4 Biologically Induced Mineralization by Bacteria

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INTRODUCTION

Bacteria are small, prokaryotic, microorganisms that are ubiquitous in surface and subsurface terrestrial and aquatic habitats. Prokaryotes comprise two Domains (Superkingdoms) in the biological taxonomic hierarchy, the Bacteria and the Archaea. They exhibit remarkable diversity both genetically and metabolically even within the same microenvironment and they are thought to play a major role in the deposition and weathering of minerals in the earth's crust. The synthesis of minerals by prokaryotes can be grouped into two canonical modes: 1) biologically induced mineralization (BIM) and 2) biologically controlled mineralization (BCM) (Lowenstam 1981; Lowenstam and Weiner 1989). In this chapter, we focus on biologically induced mineralization.

Minerals that form by biologically induced mineralization processes generally nucleate and grow extracellularly as a result of metabolic activity of the organism and subsequent chemical reactions involving metabolic byproducts. In many cases, the organisms secrete one or more metabolic products that react with ions or compounds in the environment resulting in the subsequent deposition of mineral particles. Thus, BIM is a presumably unintended and uncontrolled consequence of metabolic activities. The minerals that form are often characterized by poor crystallinity, broad particle-size distributions, and lack of specific crystal morphologies. In addition, the lack of control over mineral formation often results in poor mineral specificity and/or the inclusion of impurities in the mineral lattice. BIM is, in essence, equivalent to inorganic mineralization under the same environmental conditions and the minerals are therefore likely to have crystallochemical features that are generally indistinguishable from minerals produced by inorganic chemical reactions. In some cases, the metabolic products diffuse away and minerals form from solution. However, bacterial surfaces such as cell walls or polymeric materials (exopolymers) exuded by bacteria, including slimes, sheaths, or biofilms, and even dormant spores, can act as important sites for the adsorption of ions and mineral nucleation and growth (Beveridge 1989; Konhauser 1998; Banfield and Zhang 2001; Bäuerlein 2003).

BIM is especially significant for bacteria in anaerobic habitats including deep subsurface sites, or at oxic-anoxic interfaces. This is because under anaerobic conditions, many bacteria respire with sulfate and/or various metals including iron as terminal electron acceptors in electron transport. The metabolic products of these reductions, e.g., reduced metal ions and sulfide, are reactive and participate in subsequent mineral formation.

In BCM, minerals are usually deposited on or within organic matrices or vesicles within the cell, allowing the organism to exert a significant degree of control over the

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nucleation and growth of the minerals and thus over the composition, size, habit, and intracellular location of the minerals (Bazylinski and Frankel 2000a,b). These BCM mineral particles are structurally well-ordered with a narrow size distribution and species-specific, consistent, crystal habits. Because of these features, BCM processes are thought to be under metabolic and genetic control. Because intra-vesicular conditions (e.g., pH, Eh) are controlled by the organism, mineral formation is not as sensitive to external environmental parameters as in BIM. BCM by bacteria is discussed later in this volume (Bazylinski and Frankel 2003).

BIOLOGICALLY INDUCED MINERALIZATION ON ORGANIC SURFACES

Because of the high surface to volume ratio of bacteria, cell surfaces and the surfaces of exopolymers can be especially important in BIM processes. Negative charges on most cell and exopolymer surfaces can result in binding of cations by non-specific electrostatic interactions, effectively contributing to local supersaturation. Binding also helps stabilize the surfaces of nascent mineral particles, decreasing the free energy barrier for critical, crystal-nucleus formation. By this means, the rate of mineralization of amorphous to crystalline mineral particles can become several orders of magnitude faster than inorganic (i.e., without surface binding and nucleation) mineralization. In some cases this can result in a mineral layer that covers the cell.

Two surface BIM processes, known as passive and active, have been distinguished (Fortin and Beveridge 2000; Southam 2000). Passive mineralization refers to simple non-specific binding of cations and recruitment of solution anions, resulting in surface nucleation and growth of minerals. Active mineralization occurs by the direct redox transformation of surface-bound metal ions, or by the formation of cationic or anionic by-products of metabolic activities that form minerals on the bacterial surfaces.

Bacterial surface properties

Prokaryotes have various cell wall types whose chemistry determines the ionic charges present on the surface of the organism. In the Domain Bacteria, there are two general types of cell wall: gram-positive and gram-negative, the difference being the cell's reaction to a staining procedure used in light microscopy. The gram-positive cell wall is separated from the cytoplasm by a lipid/protein bilayer called the plasma or cell membrane and consists mainly of peptidoglycan (murein) that is rich in carboxylate groups that are responsible for the net negative charge of this structure (Beveridge and Murray 1976, 1980). Peptidoglycan forms a 15-25 nm thick sheet (Beveridge 1981) comprising multiple layers of repeating units of two sugar derivatives, N-acetylglucosamine and N-acetylmuramic acid, and a small group of amino acids. Peptidoglycan gives rigidity to the cell wall and its charged, multiple layers are mainly responsible for mineral formation (Beveridge and Murray 1976; Fortin et al. 1997; Fortin and Beveridge 2000). Additional components such as teichoic and/or teichuronic acids can be bound into peptidoglycan (Beveridge 1981). These polymers contain phosphoryl groups that further contribute to the net negative charge of the cell wall (Southam 2000).

The gram-negative cell wall is structurally more complex than, and differs from, the gram-positive type in that it has a thinner peptidoglycan layer (about 3 nm thick) and does not contain secondary polymers (Beveridge 1981). It is sandwiched between two lipid/protein bilayers, the outer and the plasma (or cell) membranes, within the space between the cell walls known as the periplasm. The outer membrane represents the cell's outermost layer. Unlike the plasma membrane, the outer membrane is not solely constructed of phospholipid and its outer face contains lipopolysaccharide (LPS) which is highly anionic. LPS consists of *O*-polysaccharide, the core polysaccharide, and lipid A. The *O*-sidechain can extend up to 40 nm away from the core polysaccharide which is attached to lipid A. Lipid A contains several strongly hydrophobic fatty acid chains that cement the LPS into the outer membrane bilayer. The core oligosaccharide and upper regions of lipid A are rich in phosphate groups that have an affinity for Mg^{2+} and Ca^{2+} (Ferris and Beveridge 1986b). The core has several keto-deoxyoctonate residues that provide available carboxylate groups while many *O*-sidechains also contain residues rich in carboxylate groups (Ferris and Beveridge 1986a). Phospholipid is mainly present in the inner face of the outer membrane. In gram-negative cells, it is the LPS that is the major factor in catalyzing mineral formation because of its high concentration of phosphate and carboxyl groups (Ferris and Beveridge 1984, 1986a).

Members of the Archaea also show gram-positive and gram-negative staining characteristics. However, the cell walls of the Archaea are very different chemically from the Bacteria and from each other (König 1988). Some gram-positive Archaea have cell walls composed of a layer of a peptidoglycan-like polymer, consisting of N-acetyltalosaminuronic acid and N-acetylglucosamine, called pseudomurein that overlies the plasma membrane. Others lack pseudomurein and have cell walls consisting of polysaccharide, glycoproteins, or protein. Some gram-negative Archaea lack a cell wall entirely but retain the plasma membrane. Thus, electrochemical charges present on the cell surfaces of the Archaea vary.

Other layers external to the bacterial cell wall that may be involved in mineral nucleation include S layers, capsules, slimes, and sheaths. S layers, very common in Archaea, are paracrystalline cell surface assemblages composed of protein or glycoprotein that self assemble and associate with the underlying wall through non-covalent interaction (Koval 1988). When S layers are present, they are the outermost layer of the cell facing the surrounding environment. S layers are acidic and possess a net negative charge thereby having an affinity for metal cations (Southam 2000). Capsules are dense, highly hydrated amorphous assemblages of polysaccharides or proteins that are chemically attached to the cell surface. They can be quite thick and extend up to 1 μm from the cell. Capsules are rich in carboxylate groups and may also contain a significant number of phosphate groups, both giving the structure a net negative charge. Because capsules are highly hydrated and cover the cell surface, there can be extensive interaction between the capsule and metal cations. In some cases, capsules are known to form in response to the presence of metal ions (Appanna and Preston 1987). Slime layers, a much more loosely packed version of the capsule, are similar to capsules chemically but are not attached to the cell so they can leave the cell entirely (Southam 2000). Sheaths are rigid hollow cylinders generally surrounding chains of cells or filamentous bacteria produced by a few species of prokaryotes (e.g, *Leptothrix*). In the Domain Bacteria, the sheath is a rigid homo- or heteropolymer of carbohydrate or carbohydrate and protein. In some organisms, the sheath is important in the nucleation of oxidized mineral precipitates and active biomineralization since it sometimes contains proteins that oxidize metals, e.g., as in the oxidation of manganese by *L. discophora* (Adams and Ghiorse 1986, 1987). In the Archaea, sheaths are composed of protein and are covalently linked to the cell wall.

Minerals known to be formed via BIM through passive surface-mediated mineralization include Fe, Mn, and other metal oxides, e.g., ferrihydrite ($5Fe_2O_3 \bullet 9H_2O$), hematite (α-Fe_2O_3), and goethite (α-FeOOH); metal sulfates, phosphates, and carbonates; phosphorite; Fe and Fe-Al silicates; and metal sulfides. Mineral formation results initially from the neutralization of chemically reactive sites on the cell, and proceeds via nucleation of additional metal ions with the initially sorbed metals (Southam 2000). Mineralization is most active at the sites of initial nucleation on the outer surface of the cell. Complete mineralization of the cell surface may eventually occur producing hollow minerals the size and shape of a bacterial cell (Southam 2000) (Fig. 1). It is interesting that nonliving cells may also form minerals in this way and, in one study, living cells of *Bacillus subtilis* bound less metal ions than nonliving cells (Urrutia et al. 1992). In this case, the membrane-induced proton motive force reduces the metal binding ability of the cell wall, most likely through competition of protons with metal ions for anionic wall sites.

There are a large number of examples of bacterial BIM resulting from active mineralization and the formation of reactive by-products. Some cyanobacteria precipitate a number of different minerals that result from the uptake of bicarbonate from solution and the release of hydroxyl anions. This causes an increase in the local pH of the cell. The S layer in some species (e.g., *Synechococcus* spp.) is the site of nucleation of gypsum ($CaSO_4 \bullet 2H_2O$) in weak light. However, during photosynthesis, an increase in pH at the S layer causes precipitation of calcite ($CaCO_3$) (Schultze-Lam et al. 1992, Fortin and Beveridge 2000). Cyanobacteria can also promote the precipitation of Fe and Mn oxides by increasing the pH and raising the O_2 concentration through oxygenic photosynthesis (Fortin and Beveridge 2000). The formation of iron sulfides by sulfate-reducing bacteria is also an excellent example of active mineralization from the formation of sulfide.

IRON AND MANGANESE MINERALIZATION PROCESSES

Biogenic iron and manganese minerals are particularly common products (Table 1) of BIM processes because of the relatively high concentrations of these elements in the earth's crust (4th and the 12th most abundant elements, respectively). Of these minerals, magnetite and maghemite are especially significant in geology because of their contribution to the magnetism of sediments. We will, therefore, emphasize BIM of iron minerals, especially magnetite. Our discussion is organized in terms of the major metabolic processes that cause deposition or dissolution of iron minerals, including metal oxidation and reduction, and sulfate oxidation and metal sulfide reduction.

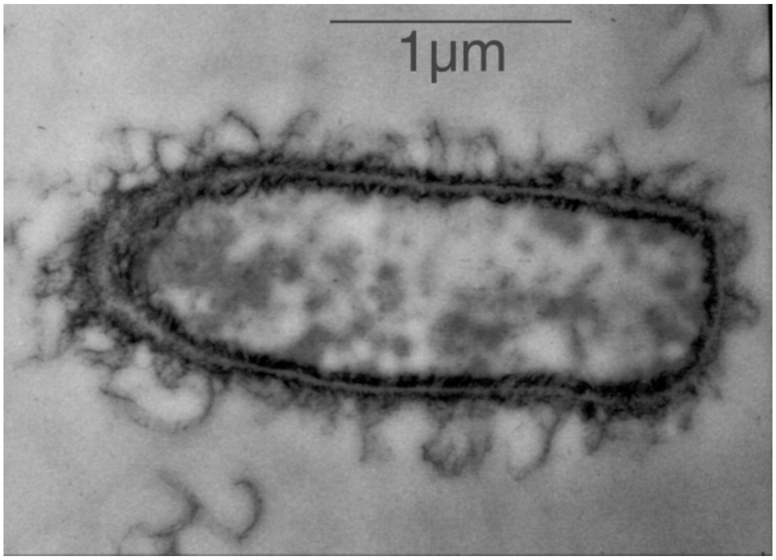

Figure 1. Unstained ultrathin section transmission electron micrograph of a "bacterial fossil" from a sulfate-reducing consortium. The cell has lysed but iron sulfide mineral encrustation has preserved the cell envelope. Figure kindly supplied by W. Stanley and G. Southam.

Table 1. Some biologically-induced iron and manganese minerals

Chemical Formula	*Mineral Name*
$Fe(OH)_3$ (approx.)	Ferric oxyhydroxide
$2Fe(OH)_3 \cdot Fe(OH)_2$ (approx.)	Green rust
α-FeO(OH)	Goethite
γ-FEO(OH)	Lepidocrocite
$5Fe_2O_3 \cdot 9H_2O$	Ferrihydrite
Fe_3O_4	Magnetite
γ-Fe_2O_3	Maghemite
$FeCO_3$	Siderite
$FePO_4 \cdot nH_2O$	Hydrous Ferric Phosphate
$Fe_3(PO_4)_2 \cdot 2H_2O$	Vivianite
FeS	Cubic FeS (Sphalerite-type)
FeS	Mackinawite (tetragonal FeS)
Fe_3S_4	Greigite
$Fe_{1-x}S$	Pyrrhotite
FeS_2	Pyrite
$KFe_3(SO_4)_2(OH)_6$	Jarosite
$Fe_8O_8SO_4(OH)_6$	Schwertmanite
$FeSO_4 \cdot 7H_2O$	Melanterite
$MnCO_3$	Rhodochrosite
$Mn_4O_7 \cdot H_2O$	Todorokite
$Na_4Mn_{14}O_{27} \cdot 9H_2O$	Birnessite

Adapted from Lowenstam and Weiner 1989.

Iron and manganese oxidation

Fe- and Mn-oxidizing bacteria are known to be responsible for the precipitation of oxides of both metals at acidic and neutral pH conditions. At low pH, where oxidized Fe(III) and Mn(IV) are soluble, active mineralization by organisms, such as the mesophilic, autotrophic Bacteria *Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*) (Kelly and Wood 2000) or *Leptospirillum* spp., that oxidize Fe(II), may be more important in iron oxyhydroxide precipitation (Fortin and Beveridge 2000; Southam 2000). The acidophiles are better known for their dissolution and bioleaching of minerals, particularly sulfide minerals such as pyrite, but are often involved in the nucleation and deposition of a secondary mineral, ferric oxyhydroxide, during Fe(II) oxidation (Fig. 2). Although mineral formation by BIM processes may not have been definitively demonstrated in every case, all Fe(II) oxidizers should be considered to have this potential. The known Fe(II)-oxidizing acidophiles are diverse and include: thermotolerant gram-positive species such as *Sulfobacillus* spp., *Acidimicrobium ferrooxidans*, and *Ferromicrobium acidophilus* (Blake and Johnson 2000); mesophilic Archaea such as *Ferroplasma* spp. (Edwards et al. 2000, 2001; Golyshina et al. 2000) that lack cell walls; and thermophilic Archaea such as *Sulfolobus* spp., *Acidianus brierleyi*, *Metallosphaera* spp., and *Sulfurococcus yellowstonensis* (Blake and Johnson 2000).

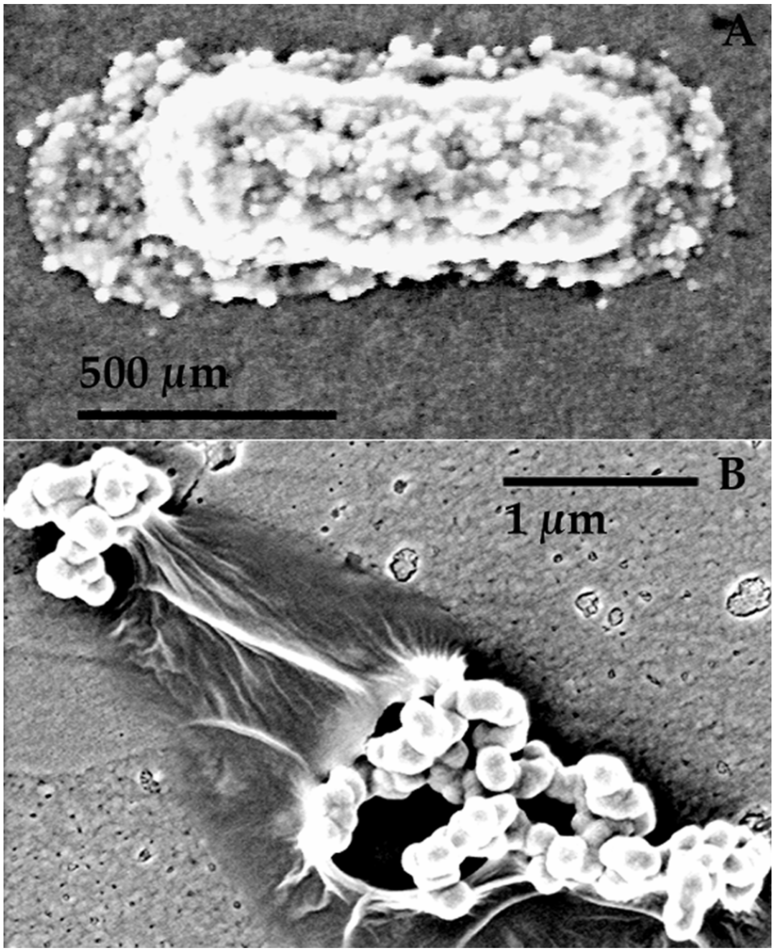

Figure 2. A) Scanning electron micrograph of a heavily mineralized cell of *Acidothiobacillus ferrooxidans* on a pyrite surface. The ferric oxyhydroxide deposits resulting from the oxidation of pyrite completely cover the cell. B) High resolution scanning electron micrograph of the ferric oxyhydroxide deposits on a cellular exopolymer. This figure was adapted from one kindly provided by K.J. Edwards.

At neutral pH, bacteria are thought to be more important in the passive formation of Fe(III) and Mn(IV) oxides although active mineralization of these oxides can also occur. There are several different physiological groups of bacteria that are known to oxidize Fe(II) at neutral pH, including both aerobes and anaerobes.

For the aerobic Fe(II) oxidizers to make a living at neutral pH, they must overcome several problems (Emerson 2000). First they must compete with inorganic oxidation of Fe(II) by O_2. Under aerobic conditions, the chemical oxidation of Fe(II) is relatively rapid. Acidophilic Fe(II)-oxidizing bacteria do not experience this problem because Fe(II) is very stable at low pH. A second problem is that the products of Fe(II) oxidation at neutral pH are insoluble Fe(III) oxyhydroxides. The cell must therefore oxidize Fe(II) at the exterior surface in order to prevent hydrolysis and precipitation of oxyhydroxides from occurring within the cell. Thus, cells must be able to transport electrons across the periplasm to the cell membrane where a chemi-osmotic potential is established. To solve the first problem, Fe(II) oxidizers grow under microaerobic conditions where the concentration of O_2 is low (e.g., oxic-anoxic interfaces), reducing the rate of inorganic iron oxidation. To solve the second problem, the Fe(II) oxidase, as well as the soluble electron transport components, are external to the cell membrane, as is apparently the case in *Acidithiobacillus ferrooxidans* (Rawlings and Kusano 1994).

Gallionella, originally described in the 1800s, was probably the first organism thought to be chemolithautotrophic based on Fe(II) oxidation (Hanert 2000a). When growing on Fe(II), each bean-shaped cell exudes a helically-twisted stalk composed mainly of ferric oxyhydroxides (Fig. 3). An organic matrix is present within the stalk (Hanert 2000a). Once formed, stalks appear to nucleate further mineralization and the iron mineral continues to accumulate on them (Hanert 2000a; Heldahl and Tumyr 1983). The stalks appear to be made of separate filaments; different species or strains synthesize different numbers of these filaments (Hanert 2000a). This is an interesting case of BIM in that there seems to be some control by the cell over the overall shape of the mineralized polymeric product and moreover, the product appears to be extruded from a specific site on the cell and is quite pure in composition. However, there is no obvious function to the structure. It is not essential for growth but Hallbeck and Petersen (1990) speculate that the stalk may represent a survival strategy. *Gallionella* appears to be a mesophilic chemolithoautotroph (Hallbeck and Petersen 1991) (it can also grow mixotrophically) and is phylogenetically associated with the β-subdivision of the Proteobacteria in the Domain Bacteria (Hallbeck et al. 1993).

Another group of Fe(II) oxidizers are also microaerophiles and grow at the oxic-anoxic interface of semi-solid O_2-gradient cultures (Emerson and Moyer 1997). These mesophilic organisms can use Fe(II) in iron sulfides or ferrous carbonate as electron donors and form Fe(III) oxides which are tightly associated with the cell wall of the bacteria. Although cells seem to encrust themselves with the metal oxides, they appear to be surrounded by a matrix where precipitation occurs. It is thought that this matrix may prevent them from being totally encased by the mineral. Phylogenetically, some of these organisms form a novel lineage within the *Xanthomonas* group in the γ-subdivision of the

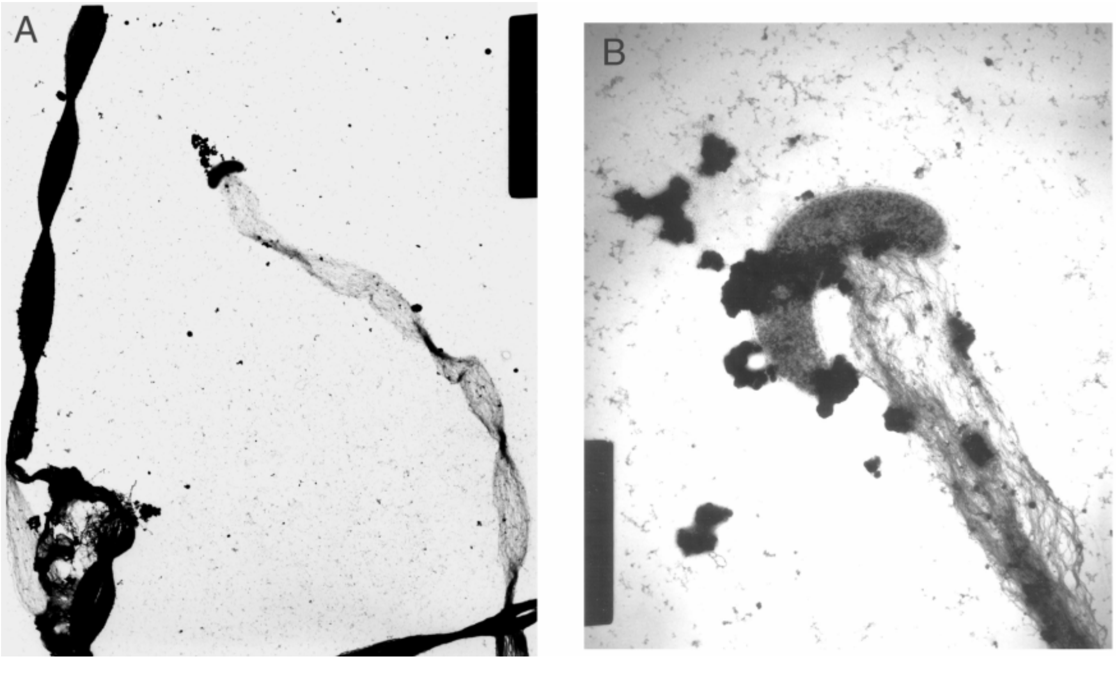

Figure 3. A) Transmission electron micrograph of unstained, whole cells of *Gallionella ferrugenia*. Contrast in electron density is primarily due to ferric oxyhydroxide mineral deposits. Newly synthesized stalk, like that attached to the cell near the upper center of the image, is composed of hair-like fibrils. Black bar along upper right edge is 5.7 μm. B) Higher magnification micrograph of a cell stained with ammonium molybdate, showing a newly synthesized stalk. Particles of ferric oxyhydroxide are attached to the cell surface and are beginning to deposit on the stalk. Black bar along lower left edge is 1.3 μm. Adapted from images kindly supplied by W. Ghiorse.

Proteobacteria (Emerson 2000). Phylotypes of these organisms have been identified from the Loihi Seamount near Hawaii (Moyer et al. 1995) where there is extensive, low-temperature, hydrothermal, venting and very large mats of hydrous Fe(III) oxides. These organisms appear to be very abundant at the site and a pure culture of a related organism has been obtained (Emerson and Moyer 2002). These organisms have also been associated with, and isolated from, the rhizosphere and Fe(III) hydroxide plaques on the roots of wetland plants (Emerson et al. 1999; Neubauer et al. 2002).

The anaerobic Fe(II) oxidizers that grow at or around neutral pH include several strains of phototrophic bacteria and some nitrate-respiring bacteria. Several freshwater strains of phototrophic bacteria are known to oxidize Fe(II) in iron sulfides, or in a mixture of ferrous carbonate and ferrous phosphate, to insoluble, rust-colored Fe(III) oxyhydroxides whose precise composition was not determined (Ehrenreich and Widdel 1994). These strains belong to the α- and γ-subgroups of the Proteobacteria. Two marine phototrophic strains of *Rhodovulum* (α-subgroup of Proteobacteria) growing on the same substrates produced the iron oxides ferrihydrite (~98%) and magnetite (trace amounts) (Straub et al. 1999). Both the freshwater and marine strains grow photoautotrophically and photoheterotrophically. The discovery of this novel type of microbial metabolism received much attention because it provided an alternative explanation for the development of the massive banded iron formations which formed in the absence of free dioxygen (Widdel et al. 1993; Ehrenreich and Widdel 1994).

An anaerobic group of Fe(II) oxidizers that uses nitrate as a terminal electron acceptor (Straub et al. 1996) includes a number of mesophilic strains belonging to the β- and γ-subgroups of the Proteobacteria (Buchholz-Cleven et al. 1997). All form rust-colored ferric oxyhydroxides from Fe(II) which probably contain considerable carbonate (Straub et al. 1996). A study using 16S rRNA-targeted probes designed from several strains showed that these organisms are quite widespread in diverse European sediments (Straub and Buchholz-Cleven 1998). This finding together with the fact that other known nitrate-reducing bacteria, including *Thiobacillus denitrificans* and *Pseudomonas stutzeri,* are also capable of Fe(II) oxidation suggests that this form of metabolism is widespread in anoxic habitats containing sufficient Fe(II) and nitrate (Emerson 2000). Chaudhuri et al. (2001) reported the isolation of *Dechlorosoma suillum* strain PS, a bacterium that is capable of oxidizing Fe(II) anaerobically with nitrate as the terminal electron acceptor. After the initiation of Fe(III) formation, the Fe(III), unreacted Fe(II), and carbonate in the medium were found to combine to form green rust which transformed into magnetite after prolonged incubation.

A hyperthermophilic member of the Archaea, *Ferroglobus placidus*, isolated from a shallow marine hydrothermal vent in Italy, is known to grow lithotrophically with Fe(II) as ferrous carbonate (Hafenbrandle et al. 1996). The optimum growth temperature of this organism is 85°C although the products of Fe(II) were not discussed. This organism can also reduce thiosulfate using hydrogen as the electron donor, and in the presence of Fe(II), produces iron sulfide minerals.

Some chemoheterotrophic bacteria also oxidize Fe(II). Two of the most well-described are the filamentous, sheathed bacteria *Sphaerotilus* and *Leptothrix*. Proteins in their sheaths catalyze the oxidation of Fe(II) and Mn(II) and nucleate the precipitation of Fe and Mn oxides, with which they are often encrusted. Members of the family Siderocapsaceae, which contains the genera *Siderocapsa*, *Naumanniella*, *Siderococcus*, and *Ochrobium*, seem to oxidize Fe(II) but the evidence is circumstantial in that most of the information about them is derived from environmental studies and enrichment cultures rather than studies with pure cultures (Hanert 2000b). In fact, it is seems questionable whether true strains of these genera actually exist (Emerson 2000).

Nonetheless they are widespread in aquatic environments and always associated with Fe(III) and Mn(IV) oxides. Many produce capsules which may be involved in mineralization (Hanert 2000b).

Several bacteria oxidize Mn(II) although none are known to grow lithotrophically with it (Emerson 2000). *Leptothrix discophora*, a mesophilic, sheathed bacterium mentioned earlier, oxidizes Mn(II) via a protein normally present in its sheath (Adams and Ghiorse 1986, 1987). Apparently, the protein is excreted by the cell and becomes associated with the sheath, resulting in the sorption of metal ions onto the sheath which eventually becomes encrusted with Mn oxides. Sheathless variants also secrete the protein; in the absence of the sheath, amorphous Mn(IV) oxides form as unattached particles in the growth medium. There are several theories concerning possible functions of the oxide crusts on *Leptothrix*; these include protection from protozoal grazing, attack from bacteriophages or UV radiation, detoxification of O_2 radicals, or sequestration of nutrients (Emerson 2000). The Mn-oxidizing protein from *Leptothrix*, MofA, has been identified and partially characterized as a multi-copper oxidase (Corstjens et al. 1997).

Two freshwater strains of the gram-negative, γ-Proteobacterium, *Pseudomonas putida,* are known to actively mineralize Mn oxide from Mn(II) (Brouwers et al. 1999). Cells deposit Mn oxide on their outer membranes and the oxidation is mediated by another multi-copper enzyme, CumA.

The dormant spores of several marine *Bacillus* species oxidize Mn(II) and become encrusted with amorphous Mn oxides (Rosson and Nealson 1982; Francis and Tebo 2002). This process also appears to be enzymatic: the oxidation of Mn(II) appears to be catalyzed by yet another multi-copper oxidase, MnxG (van Waasbergen et al. 1996).

Iron and manganese reduction

Dissimilatory metal-reducing bacteria are well recognized for their ability to utilize a number of diverse, oxidized, metal ions as terminal electron acceptors (Lovley 2000). Especially in the case of iron and manganese, this results in the dissolution of oxide minerals of these and any co-precipitated metals under anaerobic conditions (Lovley and Phillips 1986, 1988; Lovley 1991). Dissimilatory iron-reducing microorganisms respire with oxidized iron, Fe(III), usually in the form of amorphous Fe(III) oxyhydroxide (Lovley 1990, 1991) or crystalline iron oxides such as goethite, hematite, etc., under anaerobic conditions, and release reduced iron, Fe(II), into the environment. The Fe(II) can subsequently participate in adventitious interactions with anions resulting in the formation of various iron minerals. Iron-reducing bacteria are known to induce the precipitation of magnetite (Fe_3O_4), siderite ($FeCO_3$), and vivianite ($Fe_3(PO_4)_2 \cdot 8H_2O$), depending on the conditions and chemistry external to the cell (Moskowitz et al. 1989; Bazylinski and Frankel 2000a,b). For example, siderite was produced in cultures of *Geobacter metallireducens* along with magnetite when cells were grown in a bicarbonate buffering system (Lovley and Phillips 1988; Sparks et al. 1990), while vivianite, but neither magnetite nor siderite, was produced by the same organism with Fe(III) citrate as the terminal electron acceptor with a phosphate buffer (Lovley and Phillips 1988; Lovley 1990). Growing cells of the magnetotactic species, *Magnetospirillum magnetotacticum*, produced significant amounts of extracellular, needle-like crystals of vivianite (Fig. 4) while actively reducing Fe(III) in the form of Fe(III) oxyhydroxides (Blakemore and Blakemore 1990).

The biomineralization reactions described in the previous paragraph occur at neutral pH. There are a number of known chemolithoautotrophic and chemoheterotrophic, acidophilic, dissimilatory Fe(III)-reducing bacteria (Blake II and Johnson 2000) but little to nothing has been published as to any type of mineral formation at low pH.

Figure 4. Optical micrograph of extracellular crystals of vivianite, $Fe_3(PO_4)_2$, produced by cells of *Magnetospirillum magnetotacticum* in cultures containing high concentrations of Fe(III) buffered with phosphate. Under these conditions the cells reduce Fe(III) to Fe(II).

Interestingly, strictly anaerobic conditions do not seem to be required for these organisms to grow on Fe(III) although Fe(III) reduction may be most rapid under microaerobic conditions. The Archaean species *Sulfolobus adidocaldarius* reduces Fe(III) while growing heterotrophically on organic substrates. *Acidithiobacillus ferrooxidans* and *A. thiooxidans* oxidize reduced sulfur compounds coupling this reaction to the reduction of Fe(III). Cells of the α-Proteobacterium *Acidiphilium acidophilum* reduce Fe(III) with organic electron donors microaerobically. The Gram-positive, moderately-thermophilic Fe(II)-oxidizing, Bacteria *Sulfolobus* and *Acidimicrobium* also reduce Fe(III) and some are known to be capable of the reductive dissolution of Fe(III)-containing minerals (Bridge and Johnson 1998).

Many Fe(III)-reducing bacteria such as strains of *Shewanella* and *Geobacter* also reduce Mn(IV) to Mn(II). In this case, the organisms are well known for the dissolution of insoluble MnO_2; they reduce the Mn(IV) in MnO_2 to soluble to Mn(II). However, there are some instances of mineral formation during Mn(IV) reduction. Several metal-reducing strains of *Thermoanaerobacter* are known to form rhodochrosite ($MnCO_3$) during Mn(IV) reduction, uraninite (UO_2) during soluble U(VI) reduction, and gold metal during reduction of soluble Au(III) (Roh et al. 2002).

Biologically induced mineralization of magnetite

Fe(II) can react with excess, insoluble Fe(III) oxyhydroxide to form green rusts (mixed Fe(II) and Fe(III) oxyhydroxides) which can age to form magnetite. Magnetite particles, formed extracellularly by dissimilatory iron-reduction are typically irregular in shape and poorly crystallized (Moskowitz et al. 1989; Sparks et al. 1990) (Fig. 5). In addition, they have a relatively broad, lognormal, crystal size distribution with the mode in the superparamagnetic size range (< 35 nm) for magnetite. These crystal characteristics are typical of mineral particles produced by BIM or inorganic processes (Eberl et al. 1998).

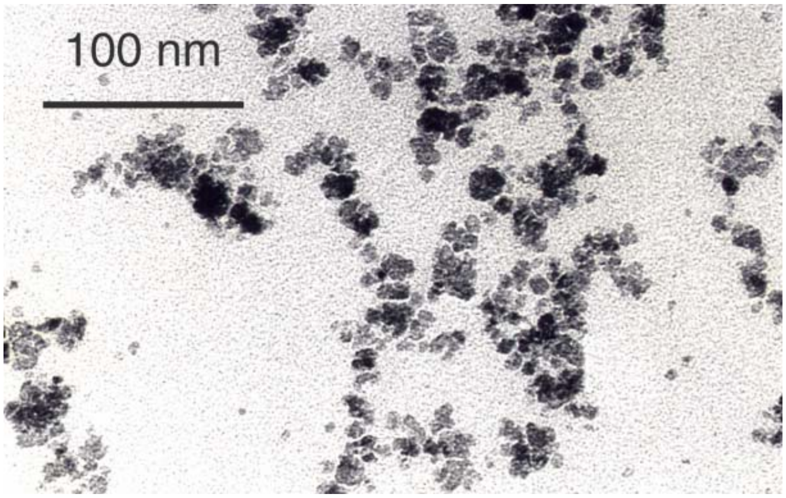

Figure 5. Magnetite crystals formed during reduction of ferric oxyhydroxide by the iron-reducing bacterium *Geobacter metallireducens.*

While many different species and physiological types of bacteria reduce Fe(III), not all conserve energy and grow from the reduction of this environmentally-abundant terminal electron acceptor (Myers and Nealson 1990) and form magnetite. *Geobacter metallireducens* and *Shewanella putrefaciens* are the most studied of this group and are phylogenetically associated with the δ- and γ-subdivisions, respectively, of the Proteobacteria (Myers and Nealson 1990; Lovley et al. 1993; Lonergan et al. 1996). *Shewanella* and *Geobacter* species are common in aquatic and sedimentary environments (DiChristina and DeLong 1993) and new species continue to be isolated (Caccavo et al. 1994; Rossello-Mora et al. 1994) suggesting that members of these genera may be the most environmentally-significant microbes involved in Fe(III) reduction and extracellular magnetite precipitation. BIM of magnetite has been demonstrated in cultures of *Shewanella*, *Geobacter* (Lovley et al. 1987; Lovley 1990), *Geothrix fermentans*, several thermophilic bacteria, including the Fe(III)-reducing bacterium strain TOR-39 (now known as a strain of the Gram-positive Bacterium *Thermoanaerobacter ethanolicus*) as well as other strains of the same genus (Liu et al. 1997; Zhang et al. 1998; Roh 2002), the Archaeon *Pyrobaculum islandicum* (Vargas et al. 1998) and the Bacterium *Thermotoga maritima* (Vargas et al. 1998). Magnetite is also formed in mixed cultures or consortia containing Fe(III) reducers (Bell et al. 1987; Liu et al. 1997; Zhang et al. 1997). It is likely that magnetite will be formed in a pure culture of any Fe(III)-reducing bacterium. Black, unidentified magnetic precipitates commonly observed in enrichment cultures or pure cultures of Fe(III)-reducing bacteria containing insoluble, amorphous, Fe(III) oxyhydroxide as the Fe(III) source (e.g., Greene et al. 1997; Slobodkin et al. 1997, 1999) probably consist primarily of magnetite. A halotolerant, facultatively-anaerobic, iron-reducing bacterium described by Rossello-Mora et al. (1994) most likely produces non-stoichiometric particles of magnetite with a composition intermediate between magnetite and maghemite (γ-Fe_2O_3) (Hanzlik et al. 1996).

The BIM magnetite particles produced by *Thermoanaerobacter ethanolicus* (strain TOR-39) have been well characterized (Zhang et al. 1998). Interestingly, like particles produced through BCM (Bazylinski 1995; Bazylinski and Frankel 2000a,b), the particles produced by *T. ethanolicus* have a size distribution that peaks in the single-magnetic-domain size range. The particles appear to be cuboctahedra with an average size of 56.2 ± 24.8 nm. *T. ethanolicus* is mildly thermophilic and growth and biomineralization experiments were performed at 65°C, raising the question of the role of temperature in size distribution of these crystals. Roh et al. (2001) later used this organism to produce metal-substituted magnetite crystals. Cobalt, chromium, and nickel were substituted into BIM magnetite crystals without changing the phase morphology. The incorporation of these metals into magnetite with the inverse spinel structure is of interest because of the unique magnetic, physical, and electrical properties of such crystals (Roh et al. 2001).

Cells of a magnetotactic species, *Magnetospirillum magnetotacticum*, have been shown to reduce Fe(III) in growing cultures and there is some evidence that iron reduction may be linked to energy conservation and growth in this bacterium (Guerin and Blakemore 1992). While extracellular BIM magnetite has never been observed in cultures of this organism, cells of *M. magnetotacticum* synthesize intracellular particles of magnetite (Frankel et al. 1979) via BCM (see Bazylinski and Frankel 2003).

Magnetite dissolution

In addition to magnetite mineralization, some iron-reducing bacteria are able to reduce ferric iron in magnetite—2 Fe(III) and 1 Fe(II) per formula unit—with release of Fe(II). *S. putrefaciens* was reported to reduce and grow on Fe(III) in magnetite (Kostka and Nealson 1995) whereas it appears *G. metallireducens* is unable to do so (Lovley and Phillips 1988). Dong et al. (2000) conducted reduction experiments in which *S. putrefaciens* strains CN32 and MR-1 respired with either biogenic or inorganic magnetite as electron acceptor and lactate as electron donor. In a medium buffered by bicarbonate (HCO_3^-), siderite ($FeCO_3$) precipitated, suggesting a dissolution-precipitation mechanism. Vivianite ($Fe_3(PO_4)_2$) precipitated in media with sufficient phosphate. The biogeochemical significance of this result is that some dissimilatory iron-reducing bacteria could utilize magnetite as an electron donor after the original pool of ferric iron, likely ferric oxyhydroxide, is exhausted. Thus it seems that some dissimilatory iron-reducing bacteria can both mineralize and dissolve magnetite under different Eh and pH conditions. Dong et al. (2000) note that magnetite is thermodynamically stable at pH 5−6.5 but is unstable at pH > 6.5. However, mineral formation that removes Fe(II) from solution tends to increase the pH range over which magnetite reduction is favorable. Thus BIM may function to shift thermodynamic equilibria in certain situations.

Sulfate reduction

Of all the metal sulfide minerals, iron sulfide mineralization is most often attributed to microbial activity (Southam 2000), more specifically to the activity of the dissimilatory sulfate-reducing bacteria. These ubiquitous, anaerobic, prokaryotes are a physiological group of microorganisms that are phylogenetically and morphologically very diverse and include species in the Domains Bacteria (δ-subdivision of Proteobacteria and Gram-positive group) and Archaea. Because all sulfate-reducing bacteria respire with sulfate under anaerobic conditions and release highly reactive sulfide ions, it is likely that all the species, regardless of phylogeny or classification, produce iron sulfide minerals through BIM under appropriate environmental conditions with excess, available, iron. Even a sulfate-reducing, magnetotactic bacterium, *Desulfovibrio magneticus* strain RS-1, is known to produce extracellular particles of iron sulfides through BIM while synthesizing intracellular crystals of magnetite via BCM (Sakaguchi et al. 1993). Sulfide ions react with the iron forming magnetic particles of greigite (Fe_3S_4) and pyrrhotite (Fe_7S_8) as well as a number of other non-magnetic iron sulfides including mackinawite (tetragonal FeS), pyrite (cubic FeS_2) and marcasite (orthorhombic FeS_2) (Freke and Tate 1961; Rickard 1969a,b). Mineral species formed in these bacterially-catalyzed reactions appear to be dependent on the pH and Eh of the growth medium, the incubation temperature, the presence of specific oxidizing and reducing agents, and the type of iron source in the growth medium. In addition, microorganisms clearly modify many of these parameters (e.g., pH, Eh) during growth. For example, cells of *Desulfovibrio desulfuricans* produced greigite when grown in the presence of ferrous salts but not when the iron source was goethite, FeO(OH) (Rickard 1969a).

Berner (1962, 1964, 1967, 1969) reported the chemical synthesis of a number of iron sulfide minerals, including marcasite, mackinawite, a magnetic, cubic iron sulfide of the spinel type (probably greigite), pyrrhotite, amorphous FeS, and even framboidal pyrite, a globular form of pyrite that was once thought to represent fossilized bacteria (Fabricus 1961; Love and Zimmerman 1961). Rickard (1969a,b) concluded that extracellular, biogenic iron sulfide minerals could not be distinguished from abiogenic (inorganic) minerals. However, in many cases, the iron sulfide minerals produced by the sulfate-reducing bacteria have not been systematically examined by high resolution electron microscopy. In addition, in many of early studies, the role of the cell in mineralization was not investigated.

More recent studies with sulfate-reducing bacteria show that mineralization proceeds initially by the immobilization of amorphous FeS on the cell surface (Fig. 6) through the ionic interaction of Fe^{2+} with anionic cell surface charges and biogenic H_2S (Fortin et al. 1994). Mineral transformations cause the production of other Fe sulfides, and eventually, pyrite (Fortin and Beveridge 2000; Southam 2000). Despite the results of Berner (1962, 1964, 1967, 1969), the bacterially-induced transformation of FeS to pyrite appears to be more efficient than that occurring under abiogenic conditions (Donald and Southam 1999).

Sulfide mineral oxidation

In addition to those bacteria that facilitate the mineralization of iron sulfides, there are bacteria that can oxidize iron sulfides such as pyrite (FeS_2) with molecular oxygen, with release of Fe(III) and sulfate (SO_4^{2-}) (Nordstrom and Southam 1997). This process is responsible for acid mine drainage and has also been put to use in enrichment and leaching of sulfide ores. The most studied organism is *Acidithiobacillus ferrooxidans*, an

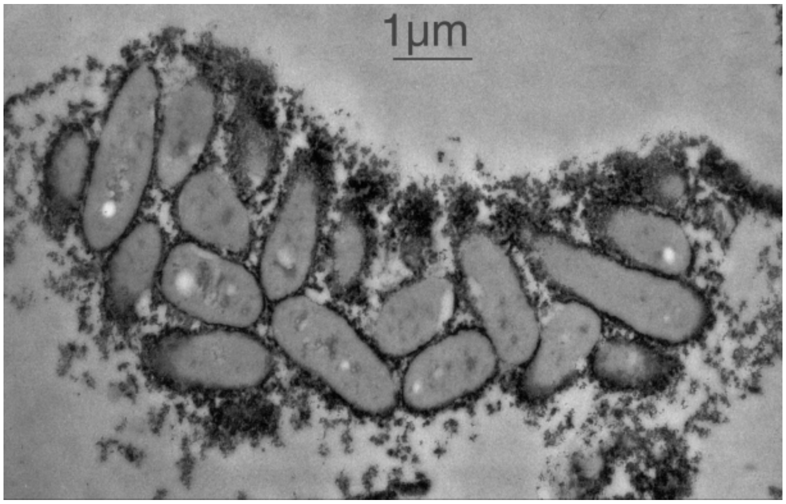

Figure 6. Unstained, ultrathin section transmission electron micrograph of a mineralized bacterial microcolony from a sulfate-reducing bacterial consortium grown with lactate in the presence of Fe(II). The cells in are encrusted with amorphous iron sulfides. Figure kindly supplied by W. Stanley and G. Southam.

acidophillic, autotrophic, bacterium. The oxidation process depends among other things on the properties of the pyrite, including grain size, crystallinity, defect structure, and trace metal impurities. Following oxidation, Fe(III) hydrolyses and initially precipitates as ferric oxyhydroxide. However, aging can result in a number of iron minerals including ferrihydrite and goethite, as well as iron-sulfate minerals jarosite ($KFe_3(SO_4)_2(OH)_6$) and schwertmanite ($Fe_8O_8SO_4(OH)_6$). Elemental sulfur is another possible reaction product. Other disulfide and monosulfide minerals can also be oxidized but result in substrate-mineral-specific products. Cells of *Acidithiobacillus* readily attach to the surfaces of sulfide minerals which maximizes the efficiency of the oxidation process. In general, microbe-mineral interactions in diverse environmental situations have become a major theme in biogeochemistry (Banfield and Hammers 1997; Fortin et al. 1997; Little et al. 1997; Edwards et al. 2001).

INTRACELLULAR BIOLOGICALLY INDUCED MINERALIZATION

Most of the examples of BIM discussed above involve extracellular deposition of minerals. However, there are several reports of intracellular deposition of minerals that seem to blur the line between BIM and BCM. For example, many bacteria have iron-storage proteins known as bacterioferritins (Chasteen and Harrison 1999). These are intracellular proteins comprising 24 identical subunits arranged in pairs that form a dodecahedral shell enclosing a 9 nm cavity. The cavity can accommodate up to 4000 iron atoms as an amorphous, ferric oxyhydroxy phosphate, with variable P/Fe ratio. The subunit pairs contain ferroxidase centers which catalyze the oxidation of ferrous iron and nucleation of the mineral in the cavity. While the organism provides an organic vesicle (the protein shell) for the deposition of the mineral, it apparently does not control the composition or crystallinity of the mineral. On the other hand, less crystallinity may allow greater access to the iron and perhaps phosphate stored as the mineral in the cavity. Addition of phosphate, a known glass former, may insure formation of an amorphous core mineral.

Intracellular iron-sulfide particles have been reported within cells of some sulfate-reducing bacteria, including *Desulfovibrio* and *Desulfotomaculum* species, when they were grown with relatively high concentrations of iron in the growth medium (Jones et al. 1976). The "particles" were randomly arranged in the cell and, based on electron diffraction, were not well-ordered crystals. They were also not separable by density gradient centrifugation. They are apparently not essential to the cell in that cells can be grown with much less iron where they do not form these structures.

Unidentified, presumably magnetic ("magnet-sensitive"), electron-dense particles were reported in cells of several purple photosynthetic bacteria including *Rhodospeudomonas palustris*, *R. rutilis* (both α-Proteobacteria), and *Ectothiorhodospira shaposhnikovii* (a γ-Proteobacterium) cultured in growth media containing relatively high concentrations of iron. The inclusions were spherical particles containing an electron-transparent core surrounded by an electron-dense matrix. The particles could be separated from lysed cells; X-ray microanalysis showed that the inclusions are Fe-rich but did not contain sulfur. The particles were arranged in a chain like magnetosomes (Bazylinski 1995) and possibly surrounded by a membranous structure (Vainshtein et al. 1997). Vainshtein et al. (2002) later showed that many other bacteria including non-photosynthetic members of both prokaryotic domains could be induced to form similar particles. Cells with the particles show a magnetic response but are not necessarily magnetotactic. The authors speculate that the particles function similarly to magnetosomes. This case of biomineralization appears to be almost intermediate between

BIM and BCM in that cells appear to control some features of these particles such as their arrangement in the cell.

Glasauer et al. (2001) reported unidentified iron oxide particles within the dissimilatory iron-reducing bacterium *Shewanella putrifaciens* grown in an H_2/Ar atmosphere with poorly-crystalline ferrihydrite (ferric oxyhydroxide) as electron acceptor. There is evidence from selected area electron diffraction that the intracellular iron oxide particles are magnetite or maghemite (γ-Fe_2O_3). Magnetite also formed outside the cell. Ona-Nguema et al (2002) found green rust with Fe(II)/Fe(III) ratio ~1 when *S. putrifaciens* was cultured under anaerobic conditions with formate as the electron donor and crystalline lepidochrocite (γ-FeOOH) as the electron acceptor. The green rust eventually remineralized as black magnetite/maghemite when the reaction culture medium was incubated at room temperature.

SIGNIFICANCE OF BIOLOGICALLY INDUCED MINERALIZATION

Biomineralization by prokaryotes is of great significance in scientific and commercial applications as well as having a major impact in microbiology, evolutionary biology, and geology. Bacterial metal sorption and precipitation can be important and useful in metal and radionuclide removal during the bioremediation of metal- and radionuclide-contaminated waters (Lovley 2000). The growth of Fe(II)- and Mn(II)-oxidizing bacteria that efficiently remove Fe and Mn ions from water by mineralization in wastewater treatment plants is promoted in France, thereby eliminating the problems of biofouling of pipelines by mineral deposits and of water discoloration (Mouchet 1992). This is a major problem in the use of groundwater sources.

Konhauser et al. (2002) have speculated that iron-oxidizing bacteria could have been responsible for the formation of the massive Precambrian banded iron formations (BIF) by BIM via oxidation of dissolved Fe(II) in the ancient ocean. Oxidation of Fe(II) to Fe(III) could have occurred by chemolithoautotrophy or by photosynthesis with Fe(II) as the electron donor. Based on the chemical analyses of BIF deposits dated to 2.5 Ga from Western Australia, they concluded that bacterial cell densities less than those found in modern Fe-rich environments would have been sufficient.

Bacterially-formed minerals, in one form or another, may be useful as biomarkers (indications of past life) when other remains of the cells or indications of the presence of the cells are no longer evident. In many situations only mineral encrustations that once encapsulated the cell are observed (Southam 2000). These biomarkers may be useful not only in determining when bacteria evolved on Earth but also as evidence of former life in extraterrestrial materials (Thomas-Keprta et al. 2000).

An interesting yardstick for the scale of bacterially-induced mineralization is provided by the wreck of the Titanic. When Robert Ballard found the Titanic in 1985, he noted rust-colored concretions hanging off the hull. The concretions have shapes similar to stalactites or icicles; hence Ballard called them “rusticles.” Rusticles can be centimeters to meters in length and have a complex internal architecture with water channels the diameters of which are distributed over many orders of magnitude. Iron minerals comprise the major constituents of the rusticle, with ferric oxyhydroxides predominating on the outer surfaces and goethite on the inside. Associated with the rusticles are a microbial consortium of over twenty species that includes iron-oxidizing and sulfate-reducing bacteria (Wells and Mann 1997). This suggests that rusticles contain a number of micro-environments from oxic to anaerobic. SEM studies show heavily mineralized bacteria organized in chains (Fig. 7). From sequential observations over a

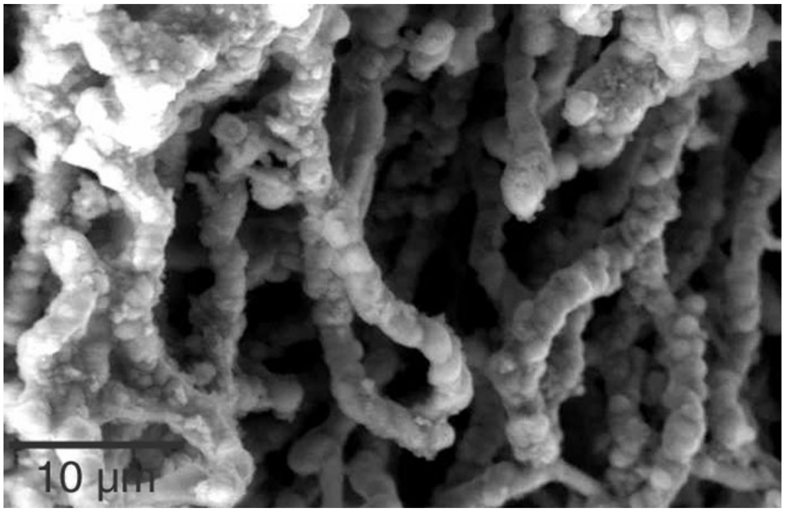

Figure 7. Scanning electron micrograph of "rusticles" recovered from the hull of the Titanic. The rusticles consist of ropes or chains of heavily mineralized bacteria. This figure was adapted from one kindly provided by H. Mann.

number of years, it has been estimated that the rusticle formation rate is about 1 ton per year over the ship. At this rate, the remaining lifetime of the hull must be measured in years, not centuries. This illustrates the fact that bacteria working over geologic time scales can effect enormous mineral transformations.

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