank, on the other hand, reported on the intimate association between fungi and roots of forest trees, which he named “mykorrhiza” (fungus root). De Bary defined symbiosis as the phenomenon in which “unlike organisms live together” and explicitly included parasitism in his definition (Kutscher and Niklas, 2005). Therefore, the original definition of symbiosis was meant to include the entire gamut of close and long-term associations between two distinct organisms, irrespective of the benefit or harm caused by the interaction. The historical definition of symbiosis was eventually replaced in many modern textbooks with a more restricted definition that included only mutually beneficial associations, making the meaning of symbiosis synonymous with “mutualism” (Wilkinson, 2001). Unfortunately, research on symbioses has been fraught with confusion and controversies, in part due to the two coexisting definitions of the term (Smith, 2001; Sapp, 2004).

Conceptually, the original definition of symbiosis makes more sense in light of the fact that “benefit” is difficult to quantify in studies of symbioses (Smith, 2001). Cost-benefit ratios of symbioses can change with environmental conditions. For example, symbiotic mycorrhizal
fungi benefit their host plants through nutrient uptake, but represent a metabolic cost to the plant under high nutrient conditions (Johnson et al., 1997). Moreover, the molecular mechanisms of communication underlying mutualistic and parasitic interactions are rather similar (Hentschel et al., 2000), and the nature of the interaction can switch during the course of evolution (Sachs and Simms, 2006). Thus, a natural continuum exists among mutualism, commensalism, and parasitism, and arguments about restricting the definition of symbiosis only serve to obscure a true understanding of the phenomenon.

**Symbiosis and evolution**

Symbioses can induce diverse physiological, morphological, and developmental modifications and thereby strongly influence the evolution of the organisms involved (Sapp, 2004). Microbial symbionts often confer novel metabolic capabilities to their hosts, allowing them to occupy new ecological niches. They can even alter the reproductive behavior of their hosts, and induce speciation through reproductive isolation of host populations (Engelstädter and Hurst, 2009). Conversely, the mode of transmission has a strong impact on symbiont evolution. Many vertically transmitted symbionts have severely reduced genomes, some of them retaining only limited genes corresponding to biosynthetic pathways for nutrients they provide to their host (Moran et al., 2008; McCutcheon, 2010).

Moreover, it is now commonly accepted that the rise of eukaryotes was intimately tied to symbiotic interactions. According to the “endosymbiosis hypothesis” popularized by Lynn Margulis (Margulis, 1970), mitochondria and plastids of present-day eukaryotes evolved from bacterial endosymbionts. Although the nature of the original host cell is still debated, molecular analyses have revealed that present-day mitochondria share a common ancestor with extant alpha-proteobacteria, while chloroplasts evolved from an ancient cyanobacterium (Kutschera and Niklas, 2005).

**Symbiosis and its relevance to geobiology**

Symbiosis research is inherently interdisciplinary in nature (Sapp, 2004), and in many cases lies at the interface between geology and biology. A few examples are given below to underscore the importance that symbioses hold for geobiologists.

**Mineral weathering by symbiotic organisms**

Bare rock surfaces are harsh environments for biological organisms, as they experience intense solar radiation, and strong fluctuations in temperature and humidity. Hence, cooperation rather than competition is favored, and many mutualistic or commensalistic interactions are observed in communities colonizing rock surfaces (Gorbushina, 2007). Perhaps the most well known of rock weathering agents are lichens (Fungi and Lichens). Lichens are usually considered to be symbiotic associations of fungi and photosynthetic algae and/or cyanobacteria, but in reality they comprise complex communities of several types of microbes (Banfield et al., 1999). Lichens contribute to rock weathering through both physical and chemical processes (Chen et al., 2000). The fungal partners produce hyphae (long, branching filamentous cells) that penetrate minerals, and in combination with freeze-thaw cycles cause mineral breakdown. In addition, lichens dissolve minerals by exuding organic acids, the production of which is enhanced by symbiotic bacteria (Uroz et al., 2009). Lichens have colonized terrestrial habitats since the early Devonian (Jahren et al., 2003), and are widely distributed, occurring even in the southernmost parts of Antarctica (Chen and Blume, 2002). They can colonize and weather the same location on a rock for decades (Uroz et al., 2009), and therefore play a profound role in rock weathering.

In soil environments, mycorrhizal fungi dominate mineral weathering processes. Mycorrhizae (from the Greek mycos = fungus, and rhiza = root), which are plant-fungal associations, are the most widespread type of terrestrial symbiosis (Smith and Read, 2008). They are broadly of two types: ectomycorrhizal fungi (EMF), in which the fungal partner remains outside the plant cells, and endomycorrhiza (which includes arbuscular mycorrhizal fungi; AMF), in which part of the fungal hyphae penetrates inside the cell. EMF aggressively weather minerals by acidifying the surrounding soil microenvironment, and by exuding organic molecules that chelate metal ions (Taylor et al., 2009). They are referred to as “rock-eating fungi” as they not only dissolve surfaces of minerals but also form tunnels inside mineral grains (van Schöll et al., 2008). On the other hand, AMF indirectly enhance weathering by their plant hosts by supporting increased plant biomass and more extensive rooting systems (Taylor et al., 2009).

The role of symbiotic organisms in biogeochemical cycles

Symbiotic organisms influence biogeochemical cycles of global importance. Methane, an important greenhouse gas, is produced in copious amounts by methanogenic symbionts of ruminants and insects (Thauer et al., 2008; Brune, 2010). In turn, Anaerobic Oxidation of Methane (AOM) in marine sediments, mediated by microbial consortia comprising intimate syntrophic associations between archaea and eubacteria, is a major biological sink of methane (Hoffmeister and Martin, 2003). AOM consortia are responsible for building massive carbonate reefs in present-day anaerobic environments such as the Black Sea and were likely important players in carbon cycling during anaerobic periods of Earth’s history (Michaelis et al., 2002). Carbonate accretion in the ocean is an important part of the global carbon cycle (Ridgwell and Zeebe, 2005), and can be enhanced by reef-building corals that depend on nutrients from their endosymbiotic dinoflagellates to maintain high calcification rates (Hoegh-Guldberg et al., 2009).
A wide variety of animals with chemosynthetic symbioses occur in diverse marine habitats. (From Dubilier et al., 2008, reprinted with permission from Macmillan Publishers Ltd.)
et al., 2007). Carbon cycling in Earth’s history was also strongly influenced by mycorrhizal symbioses, which were intimately linked to the advent of land plants over 400 million years ago, an event that caused dramatic declines in atmospheric carbon dioxide levels (Taylor et al., 2009). Mycorrhizal symbioses also impact the global nitrogen cycle, but not as directly as nitrogen-fixing plant symbionts (Jackson et al., 2008; see entry “Algae (Eukaryotic”). Bacteria belonging to the Rhizobium genus are often symbionts of leguminous plants, and can convert atmospheric nitrogen into ammonia, a process that has wide impact on the environment and on agriculture (Dixon and Kahn, 2004).

Geochemical influence on distribution of symbiotic organisms

Symbiotic organisms impact geological processes, and conversely, environmental geochemistry can influence the distribution and abundance of symbiotic fauna. Prime examples are chemosynthetic symbioses, which involve chemolithoautotrophic or methanotrophic microbes and invertebrate hosts (reviewed in Stewart et al., 2005; Cavanaugh et al., 2006; Dubilier et al., 2008; Petersen and Dubilier, 2009). Chemosynthetic microbes use chemical energy derived from reduced compounds such as sulfide and methane to fix inorganic carbon. The first case of a chemosynthetic symbiosis involving the giant tubeworm Riftia pachyptila was discovered more than 30 years ago at an eastern Pacific hydrothermal vent (see Cavanaugh et al. (2006) for details). Subsequently, chemosynthetic symbioses have been found worldwide in a large variety of marine environments including organic rich coastal sediments, Whale and Wood Falls, mud volcanoes, and Cold Seeps (Figure 1; Dubilier et al., 2008). Chemosynthetic symbioses have independently evolved multiple times, and involve at least seven different animal phyla and nine phylogenetically distinct clades of bacteria. Recently, the first case of a freshwater chemosynthetic symbiosis was discovered in a sulfur-rich limestone cave in central Italy, expanding the diversity of environments where these symbioses are found (Dattagupta et al., 2009). Since chemosynthetic microbes need to oxidize reduced compounds to derive energy, animals with chemosynthetic symbionts invariably need to bridge oxic–anoxic interfaces (Stewart et al., 2005; Cavanaugh et al., 2006). Thus, the biology and ecology of chemosynthetic symbioses are strongly interrelated with environmental geochemistry.

Summary

Symbiosis structures and influences many aspects of life on Earth. It is an important driving force in evolution, and it impacts not only the biology and ecology of organisms but also the biogeochemical processes of global importance.

Bibliography


**Cross-references**

- Algae (Eukaryotic)
- Anaerobic Oxidation of Methane with Sulfate
- Archaea
- Bacteria
- Biogeochemical Cycles
- Chemolithotrophy
- Cold Seeps
- Dinoflagellates
- Fungi and Lichens
- Hydrothermal Environments
- Methanogens
- Reefs
- Whale and Wood Falls

**SYNTROPHY**

Syntrophy is a symbiotic association, where metabolite exchange occurs between the organisms involved. See entry “Symbiosis” for further reading.
TERRESTRIAL DEEP BIOSPHERE

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Synonyms
Continental deep biosphere; Subterranean biosphere

Definition
The terrestrial deep biosphere comprises ecosystems in the marine (e.g., shelf-sediments) and continental subsurface, beneath the rhizosphere or bioturbated zone. The habitable depth is limited by space (rock porosity), the availability of water and in particular the maximum viable temperature depending on the local geothermal gradient (Gold, 1992; Pedersen, 1993, 2000, 2001, 2002; Stevens, 2002).

Introduction
Microbial life exists in, and seems to be adapted to all kinds of ecological niches on earth. From the early 1930s, increasing numbers of publications report on the occurrence of microorganisms in deep terrestrial settings, such as coal mines (Lipman, 1931, 1937; Lieske, 1932) and deep formation waters from oil drilling, as deep as 2,000 m (Ginsburg-Karagitscheva, 1933; Issatchenko, 1940). During the following decades the scientific interest in subsurface microbiology focused on the effects of microorganisms on oil and gas exploration, such as corrosion of drilling devices, oil transformation, but also the role of microorganisms in the biological origin of natural gas resources (Bailey et al., 1973; Belyaev et al., 1983; Colwell et al., 2004; Lollar et al., 2006; Zobell, 1945). Deep subsurface microorganisms have a potential impact not only on oil and gas exploration, but also on groundwater (Chapelle, 2000), long-term storage of nuclear waste (Boivin-Jahns et al., 1996; Christofi and Philip, 1997; Hersman, 1997; Pedersen, 1993, 1997; Pedersen et al., 2008), and finally, implications for the origin of life and the search for extraterrestrial life (Cockell, 2004; Faison, 2003; Stevens, 1997a).

Scientific continental drilling (ICDP), and also site investigations for the storage of nuclear waste for a better understanding of geological, hydrological, and biogeochemical processes in the deep continental crust, supported the development of contamination minimizing, drilling, sampling, and analytical techniques (Abyzov et al., 2001; Griffin et al., 1997; Pedersen, 1993 (and references therein); Pedersen et al., 2008; Russel, 1997). Using enhanced drilling and sampling techniques, a broad diversity of microorganisms living under extreme conditions (extremophiles), i.e., under elevated temperatures and high pressure conditions, was observed (Gold, 1992; Boone et al., 1995; Bonch-Osmolovskaya et al., 2003; Kotelnikova et al., 1998; Lin et al., 2006; Szewzyk et al., 1994; Sahl et al., 2008). Whether the organisms observed in the deep terrestrial subsurface are indigenous or derive from surface contamination has been a major issue since the first decades of exploration and is still (even though more advanced sampling techniques are now available), an important question to be considered (Farrell and Turner, 1932; Griffin et al., 1997; Haldemann, 1997; Lieske, 1932; Lipman, 1937; Pedersen, 1993; Phelps et al., 1989; Russel, 1997; Stevens, 2002). Furthermore comprehensive sampling (contamination controlled sampling) and careful data interpretation is matter of major importance (Hallbeck and Pedersen 2008; Lehman et al., 1995; Zhang et al., 2006).

The deep terrestrial biosphere
Numerous reports about diverse, well adapted, active microorganisms, being most likely indigenous, and
occurring down to a depth of several km, rise the questions of (a) how the organisms got down there, (b) how are they able to maintain, i.e., life and growth, (c) what are the limiting factors for life in the deep biosphere.

There are different theories about microbial migration or transport into the deep subsurface can happen. One possibility is the transport of microorganisms within groundwater aquifers as underground rivers (Karst systems) or percolating through the sediment or fracture systems. Groundwater may reach depths of several hundreds of meters and may have intervals from hundreds to thousands of years (Moser et al., 1988; Seiler and Lindner, 1995; Simpkins and Bradbury, 1992). Further, it is assumed that living microbial matter is buried with the sediments, thus migrating vertically as a part of the rock cycle. As a consequence, some microorganisms are capable to survive for millions of years. Reports on the detection and isolation of bacteria from the Taylorsville Triassic Basin from 2.7 km depth (Boone et al., 1995), and from the Piacean Basin of western Colorado from 858 m depth (Colwell et al., 1997) support this scenario.

General requirements for life are the availability of water, space, nutrients (O, C, H, N, Ca, P, and S), and trace elements (Fe, Ni, Mn, W, Mo, V, Zn, Cu, Co, Se, and Cr). Being independent from photosynthesis, subterranean organisms have to adapt their metabolism to local resources or form dormant stages, in order to survive at great depth (Amy, 1997; Dobretsov et al., 2006; Kieft and Phelps, 1997; Pedersen, 1993; Stevens, 2002). Since the discovery of the first subsurface microorganisms, and the subsequent identification of a broad variety of aerobic, anaerobic, autotrophic, and heterotrophic microbes, the potential carbon and energy sources available, and depth, were intensively debated and approved. One major theory is the deep hydrogen-driven biosphere hypothesis (Pedersen, 1997), where acetogenic bacteria, acetoclastic methanogens, and autotrophic methanogens consume abiotic, deep crustal H2 as an energy source and CO2 as carbon source. These organisms could be considered as the primary producers in the deep terrestrial biosphere. Experimental studies, calculating the production rates of radioactive H2, support the potential microbial consumption of H2 (Lin et al., 2005). Beside H2 as electron donor, chemotrophic organisms can use, for example, methane (CH4), elemental sulfur (S0), sulfite (SO32–), thiosulfate (S2O32–) with water H2O, nitrate (NO3–), sulfate (SO42–) or iron (Fe (III)), thereby forming nitrogen gas, hydrogen sulfide (H2S) and ferrous iron, respectively (Amend and Teske, 2005 and references therein; Kieft and Phelps, 1997; Stevens, 2002).

Numerous microorganisms observed in the deep subsurface are not only metabolically well adapted, they also manage to survive and reproduce under extreme conditions, such as hot or freezing temperatures, high pressure, alkaline or acidic conditions, and high salinity. Examples of these extremophiles and references are listed below in the section of the respective deep terrestrial environment. Moreover, some microorganisms are able to get into a dormancy stage by forming spores, cysts, or other types of resting cells and survive starvation, desiccation periods, exposure to extreme temperatures, and elevated background radiation (Amy, 1997; Burke and Wiley, 1937; Johnson et al., 2007; Ponder et al., 2005; Suzina et al., 2004).

Regarding these adaptation mechanisms and considering the ongoing discovery of unknown microorganisms, new metabolic pathways and adaptation mechanisms, it is difficult to define the limits of life in the terrestrial deep biosphere. These actual limits may be due to a combination of several factors: with increasing depth, the growing load of the overlying rocks or ice masses progressively decreases the pore space available, temperature rises due to the geothermal gradients and the water activity changes with increasing temperatures and pressures (Gold, 1992; Pedersen, 1993, 2000; Stevens, 2002).

Terrestrial deep biosphere environments

Pedersen (2000) divided the terrestrial deep biosphere into continental sedimentary rocks, ancient salt deposits, aquifers in igneous rocks, and caves, whereas Stevens (2002) subdivided the deep biosphere of the continental crust into sedimentary environments, permafrost, ice sheets and glaciers, and bedrock environments. Salt deposits and permafrost soils are sediments by definition, and therefore, in this chapter, they are discussed in the section sedimentary environments. Caves exhibit a great variety of different ecosystems that might serve as models for the formation of special adaptations or symbioses and may therefore represent a link between surface and subsurface environments (Dattagupta et al., 2009; Pedersen, 2000).

Sedimentary environments

Since the beginning of the last century, microorganisms in deep sedimentary environments have been frequently found and described from exploration sites, for example in coal (Burke and Wiley, 1937; Lieske, 1932; Lipman, 1931, 1937). Later when advanced drilling techniques and modern sequencing methods became available, a more precise characterization of these microorganisms is possible. A strict anaerobe, Bacillus species (Bacillus infernus) was isolated from drill core samples obtained from 2,700 m depth, from the Triassic Taylorsville basin (Boone et al., 1995). Several sediment core samples from 856 to 2,096 m from the Piacean Taylorsville basin (Boone et al., 1995). Several sediment core samples from 856 to 2,096 m from the Piacean Basin of Colorado contained Fe(III)-reducing and -fermenting bacteria (Colwell et al., 1997). Krumholz (2000) reported microbial communities within Cretaceous rocks in New Mexico, where living sulfate-reducing bacteria (SRB) and acetogens were found at the interface of porous sandstone and dense organic-rich shale. Later studies of the same site revealed high numbers of Fe(III) and S-reducing bacteria (Kovacic et al., 2006). Sass and Cypionka (2004) isolated moderately thermophilic SRB in porous sandstones from 600 to 1,060 m depth. In coal seem groundwater within 843 to 907 m depth microbial communities of
methanogenic archaea, denitrifying, acetogenic, and SRB where detected (Shimizu et al., 2007). Thermophilic archaea and bacteria, including SRB, are common in oil reservoirs and geothermal fluids (Bonch-Osmolovskaya et al., 2003; Kimura et al., 2007; Zagarese et al., 2007; Zobell, 1945). Culturing experiments and biomarker studies of 170 million years old claystone indicate the presence of active SRB within the rock (Mauclaire et al., 2007).

A special form of sedimentary environment is salt deposits: massive salt deposits have formed mainly due to marine transgression and regression cycles within epicontinental seas. Halophilic bacteria were described by Vreeland et al. (1998) from salt deposits in New Mexico and Gruber et al. (2004) isolated halophilic archaea in alpine salt deposits. Whether the halophilic microorganisms are indigenous and survived for millions of years in the salt, is still unclear (McGenity et al., 2000). However, the isolation of viable Halobacterium salinarium from brine inclusion within 9,600-years-old halite crystals (Mormile et al., 2003) indicates long-term survival of the microorganisms enclosed in salt deposits.

Permafrost soils are composed of silt, loam, peat, organic material from plants, top soil, and ice. Although temperatures in permafrost regions range from −10 and −30°C viable microorganisms have been detected in frozen sediment samples from Siberia, Alaska, Canada, and Antarctica (Vorobyova et al., 1997). Methanogenic archaea are presumably responsible for the production of high amounts of methane in permafrost soils (Rivkina et al., 2000, 2004). Gilichinsky et al. (1992) observed not only prokaryotes in Pliocene and Pleistocene sediments, even eukaryotic organisms were found in Holocene sediments.

Bedrock environments

Igneous and metamorphic rocks contain considerably less organic matter and pore space compared to sediments. Sampling of microorganisms is a problematic issue, as drilled rock material is often contaminated by drilling fluids (Pedersen, 2000; Hallbeck and Pedersen, 2008). However, investigations of a deep biosphere in igneous rocks were performed using the fracture and well water, drilling fluids, and groundwater aquifers. In deep granite aquifers of the Fennoscandian shield, anaerobic thermophilic fermenting bacteria and SRB (Swiezyk et al., 1994), methanogens, homoacetogens (Kotelnikova and Pedersen, 1998; Pedersen, 2000; Kotelnikova, 2002), yeasts (Ekendahl et al., 2003), viruses (Kyle et al., 2008), and bacteriochromes (Eybalin et al., 2009) were observed. The detection of fungi in deep biosphere environments was also reported by Retn et al. (2005), describing hyphae from unknown fungal mycelia in the Triberg Granite (Germany). In deep granite fracture water and rock cores from the Henderson mine (Colorado), iron-oxidizing bacteria and the “Henderson candidate division” were detected (Sahl et al., 2008). In deep anaerobic and alkaline aquifers within the Columbia River Basalt Group, SRB and metal-reducing bacteria were described by Fry et al. (1997). Drilling fluids from boreholes at 2,290–3,350 m depth, from the Chinese Continental Drilling Program, exhibited a huge diversity of extreme thermophilic, anaerobic chemoorganotrophs, anaerobic Fe(III) reducers, halotolerant, alkaliphilic microorganisms (Zhang et al., 2006). Lin et al. (2006) report about thermophilic SRB in deep alkaline saline groundwaters in Archaean metabasalt. A remarkable finding was a star shaped bacterial morphotype at 1,700 m depth in a South African platinum mine, observed by Wanger et al. (2008).

Ice sheets and glaciers

Microbiological investigations of ice cores obtained above the subglacial lake Vostok, Antarctica, exhibited various microorganisms from moraine material, from shallow regions of the glacier, but also presumably from the lake water (Abyzov et al., 2001). Poglazova et al. (2001) described Cyanobacteria and microalgae occurring in accreted ice of the subglacial lake Vostok. Highly diverse microbial communities in 120,000-years-old deep glacier ice cores from Greenland were described and isolated by Miteva and Brenchley (2005), Miteva et al. (2004).

General remarks

Fossil deep biosphere, evolutionary aspects, and implications for extraterrestrial life

As research on the deep biosphere continues, novel organisms, metabolic pathways, and adaption mechanisms will certainly be discovered. A look at the different subsurface environments shows that a generalization of the microbial diversity and adaption mechanisms is rather impossible as they all host their own specialized ecosystems.

Learning more about life in deep subterranean environments, may help to understand how life might have evolved and survived during meteoric impacts, increased rates of volcanic activity, or a potential global glaciation (Snowball Earth). In fact, geothermal waters, H2 and CH4 degassing volcanic or plutonic rocks may have served as local nutrient and energy sources for chemotrophic ecosystems (Dobretsov et al., 2006). Models about the habitability of the Hadean earth indicate that even the late heavy meteoric bombardment, around 3.9 Ga would not have been able to sterilize the primeval biosphere of the earth crust, considering the existence of a near and subsurface biosphere (Abramov and Mojzis, 2009). Traces of a fossil subterraneous biosphere were observed in metasedimentary rocks from Timmins (Canada) from the late Archaean (Ventura et al., 2007) and Bons et al. (2009) report about fossilized microbes within 585 Ma old calcite veins from Oppaminda Creek (Australia).

Increased interest for research in deep terrestrial, means also extreme environments, is its implication for extraterrestrial life. A better understanding of the earth’s deep biosphere allows the development of models to understand
general biogeochemical processes not only for our earth but maybe for biogeochemical processes on other planets. For example, permafrost soils on Mars may comprise recent or ancient life (Gilichinsky et al., 1992; Rivkina et al., 2000, 2004) or Jupiter’s satellites may have ice-covered lakes similar to lake Vostok (Abyzov et al., 2001) serving as a potential deep biosphere environments.

**Bibliography**


Cross-references

- Asteroid and Comet Impacts
- Astrobiology
- Biofilms and Fossilization
- Biosignatures in Rocks
- Deep Biosphere of Salt Deposits
- Extreme Environments

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**THIOESTER WORLD**

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**Definition**

Christian de Duve (1991, 1994, 1995) proposed a Hadean “Thioester World” which should have preceded the succeeding RNA world. Thioester molecules were probably very common in the proposed “primordial organic soup”. Thioesters are formed when a ‘thiol’ joins a carboxylic acid (R–COOH) under acidic and high-temperature conditions. The general form is written as an organic group (R) bonded with sulfur and hydrogen, hence R–SH. H₂O is released in this process, and forms finally a thioester: R–S–CO–R’. The “Thioester World” represents a hypothetical very early stage in the origin of life that could have provided the energetic and catalytic framework of a protometabolic set of primitive chemical reactions, like in the transaction of molecular groups, and in Redox-reactions. Thioesters were probably involved in the formation of energy-rich phosphates via acylphosphates and pyrophosphates as precursor compounds. The thioester bond is a high-energy bond and probably thioesters had the same function as ATP as an energy supplier. With the help of thioesters amino acids (AS) could also spontaneously polymerise and form first simple proteins. The “primordial organic soup” was enriched with AS may be provided by carbonaceous fluids. In laboratory experiments, imitating the Hadean conditions on Earth, it was possible to obtain cysteamine, b-alanine and pantonic acid known as ‘panetheine’ which is a precursor of coenzyme A (Keefe et al., 1995). The thioester world is an important hypothesis in the understanding of early life processes which may explain essential reactions expected in the prebiotic world.

Cross-references

Asteroid and Comet Impacts
Chondrites
Comets
Meteoritics
Origin of Life
RNA-World

THIOMARGARITA

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Definition

Colorless, sulfur and nitrate storing bacterium belonging to the gammaproteobacteria, spherical, vacuolated. *Thiomargarita namibiensis*, the largest known prokaryote, was first discovered in 1997 off the coast of Namibia and called the Namibian sulfur pearl. Together with the larger species of the genera *Beggiatoa* and *Thioploca*, they belong to a group of benthic bacteria that live by the oxidation of sulfide to sulfate with internally stored nitrate. Sulfide is first oxidized to sulfur, which can be stored by the bacteria as an energy reservoir. Nitrate is accumulated to very high concentrations (up to 800 mM) in a central vacuole, which comprises most of the cell volume. The single spherical cells are 100–300 μm in diameter and are held together in a chain by a mucus sheath (Figure 1). A new species of *Thiomargarita*, which was discovered in the Gulf of Mexico, appears as single cells without a slime sheath and can perform reductive divisions in up to three planes. These *Thiomargarita* cells resemble some of the fossils from the Doushantuo Formation in China, which have been classified as the oldest metazoan eggs and embryos.

*Thiomargarita namibiensis* inhabits the upper centimeters of fluid, sulfidic diatom ooze, which forms the sediment off the Namibian coast. Because of their large size, they can store enough nitrate to survive many years within the sulfidic sediment without getting into contact with nitrate from the open water. As *Thiomargarita* cells are not motile, they have to get suspended with their sediment, in order to refill the vacuole with nitrate. Off Namibia sediment resuspension may occur via frequent methane eruptions. Apart from sulfur and nitrate *Thiomargarita* cells can also store polyphosphate, which they may release periodically resulting in high phosphate concentrations around the cells and spontaneous precipitation of phosphorus rich minerals.

Bibliography


Cross-references

Cold Seeps
Phosphorus, Phosphorites
Sulfur Cycle
Symbiosis
Thiotrophic Bacteria

THIOTROPHIC BACTERIA

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Synonyms
Sulfur bacteria
Definition
Bacteria living off sulfur compounds.

Introduction
The term thiotrophic is derived from Greek and translates to sulfur nourishment, meaning that thiotrophic bacteria live on sulfide or other reduced sulfur compounds. Traditionally, these bacteria are often called sulfur bacteria because some of them can be recognized as sulfide oxidizers in the microscope, as they store elemental sulfur within the cells or excrete elemental sulfur into the medium. Microbiologists distinguish between colorless sulfur bacteria and green and purple sulfur bacteria, the latter two being phototrophs, which gain energy by the oxidation of sulfide to sulfur. Other major intermediates in sulfur bacteria are found within the gammaproteobacteria (Sievert et al., 2007).

Sulfide (the sum of H2S, HS−, and S2−) is a difficult energy source because it is toxic for respiratory processes, as it tends to bind iron. Therefore, even thiotrophic bacteria have to prevent very high sulfide concentrations. Microbiologists often avoid this problem by offering thiosulfate (S2O32−) instead of sulfide for cultivating thiotrophic bacteria. Most thiotrophs oxidize sulfide with oxygen. This reaction also occurs abiotically, but at much lower rates, so thiotrophic bacteria usually compete successfully with the chemical oxidation of sulfide to sulfur in the presence of oxygen. Thiobacilli mostly oxidize sulfide completely to sulfate, to gain the maximum of energy, but some thiotrophs excrete or internally accumulate the intermediate elemental sulfur. Other major intermediates of sulfide oxidation are thiosulfate, tetrathionate, and sulfate.

The oxidation of sulfide with nitrate instead of oxygen is an energy releasing reaction, which is used by some thiotrophic bacteria. One group of free-living sulfur bacteria is even a specialist on nitrate respiration, by possessing a large vacuole in which nitrate is stored in almost molar concentrations. Apart from the oxidation of reduced sulfur compounds bacteria can also gain energy by disproportionating thiosulfate or sulfur to sulfide and sulfate. This inorganic fermentation of thiosulfate or sulfur seems to be a widespread process both in marine and in freshwater sediments (Jørgensen and Bak, 1991).

Sulfidic environments
A major source of sulfide in the marine environment is the anaerobic degradation of organic materials by sulfate-reducing bacteria. Consequently, high sulfide concentrations and dense populations of thiotrophic bacteria occur in areas where a lot of organic materials settle at the sea floor, as for example in upwelling areas, in eutrophic coastal environments or at whale falls. The main source of sulfide is the sediment; only in very enclosed eutrophic basins sulfide may be produced in the open water (e.g., the Black Sea). Another source of sulfide is highly reduced fluids, which are transported to the sediment surface by advection (e.g., hydrothermal vents, seeps). These are point sources of sulfide in the ocean, which can be densely populated by free-living or symbiotic, thiotrophic bacteria.

Free-living thiotrophs
Many heterotrophic bacteria are capable of also oxidizing reduced sulfur components for energy generation and many thiotrophic bacteria can also use organic carbon as substrate for growth (Robertson and Kuenen, 2006). The classical lithoautotrophic sulfide-oxidizing bacteria are the thiobacilli, which have recently been reclassified and are now distributed to several different genera (e.g., Acidithiobacillus, Halothiobacillus). Another classical and well-studied group of thiotrophic bacteria are the members of the genus Thiomicrospira. Both groups harbor strains which can use nitrate as alternative electron acceptor with nitrogen as the end product (Thiobacillus denitrificans and Thiomicrospira denitrificans, now Sulfurimonas denitrificans). It is long known that many thiotrophic bacteria can create and survive very low pH values. A well-studied example is Thiobacillus ferrooxidans (now Acidithiobacillus ferrooxidans), which causes problems in acid mine drainage but can also be used for ore leaching. Only recently a number of alkaliphilic thiotrophic bacteria have been isolated (Sorokin and Kuenen, 2005).

Among the free-living thiotrophic bacteria, there is a morphologically conspicuous group of large or even gigantic cells with inclusions of elemental sulfur. These large sulfur bacteria are widespread in nature, but are difficult to cultivate. Beggiatoa is the longest known, best studied, and probably also the most abundant genus among the morphologically conspicuous sulfur bacteria. They form multicellular, highly motile filaments of several millimeters to centimeter length which are 1–200 μm in diameter. Other genera of morphologically conspicuous sulfur bacteria are Thioploca, Thiromargarita, Thiothrix, Achromatium, and Thiovulum (Schulz and Jørgensen, 2001). Among the genera Beggiatoa, Thioploca, and Thiomargarita, there are giant forms with vacuoles used for the internal accumulation of nitrate.
which serve as an alternative electron acceptor for sulfide oxidation. With the exception of *Thiovulam* all morphologically conspicuous sulfur bacteria are within the gammaproteobacteria. *Thiovulam* is a member of the epsilonproteobacteria and is closely related to smaller free-living thiotrophic bacteria, which only recently have been recognized to be widespread sulfide oxidizers at hydrothermal vents (Campbell et al., 2006). Habitats with very high sulfide fluxes such as hydrothermal vents often favor the growth of a vibrid epsilonproteobacterium called Candidatus Arcoabacter sulfidicus, which oxidizes sulfide rapidly to elemental sulfur. The resulting filaments of elemental sulfur are left behind (Wirsen et al., 2002).

**Endosymbionts**

Since the discovery of abundant life at hydrothermal vent systems in 1977 it took several years before it was recognized that the gutless tube worm *Riftia* found in high numbers at vents lives on energy conserved by endosymbiotic thiotrophic bacteria (Cavanaugh et al., 1981). The bacteria live on sulfide and oxygen provided by the host and use this energy source to reduce CO$_2$ to organic carbon compounds, which they share with their host. Many such tight associations between invertebrate animals and thiotrophic bacteria have been reported since the first discovery not only from vent systems but also from other sulfidic marine environments. Endosymbiosis with thiotrophic bacteria seems to be especially common among mollusca (e.g., clams and mussels) and annelida (e.g., tube worms) (Cavanaugh et al., 2006). None of the endosymbiotic bacteria could yet be grown outside of its host, but an abundance of information was obtained by using culture-independent methods. Most endosymbiotic thiotrophs are members of the gammaproteobacteria but a few symbionts belong to the epsilonproteobacteria. Usually, host animals of one species are found to contain the same or closely related bacteria, indicating a close symbiotic association. For the clam *Calypgoena* there are indications that the symbiont is transferred directly from mother to offspring (Cary and Giovannoni, 1991), whereas the tube worm *Riftia* seems to acquire symbionts de novo from the environment (Laue and Nelson, 1997). For many host animals the mode of infection with the symbiont is not clarified yet (Cavanaugh et al., 2006).

**Ectosymbionts**

Virtually every larger organism is to some degree colonized by bacteria living on their surface. These so-called epibionts may have harmful, beneficial, or neutral effects on the host. In sulfidic environments thiotrophic bacteria frequently populate organisms such as ciliates, shrimps, or gastropods (Ott et al., 2004). In some cases, it could be shown that the host is feeding on these bacteria or may even provide conditions favoring the growth of thiotrophic bacteria. In other cases, the benefit for the host seems to lie in the detoxification of sulfide by the epibionts or no obvious benefit could be observed (Ott et al., 2004).

**Summary**

Thiotrophic bacteria (or sulfur bacteria) live by the oxidation of sulfide with oxygen or nitrate as electron acceptor. This lithotrophic metabolism is widespread among bacteria and also found in some archaea. Among the gammaproteobacteria, a group of giant bacteria is specialized on oxidizing sulfide with nitrate, which is accumulated in a vacuole. Many thiotrophic bacteria live in symbiosis with higher organisms either as endo- or as ectosymbionts.

**Bibliography**


Cross-references
Beggiatoa
Chemolithotrophy
Cold Seeps
Hydrothermal Environments, Marine
Pyrite Oxidation
Sulfate-Reducing Bacteria
Sulfur Cycle
Thiomargarita

THROMBOLITES

Thrombolites (Greek: *thrombos*, clot; *lithos*, stone) are “cryptalgal structures related to stromatolites, but lacking lamination and characterized by a macroscopic clotted fabric” (Aitken, 1967, p. 1164).

Bibliography

Cross-references
Microbialites, Stromatolites, and Thrombolites

TIDAL FLATS

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Synonyms
Wadden Sea: name of the world’s largest tidal flat system in Central Europe

Definition
*Tidal Flat*. A wide and almost flat coastal area between the mean upper and lower tidal seawater levels (eulitoral) exposed to the rhythmic tidal water movements. It consists of unconsolidated sediments and is bordered toward the land by permanently emerged areas and toward the sea by the permanently submerged sublitoral zone.

Introduction
Tidal flats occur worldwide in shallow coastal regions: in the tropics, subtropics, temperate zones, and subpolar, and polar regions. In the tropics and subtropics, such as at various regions of the east coast of South America, in Africa, Bangladesh, Indonesia, and Australia, tidal flats are closely associated with mangrove ecosystems. In other places, such as Mauretania, the Persian Gulf, and the coast of Texas, USA, tidal flats are either wide and open coastal stretches or protected by barrier islands. Large tidal flat areas adjacent to marshlands exist on the Atlantic coast of the USA (North/South Carolina, Georgia) and Canada (Nova Scotia, Bay of Fundy), Korea, and in Europe along the Atlantic coast (Portugal, Spain, France, and Great Britain), and the North Sea. At the southern coast of the North Sea, the worldwide largest tidal flat system, the Wadden Sea ranges from The Netherlands (den Helder) through Germany to Denmark (Esbjerg). Subpolar and polar tidal flat systems are found in southern Chile, Alaska, and Siberia. Tidal flats may also develop in shallow estuaries, and thus their sediments may partially exhibit riverine properties.

A special type of tidal flats is wind-induced; these wind flats form along shallow coastlines with very little tidal water movements but occasionally persistently strong winds. The winds lead to the emergence or submergence of shallow areas for irregular periods, and the flats are characterized by microbial mats and sediments similar to those of regular tidal flats.

Formation and shaping forces
Along shallow coastlines with little wave action, the rhythmic incoming and outgoing tidal currents are the main driving forces for establishing and structuring tidal flats. A critical tidal range of at least 1 m is a prerequisite for the formation of a tidal flat. The incoming tide imports dissolved nutrients and particulate organic and inorganic matter, which settle out during reduced water movement at slack tide. This hydrographic energy gradient leads to a fractionation of the suspended material according to its settling properties. The finest material, the mud fraction (>50% of the material with a particle size <63 µm and the highest content of organic carbon up to 10% of dry weight) settles at the lowest current velocity and thus in the shallowest and least energetic zones. The other fractions consist of sandy (<5% mud) and mixed sediments (5–50% mud) and form the bulk of the sediment in highly and intermediate energetic areas (Chang et al., 2006). As a consequence, material and nutrients accumulate in tidal flats and provide the basis for highly productive ecosystems (Postma, 1981). Additional input of particulate organic and inorganic matter and nutrients may come from the adjacent coastal areas permanently emerged and/or by pore water (Kasten, this volume) and groundwater via tidal pumping (only inorganic nutrients and dissolved organic matter). Export of matter occurs mainly in the dissolved form and net export of particulates occurs only when re-suspension exceeds sedimentation for more than a tidal cycle, during storm events. The interplay of coastal and tidal currents, settling, re-suspension, and fractionation of suspended matter and sediment, results in the formation of typical depositional features, such as banks, islands, barrier island systems with distinct sediment structures, and drainage channels of different sizes and depths (Figure 1). Because of the ample nutrient supply, tidal flats are hot spots of marine productivity and biodiversity.
Recent studies have shown that settling of suspended material on tidal flats is not purely hydrodynamically and physico-chemically driven. Instead, bacteria associated with the suspended material actively enhance the aggregation of suspended particles, followed by the sinking of the aggregated material at slack water (Chang et al., 2006; Lunau et al., 2006). During periods of low microbial activity, such as during winter in temperate regions, aggregation is low and suspended particles that persist in the water column are more abundant and smaller in size than during the warmer, growing season. The consolidation of sediment is enhanced by biofilms/microbial mats (Neu, Reitner, this volume) at the sediment-water interface and the associated production of exopolymeric substances (EPS, see Decho, entry Extracellular Polymeric Substances (EPS), this volume), consisting mainly of polysaccharides and proteins, which glue together the fine primary sediment particles (Stal, 2003). As these biofilms are more biologically active during the enhanced temperatures of the spring and summer growing seasons, the sediments are more subject to re-suspension and erosion during periods of low temperature in fall and winter (Chang et al., 2006).

Under conditions when ice may cover large areas of tidal flats, such as during particularly cold winters in the temperate zone or during regular winters in subpolar and polar regions, tidal movement of ice flows can cause considerable erosion of the unconsolidated surface sediments.

**Geobiology of tidal flats**

The high productivity of tidal flats is reflected in their use as resting and overwintering areas for myriads of migrating birds, which feed on the infauna such as molluscs, crustaceans, and worms. The food bases for these animals that rework and often bioturbate the tidal flats are autotrophic and heterotrophic microbes thriving in the water column, at the sediment-water interface and within the sediment (Reise, 2002). Primary producers in the water column are dominated by pennate diatoms (Bacillariales) in the temperate and polar zones, and diatoms, dinoflagellates, and coccolithophorids in subtropical and tropical regions. The suspended matter in the water column is heavily colonized by heterotrophic bacteria which decompose a substantial part of the available organic matter and also affect the aggregation and thus deposition of the settling material. In the oxic layer of the biofilms/microbial mats at the sediment-water interface, the dominant primary producers are filamentous cyanobacteria (Friedl, this volume), and diatoms (Stal, 2003). Because of the high primary production rates and the import of organic matter, mineralization rates of organic matter and thus oxygen consumption in the sediment is very high, resulting in anoxic conditions. Hence, anoxic microbial processes largely dominate within sediments, greatly affecting the formation and diagenesis of these sediments (Mackenzie, this volume).

The composition of the tidal flat-associated microbial communities is a function of the available light, pore water advection, and redox conditions, i.e., the availability of organic carbon, electron acceptors, and donors. The shapes of the redox profiles within the sediments, i.e., oxygen and sulfide concentration (Böttcher, this volume), are a function of sediment structure, grain size, pore water advection, and the metabolic activities of the microbes. Anoxygenic phototrophic bacteria contribute...
to CO₂-fixation by oxidizing sulfide. When the sediment remains undisturbed, a vertical zonation of the autotrophic communities may form distinctly colored layers, the so-called Farbstreifensandwatt (Stal et al., 1985; Figure 2), with a brownish-green layer at the top (diatoms), followed by a blue-green (cyanobacteria), a pink (purple sulfur bacteria), a green (purple non-sulfur bacteria), and a black layer at the bottom (sulfate reducers). A black sulfidic zone of sulfate reduction is missing in this example because of lack of iron. Scale bar: 1 cm. (Photo: H. Cypionka, www.microbiological-garden.net.)

Mud flats versus sand flats

The composition of the inorganic sediment in the tidal flats may vary considerably but has little influence on the general grain size distribution. Depending on the geologic setting and the coastal environment, quartz, volcanic minerals, or biogenic carbonates can dominate or constitute varying proportions of the inorganic components of the sediments. Grain size and organic carbon content differ markedly in mud flats and sand flats. The smaller grain size and higher organic content of the mud flats result in a much more compact structure and a lower pore water volume and permeability. These characteristics have led to the traditional view that decomposition rates of organic carbon are much higher in mud flats than in sand flats. Recent studies, however, have shown that mineralization rates of organic carbon in sand flats are at least as high as and often higher than in mud flats (De Beer et al., 2005). In sand flats with a high pore water volume, oxygen supply is much higher than in mud flats, supporting high aerobic mineralization rates and leading to a lower content of organic carbon. Hence, the low content of organic carbon in sand flats appears not to be a result of the lower input of organic matter or reduced microbial activities as compared to mixed or mud flats but instead reflect more favorable conditions for the microbial decomposition of organic carbon.

Geologic age

Recent tidal flats are young geobiological systems on a geologic time scale and originated in the Holocene. They typically developed in the course of the sea water level rise in shallow and flat coastal regions during the last 10,000 years after the Pleistocene, when large amounts of sediments were deposited.

Related term

Sabkha: Almost flat, vegetation-free coastal areas above the mean and upper tidal seawater level in arid subtropical regions like at the Persian and Arabian Gulf. They are covered by salt deposits of the evaporating seawater and other crystalline deposits of halite, anhydrite, and gypsum, and by microbial mats of filamentous cyanobacteria.

Summary

Tidal flats are almost flat coastal areas between the mean upper and lower tidal sea water levels and a tidal range of at least 1 m and occur in all climatic regions. They are
structured by tidal channels and banks, and accumulate particulate matter which settles out at sites of reduced water movement. As a consequence, sediment layers are formed which rapidly become anoxic below the surface because of the intense microbial decomposition processes. Due to the high input of organic matter, tidal flats are one of the most productive ecosystems in the world; this productivity is fueled by microbial processes in the water column and within the sediments of the tidal flat regions.

Bibliography

Cross-references
Anaerobic Methane Oxidation with Sulfate
Biofilms
Cyanobacteria
Diatoms
Evaporites
Extracellular Polymeric Substances (EPS)
Microbial Mats
Pore Water
Sediment Diagenesis – Biologically Controlled
Sulfate-Reducing Bacteria
Sulfur Cycle

ToF-SIMS
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Definition
Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a method for chemical microanalysis of solid surfaces (Benninghoven, 1994; Belu, 2003; Vickerman and Briggs, 2001). The method can be used to obtain detailed information of the organic and inorganic composition of samples at a spatial resolution down to <1 μm. It is a highly surface-sensitive method, providing chemical information about the uppermost molecular layers only. The analysis is based on mass spectrometry and requires no preselection or labelling of the substances to be analyzed.

In a ToF-SIMS measurement, the sample is bombarded by a pulsed, focused, high-energy ion beam (primary ions) that causes atoms, molecular fragments, and intact molecules to be ejected from the sample surface. A small fraction of the ejected particles will be ionized (secondary ions). The secondary ions are extracted and directed into a time-of-flight (ToF) mass analyzer, where they are separated with respect to mass to charge ratio. The resulting mass spectrum represents a chemical fingerprint from the sample surface at the point of impact of the primary ion beam (Figure 1).

Characterization of the chemical substances present on the sample surface is done by identification of the ions corresponding to the peaks in the mass spectrum, in a similar way as in other mass spectrometric techniques, such as GC/MS and LC/MS. The high mass resolution of the ToF analyzer (typically m/Δm ~5,000–10,000) allows for detailed identification of the secondary ions.

The spatial information, i.e., the lateral distribution of the different chemical components on the sample surface, is obtained by scanning the primary ion beam over the sample surface and recording separate mass spectra from a large number of points (pixels) within the analysis area. The acquired data can be presented in different ways: (a) as mass spectra of the total analysis area or selected regions of interest, (b) as ion images, in which the signal intensity distribution from ions representing specific elements or compounds are displayed as variations in brightness over the analysis area (see Figures 1 and 2), or (c) as time evolutions of specific ion signal intensities during ion etching using a separate ion beam (depth profiling).

An important advantage of the ToF-SIMS technique, in relation to other SIMS methods, is that the ToF mass analyzer allows for so-called static SIMS analysis, i.e., analysis of the undamaged surface before the mass spectrum has been significantly altered by the molecular damage caused by the primary ions. In dynamic SIMS, the sample surface is subjected to considerably higher primary ion dose
densities, which means that the analysis is done on a molecularly damaged and continuously eroding surface. As a consequence, ToF-SIMS is capable of providing detailed molecular information of, e.g., organic substances (up to around 5,000 Da), while dynamic SIMS is limited to information on elements, isotopes, and small fragments on the surface.

For organic microanalysis using ToF-SIMS, the use of cluster ions, such as $\text{Bi}_n^+$, $n = 3-7$, $\text{Au}_3^+$, and $\text{C}_{60}^+$, as primary ions has proven advantageous because...
these ions produce high secondary ion yields for large (characteristic) organic ions in organic materials (Kollmer, 2004). In addition, it has been shown that some molecular information is retained in organic materials also after extensive bombardment by C$_{60}^+$ ions, making this ion suitable for ion etching (depth profiling) of organic materials (Wucher et al., 2007).

These capabilities make ToF-SIMS a promising tool for the investigation of organic biomarkers in different types of geobiological materials, particularly if only small sample amounts are available, or a high lateral resolution is desired. Examples are mineral phases in microbialites or microscopic samples (i.e., sections) of microbial mats (Figure 3; Thiel et al., 2007a, b; Sjövall et al., 2008). Furthermore, after hopanes and steranes have been successfully detected in different types of crude oils using ToF-SIMS (Sjövall et al., 2008; Siljeström et al., 2009), the identification of authentic biomarkers in single oil inclusions of ancient rocks now appears to be within reach (Siljeström et al., 2010).

**Bibliography**


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Synonyms
Ichnofossils; Neoproterozoic

Definition
Neoproterozoic trace fossils give us the earliest signs of animals moving on or just below the sea floor typically in the form of small horizontal unbranched trails and furrows. Despite their modest appearances, these trace fossils are important in that they provide an undisputable minimum age for the appearance of bilaterian animals. Remarkable are the recent discoveries of the Ediacaran organisms, Kimberella and Dickinsonia, in association with their trace fossils. These associations suggest exploitation of microbial mats, and a similar behavior probably explains also the simple horizontal Neoproterozoic trace fossils.

Introduction
Much of the Neoproterozoic sedimentary record is without the sign of burrows, trails, or other types of animal trace fossils. A continuous record of widely accepted trace fossil appear only after about 560 Ma ago (Figure 1), and by comparison to the Phanerozoic the diversity and complexity is low and the size and depth of bioturbation are modest and generally considered in terms of a few millimeters. Indeed, though the transition between Ediacaran and Phanerozoic ichnofossil assemblages is somewhat gradual, the difference is nevertheless striking, notably in what is missing in the former. The Ediacaran lacks any evidence of trace fossils produced by animals with arthropod-type appendages, and traces showing adjustment to sedimentation (spreiten). There is little evidence for permanently maintained open burrows, and there are essentially no complex burrow systems. The Ediacaran trace fossil record is almost exclusively known from shallow-water marine deposits. Temporal trends in Ediacaran trace fossils are as yet tentative, but it can be noted that no trace fossils are currently known from the oldest associations of Ediacara-type fossils and that the first three-dimensional burrow systems, appearing shortly before the Ediacaran–Cambrian boundary, overlapping the range of some of the youngest Ediacara-type fossils (Figure 1). Among early studies, that of Seilacher (1956) was one of the first papers in which the contrasting nature of Proterozoic and Phanerozoic trace fossils was clearly stated, and the broad synthesis of Crimes (1987) provided a summary of the literature to date. The paper by Narbonne et al. (1987) outlined the Ediacaran–Cambrian sequence of trace fossils in sections in Newfoundland and set the stage for the subsequent definition of the base of the Cambrian on the basis of trace fossils in this region. Recent overviews and discussions of Ediacaran trace fossils, where the reader can find additional references to earlier papers, can be found in Jensen (2003), Droser et al. (2005), Seilacher et al. (2005), Jensen et al. (2006), Weber et al. (2007), Seilacher (2007), and Fedonkin and Vickers-Rich (2007). Currently, one of the major areas of study of Ediacaran trace fossils is, perhaps somewhat surprisingly, in deciding what is and what is not a trace fossil. In this brief overview, we will consider Ediacaran trace fossils under three headings.

Ediacaran trace fossils: true and false

Traditional trace fossils: Meaning trace fossils that can reasonably be assigned to ichnotaxa also described from the Phanerozoic. The most common Ediacaran trace fossils are essentially horizontal, unbranched, and with few exceptions small (a few millimeters wide), grooves or ridges on the upper or lower surface of sandstone beds (Figure 2c and d). These trace fossils, which are generally referred to as Helminthoidichnites, represent transitional movement through the sediment, close to the sediment–water interface, and there may be lateral raised ridges where sediment was pressed to the sides (e.g., Droser et al., 2005). They have been interpreted as mining of decaying microbial mats (Seilacher, 1999). Other possible Ediacaran trace fossils include
small star-shaped forms and simple vertical tubes, but these are rare.

Ediacara-type organisms associated with their trace fossils: These remarkable fossils are known from northern Russia and South Australia. The possibly mollusc-grade *Kimberella* has been found associated with radial fan-shaped structures (Figure 2e and f) apparently representing raspings as well as possible traces of movement (Fedonkin et al., 2007). *Dickinsonia* has been found associated with disjunct rows of imprints (Figure 2g), perhaps representing pulses of degradation of a microbial mat (Gehling et al., 2005). These associations are all the more remarkable because associations of trace fossils with their producers are exceptionally rare in the Phanerozoic in normal marine settings.

False and doubtful Neoproterozoic trace fossils: The list of purported Ediacaran trace fossils is in fact quite extensive but many of these are now believed to be better explained as body fossils (Seilacher et al., 2005; Droser et al., 2005; Jensen et al., 2006). Common examples are *Yelovichnus* and *Palaeopascichnus*, which consist of short series of closely spaced kidney- or sausage-shaped objects (Figure 2a and b). Rather than trace fossils, these long-ranging forms, may have been algal or xenophyophore protists (Seilacher et al., 2005). Other supposed trace fossils represent the three-dimensional preservation of tubular organisms (Figure 2h) (Droser and Gehling, 2005). The evaluation of some of these purported Ediacaran trace fossil remains problematic. For example, *Nenoxites* might be an early example of a back-filled burrow (Seilacher et al., 2005). It is, however, clear that a considerable non-trace fossil Ediacaran organismal diversity is hidden here.

**Broader significance of Neoproterozoic trace fossils**

Sediment properties and paleoecology: The rise of bioturbation had dramatic effects on sediment properties as well as on seafloor ecology, as entailed in the agronomic revolution or Cambrian substrate revolution (Seilacher, 1999; Bottjer et al., 2000). The gradual appearance of deeper and more intense bioturbation and by larger animals that took place during the late Ediacaran and early Paleozoic led to a shift from a seafloor largely covered with microbial mats and with a sharp sediment–water interface to a biogeochemically more active and deeper zone of surface sediments (McIlroy and Logan, 1999; Droser et al., 2002; Meysman et al., 2006). Many late Ediacaran and early Cambrian organisms may have had a lifestyle adapted to mat grounds (including the early trace fossil-makers) and a relatively firm muddy sea floor and these would have been negatively affected by the rise of bioturbation (Seilacher, 1999; Bottjer et al., 2000; Dornbos, 2006).

Evolution of metazoans: The Ediacaran trace fossil record is important in the discussion of the appearance of metazoans and in particular that of mobile animals. An obvious but important point is that the preservation potential of a trace fossil is decoupled from that of its producer. Thus, one line of argument goes that the appearance of trace fossils is unlikely to substantially postdate the appearance of mobile benthic animals and that the
radiation of bilaterian animals would have occurred in animals of a size that would readily leave a trace fossil record (Budd and Jensen, 2000; Jensen et al., 2005). The late Ediacaran appearance of trace fossils and the subsequent radiation at the Ediacaran–Cambrian boundary fits in with evidence from diverse fossil groups (and at least some molecular clock studies) of the appearance of increasingly diverse ecosystems following the Marinoan glaciation (Peterson and Butterfield, 2005; Butterfield, 2007) (Figure 1). Organisms other than metazoans are potential trace fossil makers (Matz et al., 2008) but the Ediacaran to Cambrian trace fossil diversification can only be explained as being part of a metazoan radiation. It is not known to what extent the types of trace fossils found in the Ediacaran was the result of limited neurological sophistication, limited capability to burrow deeply and/or withstand anoxic sediments or if the type of trace fossil largely reflect a particular type of feeding adapted to microbial mats in an environment with little competition. It has been suggested that the appearance of deeper burrows were a response to increased predation (e.g., Dzik, 2005).

Summary
Animal trace fossils first appear close to the end of the Neoproterozoic era typically in the form of simple horizontal burrows or trails, a few millimeters wide; although, the exact diversity of Ediacaran trace fossils is currently a hot topic. Particularly noteworthy is the occurrence of trace fossils made by the Ediacara-type organisms Dickinsonia and Kimberella. The appearance of Neoproterozoic trace fossils and their subsequent radiation at the Ediacaran–Cambrian transition most likely correspond to the diversification of animals and ecosystems at this time.

The rise of bioturbation probably played an important role in structuring early benthic ecosystems.

Bibliography
(The list of reference is heavily biased toward the most recent literature, including recent reviews. Important earlier studies can be found by consulting these papers.)


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**Cross-references**

Critical Intervals in Earth History

Ediacaran Biota

Microbial Mats

Origins of the Metazoa

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**TUFA, FRESHWATER**

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**Synonyms**

Travertines (in definition of Pentecost, 2005)

**Definition**

Tufas are calcareous deposits formed by inorganic or microbiologically induced precipitation of calcium carbonate in a wide range of freshwater environments, including streams, rivers, and lakes. Calcium carbonate precipitates principally on the tufa surface, and the term excludes lacustrine marl in which carbonate particles are formed in water column. Tufas are commonly developed in limestone areas from tropical to temperate climatic zones (Pentecost, 1995) showing a variety of geometry from a huge riverine barrage to a small mound developed just beside water springs (Pedley, 1990). Following the definitions of Ford and Pedley (1996) that tufas are carbonate deposited in cool or near ambient temperature freshwater and in open-air conditions, the term excludes carbonate deposits in hydrothermal water (travertines) and in a limestone cave (speleothems). Some workers prefer the definition of the term carrying textural implication because the term tufa was originally derived from a Greek word *topos* which refers porous deposits, such as carbonate tufa and volcanic tuff.
Processes

Water bearing the carbonate precipitation normally originates from an underground water system in carbonate rock where relatively high $p\text{CO}_2$ raises the concentration of dissolved carbonate, but rarely from the mixture of hydrothermal water that contains a substantial amount of calcium and bicarbonate (Yoshimura et al., 2004). Tufa deposition requires supersaturation of water with respect to calcium carbonates, which are essentially achieved by $CO_2$ degassing from the water due to a large difference in $p\text{CO}_2$ between underground water and the atmosphere. The water actively degasses at sites where waterfalls or other obstructions along the passage cause water turbulence, and reaches high supersaturation ($\sim10$ times; Chen et al., 2004). Preferential deposition at the sites of high water turbulence often develops tufa mounds accompanied with water pools at the upcurrent side (Pedley, 1990). A tufa can grow at a much higher rate (typically several millimeter/year; Kano et al., 2003) than speleothems. However, continuous tufa growth has seldom lasted longer than 100 years because growth of the mounds changes watercourse in normal fluvial settings. Because of the circumsitual temperature and relatively low value of dissolved Mg/Ca, mineralogy of the most tufas is calcite. Because tufas form outside in the light, unlike speleothems, phototrophic microbes (cyanobacteria, freshwater algae, and diatoms), and mosses normally live on the surface. The microbes form biofilms and influence carbonate precipitation rate and depositional textures of tufa. Especially, cyanobacteria are most common and have been considered to activate tufa deposition by photosynthetic uptake of $CO_2$ and bicarbonate that raises supersaturation of calcium carbonate (Mert, 1992), or by providing nucleation site of carbonate minerals (Pentecost and Riding, 1986). In such tufas, the calcite is deposited as fine-grained (5–10 µm diameter) crystals encrusting a cyanobacteria sheath, which results in porous texture typically containing 50% of porosity (Kano and Fujii, 2000).

Annual lamination

Some tufas develop annual laminations consisting of both densely calcified and porous layers. Two different explanations have been proposed to account for the annual laminations. One is a seasonal change in the predominant microbial association seen in the biofilms covering the tufa surface (Freytet and Plet, 1996; Janssen et al., 1999; Arp et al., 2001). The tufa in the Franconian Alps, Germany, exhibits porous layers formed in winter–spring when diatom-dominated biofilm inhibits carbonate deposition on the tufa surface (Arp et al., 2001). However, the seasonal change in microbial association cannot always account for the lamination of some tufas, for instance, the ones from SW Japan dominated by cyanobacteria communities throughout the year (Kano et al., 2003). The annual laminations can be developed due to seasonal changes in the inorganic calcite precipitation rate (Kano et al., 2003). Under a high rate during summer–autumn, the calcite crystals thickly encrust the cyanobacterial sheathes until they become attached to neighboring encrusted sheathes and form a dense layer. In contrast, a low rate during winter–autumn results in the thinly encrusted sheathes and leaves abundant free space in a porous layer.

Climatic archive

Stable isotopes and trace elements of carbonate fraction of tufa deposits have been recently used for Holocene climatic studies (Zak et al., 2002; Makhnach et al., 2004; Garnett et al., 2004). Bulk $\delta^{18}O$ values primarily record latitude and altitude control on $\delta^{18}O$ of meteoric water (Andrews et al., 1997); although, evaporation largely affects the values in semiarid regions (Smith et al., 2004). On the other hand, $\delta^{13}C$ values are influenced by variable conditions including degassing of $^{12}C$-enriched $CO_2$ (Pentecost, and Spiro, 1990), contribution of soil $CO_2$ (Hori et al., 2008), and vegetation (C3 vs. C4 plant; Kano et al., 2007), and therefore the interpretation is often problematic.

Because of their high depositional rate and development of annual lamination, tufas are potential archives of high-resolution paleoclimatic records (Andrews and Brasier, 2005; Andrews, 2006). Studies of modern specimens have reported cyclic changes in $\delta^{18}O$ and trace elements in tufa calcite that correspond to annually laminated textures, and therefore indicate seasonal change in water temperature (Matuoka et al., 2001; Ihlenfeld et al., 2003). Tufa is also an excellent recorder of rainfall. A specimen from SW Japan exhibits a number of clay bands that were consistently correlated with heavy rainfall events of >50 mm (Kano et al., 2003). These approaches were recently applied to the Holocene tufa from Arizona (O’Brien et al., 2006).

Conclusion

Tufas are carbonate precipitates under the influence of photosynthetic microbes (cyanobacteria and algae), but the carbonate precipitation is primarily controlled by inorganic conditions, e.g., $CO_2$ degassing and raised supersaturation of the water. There are increasing evidences that stable isotopes and elemental geochemistry of the calcite fraction record clear environmental and climatic information.

Tufas provide high-resolution, but discontinuous paleoclimatic records from core and outcrop specimens, which can be dated by appropriate methods, such as U-Th dating of pure carbonate fraction, and radiocarbon composition of exceptionally preserved plant fragments. A promising approach in future research is combined investigation of speleothems and tufas from the same area. High-resolution temperature and rainfall records derived from tufas can add significant information to terrestrial paleoclimatology.
Bibliography


Cross-references

Biofilms
Calcite Precipitation, Microbially Induced Carbonates
Cyanobacteria
Isotopes and Geobiology
Waulsortian mud mounds

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Synonyms
Waulsortian banks; Waulsortian buildups; Waulsortian mounds; Waulsortian mudbanks; Waulsortian reefs

Definition
Waulsortian mud mounds are characterized by different types of complex and highly structured fabrics of carbonate muds, texturally and genetically varied (both automicrites and allomicrites occur), with a variable content in skeletal components (heterozoan assemblages). Their origin is controversial although most authors advocate a microbial origin (calcite precipitation microbially induced). They were developed throughout the northern hemisphere in many parts of the World (Europe and North America) during the Carboniferous times (Lower Mississippian, mainly in the Tournaisian). These mud mounds grew on wider ramps and within basins in tropical “calcite seas,” immersed in a period of intense global changes (tectonic activity related with the Variscan orogeny and major sea-level changes).

Introduction
The term, from the Waulsort area, was first introduced by Dupont (1863) who studied and divided the Carboniferous Limestone of Belgium in six stratigraphic units. In the 1960s and 1970s, most of the carbonate buildups of Mississippian age were collectively named as “Waulsortian,” however, not all of them can be considered as true or classic “Waulsortian” as well as the whole Mississippian bioconstruction spectrum can not be classified or assigned to the Waulsortian mud mounds (Figure 1). Some authors believe that the application of denominations like “Waulsortian,” “Waulsortian-like,” and “non-Waulsortian” buildups is of limited value because they don’t mean a fast and clear idea about the geometry, composition, and/or the possible buildup origin. In fact, the use and meanings of the term “Waulsortian” have been controversial from their origin in the 19th century and has been not always used with clarity (see historical review by Lees, 1988).

The study of the Waulsortian mud mounds have been focused in several aspects, developed not always with the same profusion: (a) facies relationships and geometries (external morphology and internal structure); (b) allochems; (c) carbonate mud types; and (d) open space structures.

Facies and geometry
Waulsortian mud mound (Mud mound is considered here as just a descriptive, nongenetic, non-morphological term, to describe a type of bioconstruction dominated by carbonate mud (>50% of the rock volume)) facies are separated normally in Waulsortian massive facies, off-mound or coeval bottom facies and flank facies, which can or not occur.

Waulsortian limestones facies are massive, although bedding intervals can occur, and characteristically pale violet-grey to dark grey in color. In the field, the major lithological variations are based on changes of the following features: (1) carbonate mud content (dominance of “muddy” fabrics); (2) presence of coarsely calcite mosaics (dominance of “sparry” fabrics); (3) in situ fenestellid bryozoan fronds; (4) skeletal debris lenses, commonly crinoids and bryozoans; and (5) other entire fossils. These characters can occur combined in different proportions,
resulting in fast laterally and vertically subfacies changes in few centimeters between: (1) bryozoan cementstones (fenestellid fronds, Figure 2a); (2) bryozoan wackestones (fenestellid fronds); (3) skeletal wackestones; (4) skeletal wackestones with “sparry fabrics” (Figure 2b); and (5) crinoidal-rich packstones.

Geometrically, they were formed by the aggregation of successive, laterally extensive banks, which developed final tabular-, knoll-, or sheet-growth morphologies (Figure 3). Growth morphologies are function of many variables, some of them related with: (1) the autochthonous carbonate mud production rate; (2) the ability of the autochthonous carbonate mud to construct depositional slopes (some of them up to 50 degrees); (3) the coeval sedimentation versus carbonate mud production relationship; (4) the sea floor topography; (5) the available accommodation space, etc.

Final dimensions vary from few meters, in the simple Waulsortian mud mounds, up to hundreds of meters in thickness and thousands of square kilometers across in some Waulsortian complexes or aggregates (Figure 3). In Ireland, the biggest Waulsortian complex crops range around 1 km in thickness and spread over 30,000 km².

Allochems
The allochems record in the Waulsortian mud mounds, in terms of volumetric importance, is fundamentally formed by crinoids, bryozoans (mainly fenestrate), ostracods, brachiopods, and molluscs (gastropods, goniatities, lamellibranches, and nautiloids). Sponge spicules (hyalosteliid) are common. Minoritary components such as foraminifers, calcified filaments of cyanobacteria, moravaminids, aoujgaliids also occur (some of them are considered as problematica taxa by some authors). Accessory allochems are trilobites, echinoid spines, serpulids, and clorophytes. Colonial rugose corals are very rare and they occurred exceptionally in the upper part of some of these mud mounds. Others as solitary forms and heterocorals are also uncommon. Non-skeletal grains as intraclasts can appear and be locally common in some parts and small, micritized radial oolites are very unusual.

Component assemblages models
First detailed systematic petrographic analyses of Waulsortian mud mounds were carried in Belgium by Lees et al. (1985) and 25 parameters were ranked according their volumetric importance. Four depth-related phases were distinguished using correspondence analysis. Later, following the same methodology, more than 30 mud mounds from Europe and North America were analyzed and they showed also the Belgium component assemblages (results summarized in Lees and Miller, 1995). Thus, four Waulsortian Phases (A–D) were established, and they have been interpreted as the mud mound growth
from deep, subtidal, aphotic marine environments through to the photic zone (Figure 4):

- Phase A: fenestellids + crinoids + ostracods
- Phase B: resembles A + hyalosteliid sponge spicules
- Phase C: resembles B + plurilocular foraminifera
- Phase D: resembles C + cryptalgal coating, micritization (cement, cavity walls, grains), and calcareous algae.

In general, there is not a close correlation between the different component-assemblage phases and a determined aspect or facies at outcrop scale. Phase A represents the “classic” idea of Waulsortian facies, characterized by the fenestellid sheets with radial fibrous cements nucleated around the fronds (fenestellid cementstones) and pockets and lenses with crinoid debris. The content in fenestellid sheets tends to decrease in abundance in the rest of phases; in fact they are rare in Phase C. Sometimes they occur again in high proportions in Phase D, but bryozoans commonly were micritized before the cement nucleation.

The relationship between the mud mound biota and the off mound facies was systematically analyzed in New Mexico, where the exceptional quality of the outcrops (Figure 5a) allows this type of comparisons. Ahr and Stanton (1996), analyzed the frequency occurrence of 26 parameters on thin sections from different mounds, included Muleshoe mound (Alamogordo, Nunn, and Tierra Blanca phases from the lower to the upper part) and the off mound facies (Figure 5a and b).

Crinoids, fenestellid hash, and ostracods are the most abundant and similarly distributed fossils in both facies. In fact, in the off mound facies, most frequent fossils are crinoids, ostracods, fenestellid hash, sponge spicules, and echinoid spines (>80% of the thin sections) followed by mollusc and brachiopods shells, fragments of trilobites, and hyalosteliid spicules (Figure 5b). However, the abundance of skeletal components was low in both settings, although they were volumetrically more important and diverse on the mud mounds. Most of the variations could be related with taphonomic bias and differences in available habitats (cryptic spaces, soft, firm, and hard substrates) more suitable in/on mud mounds.

The systematic analysis of the biotic content from the off mound record was developed by Jeffery and Stanton (1996), and four component assemblages were established from deep to more shallow conditions:

- Assemblage I: crinoid/echinoderm debris, fenestellid hash, and ostracods

Waulsortian Mud Mounds, Figure 2 Classic Waulsortian facies: (a) bryozoan cementstones (scale bar = 5 cm) and (b) skeletal wackestone with stromatoid cavities. Ballybunnion outcrops, W Ireland.

Waulsortian Mud Mounds, Figure 3 Observed growth morphologies and facies relationships in Waulsortian mud mounds, redrawn and simplified from Lees and Miller (1995). a = Waulsortian facies; b = flank facies; c = off mound facies.
Assemblage II: I + stacheins (possible algae), rare salebrids (possible bryozoan), and commonly abundant sponge spicules
Assemblage III: II + plurilocular foraminifera
Assemblage IV: III + green algae

Biologically controlled contribution
The dominant macrobiota in the Waulsortian mud mound was formed by heterozoan assemblages, which never constituted a skeletal framework. Photosynthetic groups such as calcareous algae were very rare and uncommon to colonize these mud mounds. Crinoids and bryozoans have played an important role when they colonized and stabilized the slopes and mound surfaces. As skeletal producers, they formed favorable substrates (encrusters, cement nucleation, etc.) and they had also relative importance as allomicrite bafflers. Thus, the endemic macrofauna was composed by crinoids, fenestrate bryozoans, and sponges (Figure 6). The metabolism and decay of siliceous sponges could also favored the bacterial activity producing organic micrites, which would be added to the main autochthonous carbonate production in these mud mounds (biologically induced mechanism are argued by most authors). Opportunistic epifauna and infauna biota could colonize the locally soft mound substrates and after their death contribute to the biodegradation with the skeletal debris (Figure 6).

Carbonate muds
The most important component in the Waulsortian mud mounds is the carbonate mud, paradoxically during many decades its nature has been not considered or specifically studied. In fact, the origin of the carbonate muds and its ability to maintain steep slopes has been subject of much debate during years. Some authors have advocated for an external source followed by some kind of posterior accumulation mechanisms (hydrodynamic, baffling by bryozoans and crinoids, trapping and binding by bryozoans or other organisms). From the 1980s most part of the authors suggest a probable microbial origin for the carbonate mud, and a dominant in situ carbonate mud production is assumed.

The carbonate muds appear forming mudstone to packstone, although most works coincide in wackestone as the commonest texture. They are highly organized displaying successive generations of carbonate muds in geopetal relationship. They have been separated in primary muds (M1) and later muds (M2, M3, M4, etc.), all forming together the known polymuds (name coined by Lees and Miller, 1995). The earliest mud generation (M1) can represent less than 50% of carbonate mud volume. Lees and Miller (1995) subdivided the primary muds into three different subtypes (Figure 6):

(M1a) optically dense micrites, often with filaments
(M1b) paler biomicrites
(M1c) peloidal micrites

A biofilm model has been proposed for the in situ primary mud production (Figure 6). The biofilm formed by bacterial cells, cyanobacterial filaments, and bacterial extracellular polymeric substances (EPS), induced the precipitation of high-magnesium calcite micrites (dense
**Waulsortian Mud Mounds, Figure 5**  
(a) Muleshoe mud mound, Sacramento Mountains, New Mexico. Mud mound growth phases following Kirkby and Hunt (1996). (b) Components distribution of 26 elements from both off mound levels and mud mounds (Alamogordo Member mounds and Muleshoe mound – Alamogordo, Nunn, and Tierra Blanca Members). Modified from Ahr and Stanton (1996). % Frequency of occurrence; $R =$ rank position.

<table>
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<tr>
<th></th>
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<th>A. Mbr. (Mounds)</th>
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**Number of thin sections**

|                           | 83 | 103 | 154 |

**MULESHOE MUD MOUND: GP Growth Phases**  
TB. Mbr. = Tierra Blanca Member GP III, IV and V  
N. Mbr. = Nunn Member GP II  
A. Mbr. = Alamogordo Member GP I
Waulsortian Mud Mounds, Figure 6  Biofilm model with the process-response mechanisms proposed to Waulsortian mud mounds. (Simplified and modified after Lees and Miller, 1995.)
and peloidal ones). Biomicrites were formed by both reworking of dense or/peloidal micrites and the skeletal debris input (Figure 6). Additionally, the biofilm could be used by other mud mound colonizers as a source of organic carbon. Carbonate mud production was not only reduced to the active biofilm mound surface, but also continued below the surface favored by the biofilm degradation during early burial (Figure 6). Dewatering, shrinkage, carbonate dissolution, and reworking processes produced the collapse of carbonate mud matrix. The collapse and successive reorganizations of the carbonate muds through the cavity system resulted in the final polymud fabrics. Later fractures and fissures could also have favored sediment inputs through the mound void system (Figure 6).

There are no available data on the quantitative distribution/contribution of the different primary muds through the Waulsortian phases. Filaments, characteristic in dense micrites (M1a) donot occur in phase A, whereas they are abundant in the rest of phases. As well as, cavities from phase D contain important volumes of geopetal peloids intercalated with early calcite cements. Thus an early, deep, non-filamentous, non-photosynthetic cyanobacterial community has been suggested for the carbonate mud production in the Waulsortian phase A (Miller, 1986). In the same way, several microbial communities may have controlled the distribution/production of the different primary non-reworked mud textures (dense or peloidal micrites).

The distribution and typologies of carbonate mud, from the transitional facies to Waulsortian mud mound nucleation, were analyzed by Devusyst and Lees (2001), in western Ireland. Precursor muds are differentiated in function by their grumous or non-grumous character, bioclastic content, and contact relationship. They were grouped into two major types with six subtypes:

- Grumous types on basis of their apparent optical density, with three intergrading subtypes (codified as 1, 2 and 3). Types 1 and 2 are normally poor in skeletal debris; however, the dark optically dense type 3 contains abundant sponge spicules. All of them have been interpreted as automicrites. The grumous types are equivalent to the peloidal micrites (M1c) of Lees and Miller (1985, 1995).
- Non-grumous types: (1) gradational (into grumous muds) bioclastic wackestones; (2) distinct bioclastic wackestones; and (3) packstone matrix. The first subtype is also interpreted as automicrite whereas the others are seen as loose sediment.

The different precursor muds and other features were analyzed by correspondence analysis and a compositional gradient or trend was detected (Figure 7). The precursor muds display different textural and genetic types and culminate in the Waulsortian polymuds that contain both grumous and non-grumous muds (mainly non-grumous).

Open space structures

Waulsortian mud mounds were originally interpreted as reefs by Dupont (1881) who coined the term *Stromatactis* in 1881 to describe and interpret the “sparry masses” from Devonian mud mounds as recrystallized fossils. The stromatactis are a special type of cavities whose origin is still controversial. They have a characteristic shape, with a flat to undulose floor and irregular digitate roof and are filled by centripetal calcite cement, although internal sediment can also occur. Other terms like stromatactoid or stromatactis-like cavity are commonly found in the literature to describe similar features. They are common through the Precambrian up to the Jurassic mud mounds, (Bosence and Bridges, 1995) although can also appear in other types of limestones not just in muddy ones. A different hypothesis has been proposed to explain the stromatactis, which can be grouped in organic or inorganic models (see reviews by Bathurst, 1982; Flajs and Häusser, 1993; Monty, 1995; Flügel, 2004, p. 194).

In Belgium, some mud mounds facies contain those described as undulating or tortuous “vein bleues” which were used, for many years, as a traditional criterion to characterize the Waulsortian facies and but was later abandoned (Lees, 1988).

Sheet-form cavities, similar as known zebra cavities, just few centimeters thick but several meters in length, are also typical in Waulsortian mud mounds. Their roofs
are not clearly associated with skeletal support and they have been interpreted as the result of dewatering and shear stress processes in the firm, gel-like consistence muds.

Stromatolites, the sheet-form and irregular shelter cavities have been considered early-formed cavity systems (Lees and Miller, 1995). Dissolution as well as physical processes like fracturing and internal erosion produce secondary cavities included fissures (Figure 6).

The size of the cavities tends to decrease through B and C phases and gets much complex in the D than in the others. Thus, “sparry” fabrics are common in phase A, whereas in B and C they tend to be muddier.

The shape and heterogeneity of the cavities seem to mainly be related with the skeletal content as well as the different carbonate mud textures and their distribution. Fillings as polymuds, cements (marine fibrous calcites are common), and their diageneric sequences are also diverse (Meyers, 1974; Miller, 1986).

Conclusions
The understanding of the Waulsortian reefs, carbonate mudbanks, mounds, buildups, or mud mounds has evolved from the last two centuries.

The application of Waulsortian term is generally related to a group of diagnosis criteria: complex carbonate mud fabrics (polymuds), stratigraphic position (Middle-Upper Tournaisian-Lower Viséan), facies, component assemblages (Waulsortian phases), and the associated macrofaunas.

The Mississippian seas were colonized by a wide bioconstruction spectrum and not by Waulsortian mud mounds alone. Bacteria and cyanobacteria are suggested as primary producers in Waulsortian microbial mud mounds. The hypothesis about the distribution of Waulsortian mud mounds has been related with upwelling areas and methane seeps. Carbon sources and convincing models to explain such large volumes of radiaxial fibrous calcite cements and continuous regional production of polymuds are still unrevealing.

Most part of the studies has been focussed on the allochems distribution that has been considered in detail by many authors. Few works are specifically dedicated to the analysis of the carbonated mud, main component in the Waulsortian mud mounds. However, more work would be necessary to analyze, quantify, and decipher the relationship between the allomicrite input and the automicrite production in mud mounds, particularly in Waulsortian ones, where clear microbialite frameworks are not always evident.

Summary
Waulsortian mud mounds are probably the most famous carbonate bioconstructions. They were described first from Belgium outcrops, in the locality of Waulsort, two centuries ago. Later, they have been recognized and analyzed from several areas of Europe and North America.

The growth of these bioconstructions was controlled by the dominant autochthonous production of carbonate mud which has been explained by the activity of marine microbial benthic communities. The associated biota which colonized mud mound substrates was composed by heterozoan assemblages dominated by fenestrate bryozaans and crinoids. Detailed studies of these component assemblages have been a key to reconstruct regional gradients from deep subtidal, aphotic marine environments through the photic zone.

Today, there are no equivalent analogs to compare with such a spectacular mud mound development during the Tournaisian-lowermost Viséan period. Thus, they represent an excellent record to reconstruct and understand the paleoenvironmental and paleoecological relationships between microbial and nonmicrobial benthic communities through deep ramp to basin settings along the northern hemisphere during Lower Mississippian times.

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Definition

Whale fall: Carcass of a large marine mammal of the order Cetacea (whales) that has sunken to the seafloor.

Wood fall: A piece of wood that has sunken to the seafloor.

History

The first examples of specialized invertebrates inhabiting bones and wood in the deep sea (qv) were recognized during the deep-sea expeditions in the early to mid 1900s. Because these samples were recovered by dredging, the complexity of whale- and wood-fall ecosystems was not recognized before the advent of deep-sea research vessels. Pioneering experimental work on wood-falls was carried out in the early 1970s by Ruth Turner (1914–2000), using the deep-sea research vessel Alvin. This research came to a halt in the late 1970s when deep-sea hydrothermal vent systems (qv) and their fascinating fauna were discovered and Alvin was occupied by exploring these systems. The first deep-sea whale-fall ecosystem was accidentally discovered by Craig Smith during an Alvin dive in 1987 and consisted of a whale skeleton associated with many chemosymbiotic taxa that were also known from hydrothermal vents (Smith et al., 1989). Nowadays, whale-fall ecosystems are mainly investigated by sinking stranded whales which are then studied by submersibles and/or ROVs. The last major discovery in this field was Osedax, a “bone-eating” siboglinid worm living in symbiosis with bacteria that degrade bone lipids and cartilage (Rouse et al., 2004).

Bivalve-bored wood has been known from the fossil record for centuries, but reports on other invertebrate groups that utilized wood falls in the geologic past are rare, and entire fossil wood-fall ecosystems have only been described very recently (Kiel and Goedert, 2006b; Kiel et al., 2009). Fossil examples of deep-sea whale-fall ecosystems were discovered soon after the discovery of the modern ones and are especially common in uplifted deep-water sediments around the North Pacific margin (Squires et al., 1991; Kiel and Goedert, 2006a). Similar ecosystems were recently found on two late Cretaceous plesiosaur (a marine reptile) skeletons in Japan (Kaim et al., 2008).

Whale falls

Whale falls are the basis for the most species-rich deep-sea ecosystem, with more than 400 species known to date (Baco and Smith, 2003). Whale falls pass through three ecologic stages that vary in length and can overlap. At first the flesh of the whale carcass is removed by sleeper sharks, hagfish, and other scavenging deep-sea fishes. In the second stage small annelids and crustaceans consume the remaining particulate organic matter. The main source of nutrients during the third stage is the hydrogen sulfide (H₂S) generated by the anaerobic decay of bone lipids, which is utilized by species harboring chemotrophic endosymbionts or by species that graze on sulfur-oxidizing...
bacteria (Smith and Baco, 2003). While this “sulfophillic stage” can last up to 100 years in oxygen-poor ocean basins, it can be as short as a few years in areas where *Osedax* rapidly consumes and destroys the bones (Braby et al., 2007; Fujiwara et al., 2007).

Whale bones consist of up to 65 wt% of lipids. These lipids are consumed by *sulfate-reducing bacteria* (qv) at the oxic–anoxic interface in the bone, using seawater as sulfate source and emitting H$_2$S as metabolic byproduct. This H$_2$S is then consumed by *Beggiatoa* (qv) and other sulfur-oxidizing bacteria on the surface of the skeleton (Figures 1 and 2). Bone lipids and other whale remains entering the mats, or covered by, sediment are also consumed in this way. The resulting H$_2$S is either consumed by bacterial mats surrounding the whale carcass, or by invertebrates harboring sulfur oxidizing bacteria. These invertebrates include siboglinid tube worms, solemyid, lucinid, and vescomyid clams, and bathymodiolin muscles. Bacteria-grazing taxa include provannid, limpet-like and various other small-sized gastropods (“skeneimorphs”), and polynoid annelids (Smith and Baco, 2003).

Fossil whale fall communities are known from to Pliocene sediments. They preserve mollusk associations resembling those at modern whale falls, and fossil traces of *Osedax* (Kiel et al., 2010).

**Wood falls**

Wood falls in the deep sea support complex ecosystem of 40 or more invertebrate species, some of which are endemic to this habitat. The wood is utilized by several xylophagous (wood-eating) invertebrate groups. Most important are wood-boring bivalves of the heterodont families Teredinidae (also known as shipworms; mainly in shallow water) and Xylophagainae (mainly in deep water below 100 m) which rapidly consume and destroy the wood, presumably aided by cellulolytic symbiotic bacteria (Turner, 1973; Distel and Roberts, 1997). Shallow water wood falls are also utilized by wood-boring isopods of the family Limnoriidae. In deeper water exists a small number of xylophagous gastropod limpets and chitons (polyplacophorans), which are preyed upon by gastropods, annelids, and crustaceans. The microbial fauna on wood falls consists of a variety of fungi and bacteria (Kohlmeyer and Kohlmeyer, 1979; Palacios et al., 2006) and these microbes are grazed upon by gastropods and chitons. Chemosymbiotic species which are phylogenetically related to those at whale falls are also found on sunken wood. They probably rely on sulfide from the anaerobic decay of fecal pellets of the shipworms, or from the decaying wood itself (Figures 3 and 4; Kiel and Goedert, 2006b; Pailleret et al., 2007). The fossil record of deep-sea wood-fall communities extends back into the late Cretaceous.

**Summary**

Whale and wood falls in the deep sea harbor species-rich ecosystems that rely to a certain extent on H$_2$S from the decay of the bones and the wood. Many of the species inhabiting these habitats are endemic. Especially the chemosymbiotic and bacteria-grazing taxa are phylogenetically related to those living at hydrothermal vents (qv) and cold seeps (qv). The main H$_2$S source for the chemosymbiotic taxa at whale falls is the anaerobic breakdown of bone lipids by sulfate-reducing bacteria. In the case of the wood falls, it is the anaerobic decay of excrements of wood-boring bivalves (Xylophagainae). Wood-fall communities with a fauna that is taxonomically comparable to the modern wood-fall fauna are known from the late Cretaceous. Whale-fall communities are as old as ocean-going whales (late Eocene). Similar ecosystems with bacteria-grazing gastropods on plesiosaur bones are known from the late Cretaceous.
Bibliography


Cross-references

- *Beggiatoa*
- Cold Seeps
- Deep Biosphere of the Oceanic Deep Sea
- Hydrothermal Environments, Marine
- Sulfate-Reducing Bacteria
ZINC

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Synonyms
Spelter (nonscientific)

Definition
Physicochemical characteristics
Zinc (Zn) is the 23rd most abundant element in the Earth’s crust, and exists as a blue-whitish metal relatively weak with a melting point of 419.5°C and a boiling point of 907°C, with a density of 7.133 g/cm³ (Henkin, 1984). Zn consists of a mixture of the five stable isotopes 64Zn (48.6%, atomic mass 63.9), 66Zn (27.9%, atomic mass 65.9), 67Zn (4.1%, atomic mass 66.9), 68Zn (18.8%, atomic mass 67.9), and 70Zn (0.6%, atomic mass 69.9) (Coplen et al., 2002). Moreover, six synthetic radioactive isotopes are known: 62Zn, 63Zn, 65Zn, 69Zn, 72Zn, and 73Zn. The atomic number is 30. Zinc metal is highly reactive and produces various different salts, but it is only stable in water as the Zn²⁺ ion and associated complexes and minerals. Its sulfates and chlorides are water-soluble and its sulfides, oxides, carbonates, phosphates, silicates, as well as organic complexes are water-insoluble (Henkin, 1984). Zinc, like many other metals, also exists as dissolved metal-sulfide cluster complexes (particularly Zn₃S₅²⁻ and Zn₅S₆⁴⁻), which can exist even in oxic river waters for extended periods and account for up to 20% of the total dissolved Zn in solution (Rozan et al., 2000). Zinc as an element is not available as a metabolic component as it only exists in the Zn²⁺ state in water, but the strong bonds formed with dissolved sulfide ions result in highly insoluble ZnS mineral formation, which is strongly linked to sulfur-based microbial activity.

Abundance and distribution in the environment
Zinc is one of the world’s principle ores and stands fourth among all metals in world production (behind iron, aluminum, and copper), used in a variety of ways from metal products to rubber production to medicines (USGS, 2008). The world’s total reserve of this widely distributed metal is approximately 460 million tons in the year 2006 (Cohen, 2007). More than 80 zinc minerals are known; ZnS (as cubic sphalerite and hexagonal wurtzite polymorphs) and its weathering products are the most important minerals for commercial use (Henkin, 1984), with smithsonite (ZnCO₃) and willemite (Zn₂SiO₄) being important non-sulfide forms.

Zn ore deposition
The genesis of many types of ZnS ore deposits are often attributed to purely physicochemical processes, and the distinction between abiotic and potentially biotic process is often made based on the temperature of sulfide formation and subsequent ZnS mineral formation. At low temperatures (below 150–175°C), thermochemical sulfate reduction (TSR) is very slow (Goldhaber and Orr, 1995; Ohmoto and Goldhaber, 1997; Thom and Anderson, 2008), whereas the upper temperature limit for microbial sulfate reduction overlaps with the formation of many low-temperature deposits (Ledin and Pedersen, 1996; Druschel et al., 2002). ZnS ore depositional processes without microbial influence were, for example, probably due to depositional environments outside the range of temperatures where microorganisms can survive, and TSR is sufficiently fast for significant ore formation, such as observed for the formation of high temperature hydrothermal Pb–Zn ore deposits as, e.g., described for the Rhodopian metallogenic region.
(Tarkian and Breskovska, 1989; Kaiser-Rohrmeier et al., 2004). On the other hand, Mississippi Valley-Type (MVT) deposits also form from highly saline brines, between 50°C and 200°C, and span depositional environments where abiotic and biotic factors may be responsible for zinc sulfide mineralization (Bastin, 1926; Druschel et al., 2002; Thom and Anderson, 2008). Siebenthal (1915) suggested, as early as 1915, that microbial sulfate reduction contributed to the formation of low-temperature strata-bound zinc sulfide deposits. However, there is considerable controversy associated with the interpretation of the complex paragenetic sequences responsible for Zn ore deposit formation, and it is entirely possible that some deposits experience both biological and abiotic ZnS formation at different times and in different places. Trudinger et al. (1972) reviewed the feasibility of biogenic ore formation and addressed three issues: (1) the environmental limits of biogenic sulfate reduction; (2) whether the age of biological sulfate reduction was coextensive with the ages of ancient deposits; and (3) whether the rates of sulfate reduction are sufficient for ore formation. Trudinger et al. (1972) concluded that the information at the time was not adequate to address these questions. However, in the last 30 years significant progress has been made on each of these issues to better evaluate the role of microorganisms in ore deposit formation (Druschel et al., 2002; Spangenberg and Herlec, 2006). Organic geochemical and isotopic indicators of biological sulfate reduction have been recently reported from some Pb and Zn deposits (Hu et al., 1998; Bechtel et al., 1998, 1999; Spangenberg and Herlec, 2006). Moreover, modern in situ formation of pure nanocrystalline ZnS deposits at low temperature from complex groundwater solutions by SRB biofilms has been documented (Labrenz et al., 2000; Labrenz and Banfield, 2004; Moreau et al., 2004). Druschel et al. (2002) modeled the source-to-sink geochemistry of this modern ZnS-forming system and reviewed the relevance of the findings to ZnS ore deposit formation. Aggregation of those 1–5 nm sphalerite and wurtzite crystals additionally emphasizes a size-dependent stability shift in sphalerite–wurtzite stability and the role of extracellular proteins in the aggregation of ZnS nanocrystals (Zhang et al., 2003; Moreau et al., 2007). Luther et al. (1999) also showed the importance of zinc-sulfide molecular clusters (particularly Zn₃S₃ and Zn₈S₆⁴⁻) on the speciation of dissolved zinc and their importance in ZnS nanocrystal formation.

Oxidation of ZnS minerals can occur abiotically in the presence of oxygen or other oxidants (Rimstidt et al., 1994), but is oxidized much faster via the activity of oxidizing microbes. Oxidative dissolution of ZnS and other metal sulfides is the principle cause of acid mine drainage (AMD), forming acidic, metal-rich waters (Nordstrom, 2000; Druschel et al., 2004). ZnS mineral oxidation releases dissolved Zn²⁺ ions and associated complexes into aqueous solutions. When AMD waters containing Zn²⁺ are neutralized, zinc can precipitate as a carbonate or oxide form, but is more commonly associated with iron oxide and oxyhydroxide minerals due to sorption of Zn²⁺ to those mineral surfaces, which can remove the majority of dissolved zinc from solution at circumneutral pH (Jönsson et al., 2006).

**Biological effects of Zinc: Zn contamination and bioremediation**

As a micronutrient Zn(II) is an ubiquitous essential metal ion playing an important role in organisms throughout the three domains *Bacteria, Archaea*, and *Eukarya*. It is essential for numerous physiological processes, serves as a cofactor in members of all six major functional classes of enzymes and is especially important in the maintenance of protein structure (Blencowe and Morby, 2003). In excess it can have toxic effects, but usually Zn deficiency is more critical for most of the higher organisms (Henkin, 1984).

Usually due to anthropogenic activity zinc has been shown to exist at significantly elevated levels in groundwaters and in soil and sediments (Sani et al., 2001). It has repeatedly been demonstrated by cultivation-dependent as well as independent methods that this contamination can have ecotoxicological effects on, e.g., soil microorganisms, and can change the microbial community composition and activity (Hanbo et al., 2004; Smolders et al., 2004). Unfortunately, critical thresholds for toxic effects of Zn on microorganisms are not easy to develop because these can be influenced by the exposure time of Zn to the environment (Mertens et al., 2006) and the potential adaptation of microbial populations to changing Zn concentrations over time.

Great interest in Zn–microbe interactions has arisen in recent years as scientists and engineers try to remove, recover, or stabilize Zn in soils, contaminated water, or waste streams (Sani et al., 2001). In general, specific metabolic pathways leading to precipitation of heavy metals as metal sulfides, phosphates, or carbonates possess significance for possible biotechnology applications (Kotbra and Ruml, 2000) and, analogous to their potential role in low-temperature Zn ore deposition, SRB are already commonly used for the bioremediation of metal-contaminated soil or water. White et al. (1998), for instance, described an integrated microbial process for the bioremediation of soil contaminated with toxic metals using microbially catalyzed reactions. In this process, bioleaching of Cd, Co, Cr, Cu, Mn, Ni, and Zn via sulfuric acid produced by sulfur-oxidizing bacteria was followed by precipitation of the leached metals as insoluble sulfides by the action of SRB.

**Conclusion**

Zinc is a common element throughout the world, mainly concentrated in zinc sulfide deposits, and has an important biological role as an essential metal ion in organisms of all three kingdoms. Though microorganisms do not directly metabolize forms of zinc, the mobility and in part the deposition of Zn²⁺ as ZnS minerals is strongly dependent on the activity of sulfur-reducing and sulfur-oxidizing microorganisms. Indications exist for the active
involvement of ancient sulfate-reducing bacteria in the genesis of low-temperature zinc ore deposits and a potential biosignature capacity of nanocrystalline sphalerite for this process; however, these aspects will need further investigation.

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Biosignatures in Rocks
Divalent Earth Alkaline Cations in Seawater
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