Diversity of bacterial iron mineralization

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Abstract

Bacterial cells, growing naturally in freshwater and marine environments or experimentally in culture, can precipitate a variety of authigenic iron minerals. With the vast majority of bacteria biomineralization is a two-step process: initially metals are electrostatically bound to the anionic surfaces of the cell wall and surrounding organic polymers, where they subsequently serve as nucleation sites for crystal growth. The biogenic minerals have crystal habits and chemical compositions similar to those produced by precipitation from inorganic solutions because they are governed by the same equilibrium principles that control mineralization of their inorganic counterparts. As the latter stages of mineralization are inorganically driven, the type of biominal formed is inevitably dependent on the available counter-ions, and hence, the chemical composition of the waters in which the microorganisms are growing. In oxygenated waters, iron hydroxides are a common precipitate and can form passively through the binding of dissolved ferric species to negatively charged polymers or when soluble ferrous iron spontaneously reacts with dissolved oxygen to precipitate as ferric hydroxide on available nucleation sites (e.g. bacteria). Alternatively, the metabolic activity of Fe(II)-oxidizing bacteria can induce ferric hydroxide precipitation as a secondary by-product. Ferric hydroxide may then serve as a precursor for more stable iron oxides, such as goethite and hematite via dissolution--reprecipitation or dehydration, respectively, or it may react with dissolved silica, phosphate or sulphate to form other authigenic mineral phases. Under suboxic to anoxic conditions, ferric hydroxide may be converted to magnetite, sidetite, and iron sulphides through various reductive processes associated with organic matter mineralization. Under biologically controlled conditions, where mineralization is completely regulated, magnetotactic bacteria form magnetite and greigite as navigational tools to guide themselves into their preferred habitat. In general, the formation of iron biominerals is not difficult to achieve, bacteria simply provide charged surfaces that bind metals and they excrete metabolic waste products into the surrounding environment that induce mineralization. The ubiquitous presence of bacteria in aquatic systems and their inherent ability to biomineralize, therefore, makes them extremely important agents in driving both modern and ancient geochemical cycles. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Bacteria are found throughout nature, inhabiting every conceivable environment where liquid water is freely available. This includes extremely harsh surroundings where they often represent the only life forms, such as deep-marine sediment (Parkes et al., 1994), in petroleum reservoirs (Grassia et al., 1996), some deep-sea hydrothermal vents (Juniper and Tebo, 1995), deep subterranean groundwater, rock fractures and clay sediment (Pedersen, 1993; Stevens and
McKinley, 1995; Boivin-Jahns et al., 1996), polar environments (Vincent and James, 1996), deserts (Adams et al., 1992), highly polluted groundwater (Bottrell et al., 1995), hypersaline lakes (Steinhorn and Gat, 1983), acid mine drainage (Clarke et al., 1997), and more specifically a nuclear reactor core (Booth, 1987) and possibly even ancient Mars (McKay et al., 1996). Moreover, fossilized microbial remains, stromatolites, chemical fossils and biogenic minerals have been reported from early Archaean rocks (Awramik et al., 1983; Hayes et al., 1983; Byerly et al., 1986; Ohmoto et al., 1993), some at least 3500 million years old (Schopf, 1993). The construction of stromatolites implies that these microbes were morphologically well-developed, and that even at this early stage in Earth’s evolution relatively advanced prokaryotes were already in existence (Awramik, 1992; McNamara and Awramik, 1992). Life may have originated possibly as far back as 3800 Ma, if the isotopically-light carbonaceous inclusions withinapatite grains of the Isua supracrustal belt, West Greenland are indeed indicative of biological activity (Mojzsis et al., 1996). Not only are bacteria ubiquitous today, but they are found in enormous numbers, usually more than $10^8$ cells/g in garden soil and marine mud, $10^5$ cells/ml in lake water (Ehrlich, 1990), $10^5$ cells/ml in river water and $10^8$ cells/cm$^2$ in biofilms (Geese et al., 1978), and up to $10^9$ cells/ml in the waters emanating from warm hydrothermal vents (Jannasch and Mottl, 1985). Invariably this abundance dictates that bacteria will control many modern biogeochemical cycles, including in particular, the formation of a wide array of biominerals from dissolved components in their immediate surroundings.

In terms of biomineralization, there appear to be two different modes of mineral formation (Lowenstam, 1981). The first is characterized by the development of an organic framework (either intracellularly or extracellularly) into which specific ions are actively introduced (Fig. 1A). This ‘biologically controlled mineralization’ process is completely regulated, allowing the organism to precipitate physiologically essential minerals from the external environment (Mann, 1983). Because the mineralization site is separated from outside the cell by a barrier through which ions cannot freely diffuse, mineralization may proceed under external condi-

tions that are normally thermodynamically unfavourable (i.e. magnetotactic bacteria that produce magnetite inside magnetosomes). By contrast, ‘biologically induced mineralization’ is where minerals are formed incidentally as a by-product of interactions between the activity of the microorganisms and their surrounding environment (Lowenstam, 1981). Minor perturbations such as the introduction of biologically produced metabolic end-products (e.g. $\text{OH}^-$, $\text{CO}_2$, $\text{H}^+$, and $\text{NH}_3$), the release of cations by the cell, or the development of a charged surface (Fig. 1B) can all induce the nucleation of minerals with crystal habits and chemical compositions similar to those produced by precipitation from inorganic solutions. This is to be expected since biomineralization is governed by the same equilibrium principles that control mineralization of their inorganic counterparts (Lowenstam, 1981; Lowenstam and Weiner, 1989). Biologically induced mineralization is the dominant process among bacteria, and one particu-

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Fig. 1. Schematic figure showing intracellular and extracellular biominalization. (A) Formation of magnetite or greigite within magnetotactic bacteria. The specificity of morphology and size of the mineral is presumably predetermined by the membrane of the magnetosome (arrow). (B) Formation of epicellular iron silicate phases similar to those found in hot spring bacterial mats. Note the initial electrostatic interaction between dissolved iron and the anionic cell wall (carboxyl and phosphoryl groups), and the subsequent reaction of the bound iron with silicic acid.
larly characteristic feature is that the type of mineral formed is a function of the environmental conditions in which the microorganism lives; conversely the same microorganism in different environments can form different minerals (Lowenstam and Weiner, 1989).

At the normal growth pH (between 5 and 8), structural polymers that reside in the cell wall and surrounding fabric of bacteria are ionized and naturally anionic (see Beveridge, 1989a for details about bacterial structures). By virtue of their small size, bacteria also have the largest surface area to volume ratio of cellular life forms (Beveridge, 1989b), with different cell shapes having different surface area to volume ratios (i.e. rods, 10:1; spheres, 5.8:1; and spiral-shaped, 16:1) (Beveridge, 1988). This property offers bacteria a remarkable potential to sequester and accumulate an assortment of metals onto their surfaces (Marquis et al., 1976; Beveridge, 1978; Beveridge and Murray, 1980; Doyle et al., 1980; Beveridge and Koval, 1981; Beveridge et al., 1982; Hoyle and Beveridge, 1983; Ferris and Beveridge, 1984; Mullen et al., 1989; Krueger et al., 1993). The cells do not even have to be viable: dead cells have greater binding capacity than living ones because cellular degradation increases the availability of functional groups capable of binding metals (Ferris et al., 1988) and the protons generated by membrane respiration in living cells no longer compete with dissolved metals for binding sites (Urrutia et al., 1992). Some bacteria show extreme selectivity in binding one metal from a range of competing cations to fulfill essential physiological functions (Williams, 1981; Hughes and Poole, 1989), whereas other bacteria react to dissolved ions as if they were an open ion-exchange resin (Marquis et al., 1976), presumably a function of living in a concentrated solution where salts abound (Beveridge, 1989c). In this regard, it is not surprising that biofilms are considered ideal cation scavengers (Geese et al., 1988). In fact, this metal-binding property has made microorganisms ideal in biorecovery of economically valued metals (Dyer et al., 1994; Southam and Beveridge, 1994; Basnakova and Macaskie, 1997) and bioelimination of both toxic metals (Scott et al., 1988; Brierley et al., 1989; White et al., 1995) and radionuclides (Khvorychev et al., 1994; Macaskie et al., 1996; Yong and Macaskie, 1997).

Based on this inherent metal-binding capacity, Beveridge and Murray (1976) proposed a two-step mechanism for the development of authigenic mineral phases in association with bacterial cells. The first step involves a stoichiometric interaction between metals in solution and the cell’s negatively charged reactive chemical groups. These include carboxyl and phosphoryl groups in the cell wall and outer membrane and carboxyl and hydroxyl groups in the surrounding capsules (Beveridge and Fyfe, 1985). Transition metals show extremely high affinities for the polymeric material (Beveridge, 1978) due to their valences, hydrated radii, hydration energies and electronegativities (Ferris and Beveridge, 1986). Iron in particular is commonly bound to organic sites (Reuter and Perdue, 1977), a finding that may in part be related to its greater concentration in natural waters compared to other trace metals (Bowen, 1979). Once bound, these metals reduce the activation energy barriers to nucleation by providing sites where strong surface chemical interactions can take place and more soluble components can be sorbed (Mann, 1988; McLean et al., 1996). Those sites within the interstices of the wall develop only fine-grained precipitates (a few nanometres thick), because the space between wall polymers constrains mineral growth. Surface sites (e.g. capsules and sheaths) have no such physical constraints, and with time and a sufficient supply of soluble components, very large epilayer mineral grains (several micrometres in diameter) can develop (Beveridge, 1989b). The end result is a mineralized cellular matrix containing detectable concentrations of metal ions that are not easily redissolved (Beveridge and Fyfe, 1985).

In recent years, high-resolution studies on bacterial communities, using transmission electron microscopy (TEM), coupled with energy-dispersive X-ray spectroscopy (EDS) and selected area electron diffraction (SAED) have shown the almost ubiquitous presence of fine-grained iron minerals associated with bacterial cells. Because most bacteria precipitate minerals through biologically induced mineralization, of which the second step is inorganically driven, the biominerals formed will be largely dependent upon the available counter-ions, and hence, the chemical composition of the waters in which they are growing. The formation of these iron biominerals is not difficult to achieve, bacteria simply provide
charged surfaces that bind metals and they excrete metabolic waste products into the surrounding environment that induce the mineralization process. The ubiquitous presence of bacteria in aquatic systems and their inherent ability to biomineralize, therefore, makes them extremely important agents in driving both modern and ancient geochemical cycles. This paper reviews some of the environments where iron biominerals have been observed, and outlines some common geochemical conditions associated with bacterial biomineralization.

2. Iron mineral formation

2.1. Hydroxides

The microbial precipitation of ferric hydroxide (e.g. ferricydrite) is widespread in nature. It has been observed directly associated with bacterial cells growing in acid mine drainage environments (Ferris et al., 1987, 1989a,b; Clarke et al., 1997; Bottrell et al., 1998), river sediment (Konhauser et al., 1993, 1994a, 1998), lake detritus and sediment (Ghirose and Chapnick, 1983; Heldal and Tumyr, 1983; Fortin et al., 1993; Tessier et al., 1996), deep subterranean groundwater (Brown et al., 1994; Doig et al., 1995; Sawicki et al., 1995), water distribution systems (Trafford et al., 1973; Ivarson and Sojak, 1978; Ridgway and Olson, 1981; Ridgway et al., 1981), geyser outflow channels (Ferris et al., 1986; Holm, 1987; Konhauser and Ferris, 1996), marine sediments (Hanert, 1973; Holm, 1987, 1989; Konhauser et al., 1996), marine detritus (Cowen and Silver, 1984; Cowen and Brunland, 1985), around deep-sea vents and in hydrothermal plumes (Jannasch and Wirsen, 1981; Alt, 1986, 1988; Cowen et al., 1986; Tunnnicliffe and Fontaine, 1987; Karl et al., 1988, 1989; Juniper and Tebo, 1995; Juniper et al., 1995), and on exposed rock surfaces (Dorn and Oberlander, 1981; Adams et al., 1992; Konhauser et al., 1994b). TEM analyses commonly indicate bacterial cells partially to completely enclosed within iron-rich epitelular matrices (Fig. 2A) or with intracellular precipitates (Fig. 2B), where the cytoplasmic material has been completely replaced once the cell had lysed. Not only do the bacteria serve as templates for iron deposition, but in the acid mine drainage sites, for example, their organic remains were also incorporated into the mineral precipitates during crystal growth (Ferris et al., 1989a).

The biologically induced formation of epicellular iron hydroxide by bacteria can occur either passively or actively. In the first instance, the oxidation and hydrolysis of cell-bound ferrous iron, the binding of ferric species [e.g. Fe(OH)\textsuperscript{2+}, Fe(OH)\textsuperscript{3+}] or cationic colloidal species [e.g. Fe(H\textsubscript{2}O)\textsubscript{6}(OH)\textsubscript{2}(OH)\textsuperscript{2+}] to negatively charged polymers, and the alteration of local pH and redox conditions around the cell due to their metabolic activity can all induce the formation to insoluble hydroxide forms (MacRae and Edwards, 1972; Ghirose, 1984; Ferris et al., 1989b; McLean et al., 1992). Alternatively, ferrous iron transported into an oxygenated environment spontaneously reacts with dissolved oxygen (at circumneutral pH) to precipitate rapidly (abiotically) as ferric hydroxide on available nucleation sites. Bacteria passively act as such sites, and over a short period of time the microbial mats can become completely encrusted in amorphous iron as abiological surface catalysis accelerates the rate of mineral precipitation (Ghirose, 1984). It has even been suggested that under circumneutral conditions any bacterium that produces acidic, extracellular polymers will nonspecifically adsorb positively charged Fe-hydroxides (Ghirose, 1984). This is not unexpected since the point of zero charge (the pH at which the mineral’s charge becomes zero) of Fe-hydroxides is 7 to 8; therefore, anionic groups of the cell easily scavenge ferric iron from the surrounding waters (Juniper and Tebo, 1995). Indeed, ferricydrite also develops on the organic remains of dead cells, implying that iron mineralization can occur independent of cell morphology, trophic classification or physiological state (Ferris et al., 1989a).

There are several well documented examples of iron-depositing bacteria in aquatic systems, such as *Sphaerotilus, Leptothrix, Crenothrix, Clonothrix*, and *Hyphomicrobium* spp., that bind and precipitate ferric iron non-specifically (passively) onto their cell surfaces to form ochreous and ferromanganese precipitates (see Ghirose, 1984; Ehrlich, 1990; Ghirose and Ehrlich, 1992 for reviews). *Leptothrix* and *Sphaerotilus* spp. deposit copious amounts of iron hydroxide as encrusted sheaths in the various environments where they grow (Ghirose, 1984). The
Fig. 2. TEM image of epilithic bacterial cells (stained with uranyl acetate and lead citrate) from a hot spring effluent channel at Lýsuhöll, Iceland. (A) Bacterial cell surrounded by a dense, iron-rich capsule of amorphous ferric hydroxide. Scale bar = 240 nm. (B) Bacterial cell with intracellular iron mineralization. Scale bar = 2.7 μm. (B) Reprinted with permission of Geology, the Geological Society of America, Boulder, Colorado, USA © 21286 (Konhauser and Ferris, 1996).

precipitation of iron hydroxide by *Sphaerotilus natans* is generally thought to be a passive process (Ghiors, 1984), while *Leptothrix* spp. may enzymatically oxidize ferrous iron (Fig. 3). This hypothesis, however, rests mainly on the cell’s requirement for ferrous iron during growth and on its subsequent oxidation to ferric iron (Ehrlich, 1990). Other bacteria such as *Crenothrix* spp. (Wolfe, 1960a) and
Clonothrix spp. (Wolfe, 1960b) frequently occur in large masses of morphologically distinct filaments that become encrusted in iron hydroxide, while Hyphomicrobium spp. thrives in deep-sea hydrothermal vents (e.g. Galapagos Rift spreading zone, depth 2550 m) where iron coating occurs on both living and dead cells (Jannasch and Wirsen, 1981). In the case of Hyphomicrobium spp., metal deposition may simply have resulted from the relatively high concentration of reduced metals in the hydrothermal waters that reacted with oxygenated seawater to precipitate indiscriminately on the bacterial surfaces (Jannasch and Wirsen, 1981).

A variety of other heterotrophic bacteria, from diverse environments, have also been shown to deposit iron (Ghiorsce, 1984). Cells of Caulobacter, Micrococcus, Pseudomonas, Mycobacterium, Escherichia, Klebsiella, Corynebacterium and Acinetobacter spp. all non-specifically precipitated positively charged ferric-iron sols when grown in culture (MacRae and Edwards, 1972; MacRae and Celto, 1975). In fact, the iron-coating on the cells was sufficiently dense to visualize the bacteria under the TEM without the standard use of metal stains (MacRae and Edwards, 1972). Interestingly, the respiration rate of Acinetobacter spp. was reduced during iron encrustation, suggesting that the iron formed a barrier inhibiting oxygen transfer and the elimination of waste products (MacRae and Celto, 1975). Other heterotrophs, present in desert varnishes (the iron and manganese coatings found on rock surfaces in arid and semiarid environments), have been implicated in the mobilization of ferric iron (via siderophore dissolution of source material), its concentration within cellular material, and ultimately the formation of mineralized coatings (Dorn and Oberlander, 1981; Krumbein and Jens, 1981; Adams et al., 1992; Staley et al., 1992). In terrestrial and marine hydrothermal settings, biological Fe-hydroxide formation occurs on a consortium of bacteria (Konhauser, 1998). In microbial mats from Iceland (Konhauser and Ferris, 1996) individual bacterial cells and microcolonies were frequently surrounded by iron-rich capsular material or fine-grained (< 100 nm in diameter) Fe-rich spheroids. In marine hydrothermal environments, Baross and Deming (1985) provided evidence that iron was deposited in significantly greater amounts on rock surfaces covered with microbial mats than on uncolonized rocks. Unidentified sheathed bacteria have been observed colonizing surfaces of vestimentiferan tubes on the southern Juan de Fuca Ridge where they accumulated iron hydroxide (as Fe-rich spheres 6 to 8 μm in diameter) around worm tubes (Tunnicliffe and Fontaine, 1987).

Bacterial filaments growing in oxide mud at Red Volcano near 21°N, East Pacific Rise (Alt, 1986),
the Larson Seamount, East Pacific Rise (Alt, 1988), and on the Loihi Seamount, Hawaii (Karl et al., 1988, 1989) also accumulated iron oxides around their cells. In some of these examples, it appeared as though the microbes played a direct role in Fe-oxidation since the hydrothermal effluents were slightly acidic (pH 5 to 6) and low in dissolved O₂. Therefore, spontaneous precipitation of iron would not occur immediately adjacent to vents without bacterially catalyzed oxidation (Alt, 1988; Juniper and Tebo, 1995).

The growth of iron-depositing microbial populations in environments where they are constantly covered in amorphous iron hydroxide implies that these sites must provide the bacteria with favourable growth conditions. Quite possibly the continual supply of iron and trace metals, which adsorb or co-precipitate directly onto iron hydroxides (German et al., 1991) or indirectly onto the hydroxides (in low pH waters) via organic coatings on the hydroxide surfaces (Tessier et al., 1996), may serve as an ideal nutrient source in close proximity to the cells (Fig. 4). For *Gallionella ferruginea* the formation of ferric hydroxide around the cell’s stalk may also protect them from the reducing capacity of ferrous iron as it becomes unstable in an oxidizing environment (Hallock and Pedersen, 1990), or it may serve as a defense mechanism against oxygen toxicity (Ghiorsie, 1984). The mineralized stalk, therefore, gives *Gallionella ferruginea* a unique possibility to colonize and survive in habitats with high iron content, inaccessible for bacteria without a defense mechanism against iron toxicity (Hallock and Pedersen, 1995).

The active process by which iron hydroxides form stems from the ability of Fe(II)-oxidizing bacteria to oxidize ferrous iron as an energy source. Most enzymatic oxidation of Fe(II) occurs at extremely low pH, ranging from 1.5 to 3.5 (Ghiorsie and Ehrlich, 1992), and in acid mine drainage environments, the activity of *Thiobacillus ferroxidans* may promote iron hydroxide or iron hydroxysulphate precipitation (Crerar et al., 1979; Lazaroff et al., 1982, 1985; Bigham et al., 1990, 1994, 1996). Because very little energy is generated in the oxidation of ferrous to ferric iron (ΔG at pH 2.5 = 6.5 kcal/mol with oxygen as the electron acceptor), these bacteria must oxidize large quantities of iron in order to grow (Lees et al., 1969; Ehrlich, 1990). For example, it has been estimated that a consumption of 90.1 mol of Fe²⁺ is required to assimilate only 1.0 mol of carbon (Ehrlich, 1990). Consequently, even a small number of bacteria can be responsible for precipitating vast amounts of iron (Brock et al., 1984). At
neutral pH, Fe(II)-oxidation by *Gallionella* genera occurs under partially reduced conditions, with an Eh range of +200 to +320 mV and oxygen levels of 0.1–1.0 mg of O₂ per litre (Ehrlich, 1990). The existence of these microaerophilic bacteria relies on their oxidizing efficiency relative to abiotic oxidation at low oxygen fugacity. Although there is no conclusive evidence that iron bacteria other than the acido-philic derive energy from Fe(II)-oxidation, *Gallionella ferruginea* grows both autotrophically and mixotrophically with Fe²⁺ as its sole energy source at a pH just below 7 (Hallbeck and Pedersen, 1991). These bacteria produce an extracellular stalk and sheath that becomes heavily encrusted with amorphous iron hydroxide (Fig. 5) (Ghiorse and Ehrlich, 1992). This is illustrated by the presence of iron precipitates in environments where they commonly grow (i.e. springs, wells, water pipes and field drains) (Trafford et al., 1973; Ivarson and Sojak, 1978; Ridgway and Olson, 1981; Ridgway et al., 1981; Hallbeck and Pedersen, 1995). The copious amounts of iron precipitated often cause serious problems in water distribution systems through the impairment of water flowing through pipes (Trafford et al., 1973). Some *Gallionella* spp. have even been shown to inhabit relatively inhospitable environments, such as hydrothermal sites (Hanert, 1973; Holm, 1987, 1989) and on tunnel surfaces in an underground hard rock laboratory, Äspö, Sweden (Pedersen and Karlsson, 1995). Ferrous iron has also been observed to undergo microbial oxidation under anoxicogenic conditions. Widdel et al. (1993) and Ehrenreich and Widdel (1994) have described purple, non-sulphur bacteria that combined ferrous iron oxidation to CO₂ fixation for cellular material, with light as the energy source. The ferrous iron oxidation takes place on the outer membrane so that the insoluble ferric hydroxide remains outside the cell (Ehrenreich and Widdel, 1994). These results indicate that ferrous iron oxidation only requires Photosystem 1 (reaction 1) in anoxicogenic phototrophs (Widdel et al., 1993):

\[
4\text{Fe}^{2+} + \text{CO}_2 + 11\text{H}_2\text{O} \rightarrow 4\text{Fe(OH)}_3 + \text{CH}_2\text{O} + 8\text{H}^+ 
\]

Straub et al. (1996) also demonstrated that the biological oxidation of ferrous iron in the absence of oxygen was possible by light-independent, chemotrophic activity with nitrate as the electron acceptor. In their study, the observation that nitrate reducers, which had never been previously grown in iron media, exhibited the capacity for ferrous iron oxidation implied that this form of microbial oxidation may be common in the suboxic zone of aquatic environments.

2.2. Oxides

In most natural systems, ferrihydrite serves as a precursor to more stable iron oxides, such as goethite and hematite. The transformation into more crystalline minerals proceeds through: (1) internal aggregation and rearrangement leading to hematite; and (2) dissolution–reprecipitation leading to goethite (Cornell et al., 1987). The end-product depends
largely on pH, with maximum hematite formed experimentally between pH 7 and 8, and maximum goethite at pH 4 and at pH 12 (Schwertmann and Murad, 1983). In two Canadian studies on surficial sediments from an acid mine drainage repository in northern Ontario (Ferris et al., 1989a) and subterranean biofilms in an underground research laboratory, Manitoba (Brown et al., 1994; Sawicki et al., 1995), ferrihydrite and poorly ordered hematite were found associated with individual bacterial cells. It was conjectured that in both environments hematite formed through dehydration of the ferrihydrite precursor. Extracellular grains of acicular goethite (Fig. 6) were identified on bacterial surfaces collected from microbial mats lining a geyser outflow channel in Lýsuhóll, Iceland (Konhauser and Ferris, 1996), while in sediments from the shallow waters of the ancient caldera of Santorini, Holm (1987) observed goethite beneath iron hydroxide layers. Goethite re-precipitation is commonly favoured after ferrihydrite dissolution, particularly in the presence of organic compounds capable of complexing Fe$^{3+}$ and keeping its activity in solution low (Schwertmann and Fitzpatrick, 1992). Lepidocrocite, a polymorph of goethite, is also widespread in surface environments and is commonly associated with goethite and ferrihydrite (Schwertmann and Fitzpatrick, 1992). TEM analysis of a bacterium growing in a Fe-containing seawater medium revealed lepidocrocite particles in association with the cell’s extracellular polymers after two weeks of incubation (Ghirose, 1984). In a study of ‘microbial blooms’ from a uranium mining tailings ditch in Elliot Lake, Ontario, Mann et al. (1987) similarly observed lath-shaped intracellular and epicellular lepidocrocite precipitates associated with *Euglena* spp. (a unicellular alga). Some pure isolates contained 40–60% Fe by dry weight (H. Mann et al., 1987).

Ferromanganese oxides can be found on the ocean floor, in soils, and lake sediment as concretions/nodules (see Lundgren and Dean, 1979; Ehrlich, 1990 for reviews). Their formation involves a series of microbially catalyzed reactions that may include: (1) the concentration of metals by microorganisms growing in the water column; (2) their transport to the bottom sediment as the microbes die; (3) their release through organic matter mineralization; and (4) subsequently the bacterial oxidation of the Mn$^{2+}$ and Fe$^{2+}$ into nodules (Lundgren and Dean, 1979; Dubinina, 1981). Microscopic examina-

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Fig. 6. TEM image of a bacterial cell (stained) with crystalline, acicular goethite associated with the cell wall. Grains around cell may have been shed (arrow). Scale bar = 130 nm. Reprinted with permission of Geology, the Geological Society of America, Boulder, Colorado, USA © 21286 (Konhauser and Ferris, 1996).
tion has shown the presence of a consortium of bacteria on the surfaces and within the nodules (Ghiorse, 1980; Burnett and Nealson, 1981, 1983; Mustoe, 1981; Ghiorse and Hirsch, 1982; Ghiorse et al., 1983), with for example, inoculation on a nutrient-rich medium producing over $10^4$ viable cells of *Hyphomicrobium* spp. per gram of nodule (Dubinina, 1981). The finding of iron and manganese precipitating bacteria associated with ferromanganese nodules suggests that these microorganisms play a direct role in their formation (Ghiorse and Hirsch, 1982; Ehrlich, 1990; Ghiorse and Ehrlich, 1992). A similar conclusion was made from analyses of surface films, in a swamp surface in Ithaca, New York, which showed that viable *Leptothrix* spp. cells controlled the distribution of Fe and Mn in surface waters (Ghiorse and Chapnick, 1983). Other heterotrophic microbes may passively cause mineral precipitation via adsorption of iron oxides and hydroxides onto their cell surfaces (Burnett and Nealson, 1983). For example, Ghiorse et al. (1983) found that 20–40% of the cultured heterotrophic bacteria in Oneida Lake were identified as being capable of precipitating iron-manganese oxides in their colonies.

2.3. Magnetite

The potential for bacteria to contribute to the stable remnant magnetism in soils and sediments has recently generated a great deal of interest (Kirschvink, 1982; Kirschvink and Chang, 1984; Chang and Kirschvink, 1985; Stolz et al., 1986; Banerjee, 1988; Maher and Taylor, 1988). Biogenic magnetites are commonly single domain, with a high natural magnetic remanence, and are known to proceed under both 'biologically controlled' and 'biologically induced' conditions (see Blakemore and Frankel, 1989; Blakemore and Blakemore, 1990 for reviews). In the first instance, magnetotactic bacteria produce intracellular chains of single, pure magnetite crystals (Fig. 7), under precise biochemical, chemical and probably genetic control (Bazylinski, 1995). The magnetite particles, called magnetosomes (Balkwill et al., 1980), are enveloped within an intracytoplasmic membrane consisting of a lipid bilayer admixed with proteins (Gorby et al., 1988). The membrane presumably serves as the locus of control over the specificity of their morphology and size (Bazylinski, 1995, 1996).

Fig. 7. Transmission electron micrograph of a negatively stained cell of a magnetotactic marine spirillum, designated strain MV-4, that produces a single chain of parallelepipedal crystals of magnetite that longitudinally traverse the cell. Scale bar = 360 nm. Reprinted from cover of ASM News 61 (7), with permission of Dennis Bazylinski and ASM News, the American Society for Microbiology.
Three general morphologies of magnetite have been observed, including cuboidal (Frankel et al., 1979; Blakemore, 1982; Stolz et al., 1986), parallelepipedal (rectangular in the horizontal plane, but could be hexagonal or octahedral on end; Towe and Moench, 1981; Matsuda et al., 1983; Stolz et al., 1987; Bazylinski et al., 1988; Sparks et al., 1990), and arrow-head or tooth-shaped crystals (Stolz et al., 1986; H. Mann et al., 1987; Bazylinski et al., 1993, 1995). Irregular shapes (Mann et al., 1984a; Stolz et al., 1986; Sakaguchi et al., 1993) and clumps of crystals (Fassbinder et al., 1990) have also been observed; the latter forms reflecting corrosion or dissolution of magnetite after cell lysis (Vali et al., 1987; Vali and Kirschvink, 1989), when chemical reactions with dissolved sulphide (Canfield and Berner, 1987) or microbially mediated dissolution and Fe(III)-reduction (Kostka and Nealson, 1995) occurred during sediment burial. Magnetotactic bacteria also precipitate magnetite within a narrow size range, from approximately 35 to 120 nm (Bazylinski et al., 1994). This establishes stable single magnetic domains, which arranged in a chain that traverses the cell along its long axis, provide the microorganisms with the ability to orient and swim along geomagnetic field lines (Blakemore, 1975; Frankel et al., 1979; Frankel and Blakemore, 1980, 1989). A magnetotactic cell can have only one of two possible magnetic polarities (Bazylinski, 1996); populations of magnetotactic bacteria collected from the Northern Hemisphere contain >99.9% north-seeking cells, south-seeking cells predominate in the Southern Hemisphere (Blakemore et al., 1980), while approximately equal numbers of cells of both polarities exist at the Equator (Frankel et al., 1981). These observations imply that the vertical component of the geomagnetic field selects the predominant polarity type among magnetotactic bacteria in natural environments, with downward-directed motion advantageous for survival (Frankel et al., 1981). This is particularly useful as a navigational tool, guiding them to preferred chemical and/or redox gradients in the water column or sediment, away from high oxygen concentrations at the surface (Frankel et al., 1981; Blakemore, 1982; Frankel and Bazylinski, 1994; Bazylinski, 1995, 1996).

Magnetotactic bacteria are common in aquatic habitats, their greatest abundance being at the oxic-anoxic boundary (Bazylinski, 1995, 1996). Obligately microaerophilic bacteria, such as *Aquaspirillum magnetotacticum* strain MS-1, use oxygen, nitrate (Bazylinski and Blakemore, 1983) and possibly ferric iron (Blakemore and Frankel, 1989) as terminal electron acceptors, whereas the marine bacterium strain MV-1 uses nitrous oxide (Bazylinski et al., 1988) and the freshwater bacterium strain RS-1 uses sulphate (Sakaguchi et al., 1993) as terminal electron acceptors. The latter findings now extend the habitat of magnetotactic bacteria down into anoxic sediments, precluding the previously held belief that magnetogenesis occurred only in partially oxygenated environments.

The crystallization of magnetite involves the reaction of a hydrous ferric iron precursor (i.e. ferrhydrite) with dissolved Fe$^{2+}$ ions within a localized region of the cell (Frankel et al., 1983), with the aqueous Fe$^{2+}$ arising from either partial reduction of the hydrous ferric iron phase at the solid-state interface or transported directly from the cytoplasm or periplasmic space into the designated crystallizing area (Mann et al., 1984a). The subsequent adsorption of Fe$^{2+}$ ions on the solid ferric iron surface has been suggested as the possible trigger for magnetite formation (Mann et al., 1984b), with the solid-state rearrangement shown as a growing crystal front of magnetite extending into the amorphous iron oxide (Mann et al., 1984a). The presence of hydrous ferric oxide in anaerobically grown cells of MV-1 similarly suggests its role as a precursor to magnetite formation (Bazylinski et al., 1988; Bazylinski, 1990).

Non-magnetotactic bacteria (biologically induced mineralizers) generate magnetite strictly under anaerobic conditions. Dissimilatory Fe(III)-reducers (e.g. *Geobacter metallireducens*; previously designated GS-15) have been shown to produce magnetite in growth culture as a by-product during the oxidation of organic compounds coupled to the reduction of poorly crystalline, Fe(III)-oxide (see Lovley, 1990 for review of magnetite formation and Fredrickson and Gorby, 1996 for review of iron-reduction). In wet mounts *Geobacter* did not orient itself in response to an applied magnetic field (Lovley et al., 1987). Because magnetite formation is an end-product of an energy-generating metabolism, on a per-cell basis, *Geobacter* typically generates 5000 times more magnetite than a magnetotactic bacterium (Frankel,
1987), with the amount of magnetite produced primarily limited by the amount of available Fe(III)-oxide (Lovley, 1990).

The morphology and size of the biologically induced magnetite is quite different from those formed under controlled conditions (Fig. 8). The magnetite is extracellular, there is no evidence of cellular material associated with it, and the crystals typically consist of a mixture of round and oval particles that range in size from 10 to 50 nm (Lovley et al., 1987); most are found at the lower end of this size range (Sparks et al., 1990). Although the Fe(II) produced during iron reduction non-enzymatically (i.e. inorganically) reacts with some of the remaining solid Fe(III) to precipitate magnetite, the metabolism of the Fe(III)-reducing bacteria may contribute more than just Fe(II) to magnetogenesis (Lovley, 1990). Magnetite formation is favoured by high pH; a condition met during Fe(III)-reduction (reaction 2):

\[
\text{CH}_3\text{COO}^- + 8\text{Fe(OH)}_3 \rightarrow 8\text{Fe}^{2+} + 2\text{HCO}_3^- + 15\text{OH}^- + 5\text{H}_2\text{O}
\] (2)

Therefore, the appropriate combination of a high Fe(II)-concentration and high pH at the contact of the Fe(III)-solid provides an ideal interface for secondary magnetite formation (Lovley, 1990).

The characteristic properties of both intracellular and extracellular magnetite are often clearly recognizible in both recent and ancient sedimentary environments. Extant magnetotactic bacteria have been recovered from freshwater swamps and ponds (Frankel et al., 1979; Matsuda et al., 1983), river sediment (S. Mann et al., 1987), anoxic freshwater sediment (Sakaguchi et al., 1993), soils (Fassbinder et al., 1990), marine salt marshes and estuaries (Blakemore, 1975; Bazylinski et al., 1988; Heywood et al., 1990; Mann et al., 1990), marine water column (Bazylinski et al., 1993, 1995), marine sediments (Frankel et al., 1981; Chang and Kirschvink, 1985; Stolz et al., 1986; Karlin et al., 1987) and hypersaline stromatolites (Stolz et al., 1987), while fossil magnetotactic bacteria have been found in deep-sea sediment (Kirschvink and Chang, 1984; Petersen et al., 1986) and Precambrian stromatolites (Chang et al., 1989). Magnetofossils extracted from the 2000 Ma old Gunflint Iron Formation represent the oldest evidence of controlled biomineralization (Chang et al., 1989). In modern anoxic marine and freshwater sediments, much of the magnetite has morphologies similar to that produced during Fe(III)-reduction (Lovley et al., 1987; Lovley, 1990), while \(^{13}\)C analyses suggested that the extensive deposits of magnetite in some Precambrian banded iron formations (BIFs) may be the result of the organic carbon oxidation coupled to Fe(III)-reduction (Perry et al., 1973; Walker, 1984; Baur et al., 1985). Fur-

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Fig. 8. (A) Micrograph of extracellular magnetite precipitates. (B) Cells of Geobacter metallireducens (GS-15) with extracellular magnetite particles. Scale bars = 1 \(\mu\)m. Reprinted with permission of the author and Nature, Macmillan Magazines Ltd. © 1997 (Lovley et al., 1987).
thermore, extracellular magnetite has been found associated with samples of solid bitumen (McCabe et al., 1987) and around hydrocarbon seeps in overlying rock (Donovan et al., 1978). The reduction of Fe(III) in the presence of hydrocarbons has been attributed to microbial biodegradation (Elmore et al., 1987), and the formation of biogenic magnetite is supported by findings of magnetite accumulation during toluene oxidation coupled to Fe(III)-reduction by *Geobacter metallireducens* (Lovley and Lonergan, 1990).

### 2.4. Silicates

Examination of hot spring sediments from Yellowstone National Park, USA (Ferris et al., 1986) and from microbial mats growing in hot spring outflow channels, Krisuvik, Iceland (Konhauser and Ferris, 1996) have revealed bacterial cells completely encrusted with granular and spheroidal crystallites. In the Yellowstone samples, both intact and lysed cells were mineralized with grains typically smaller than 100 nm (Ferris et al., 1986). In one cell that appeared to have undergone an extended episode of iron–silica growth and compaction, an interlocking mosaic was produced that completely encrusted the microorganism. In Krisuvik, some bacterial cells had spheroidal grains (~200 nm) embedded in dense Fe-rich capsular material. In other cells from the same sample site, larger amorphous precipitates (~500 nm), consisting of iron and silica in approximately equal proportions, were evident directly on the cell wall (Fig. 9). These crystallites presumably formed when iron was initially bound to anionic cellular sites, after which dissolved silica (found in saturated conditions with respect to solid amorphous silica after cooling of the hydrothermal waters) was added to the growing mineral via hydrogen bonding between hydroxyl groups.

Iron–silica deposits have also been identified from both active and inactive deep-sea hydrothermal sites. One active site at the Philosopher Vent on Explorer Ridge in the northwest Pacific Ocean, where the hydrothermal fluids were enriched in iron and silica over ambient seawater (Tunnicliffe et al., 1986), had iron–silica deposits with a filamentous morphology (Juniper and Fouquet, 1988). The resemblance of these filaments to those produced by microorganisms, together with the presence of organic carbon.

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Fig. 9. TEM image of bacterial cell (stained) from Krisuvik, Iceland with (i) siliceous spheres embedded within dense, iron-rich capsule and (ii) amorphous iron–silicate grains (arrow). Scale bar = 920 nm. Reprinted with permission of Geology, the Geological Society of America, Boulder, Colorado, USA 5 21286 (Konhauser and Ferris, 1996).
(1.3%) and filamentous bacteria (from one vent sample) suggested that microorganisms may have provided substrata for mineral nucleation. Transmitted light microscopy of these iron-silica deposits further identified intense iron accumulation on the filaments, upon which silica appears to have precipitated. Extensive accumulation of microbial-mineral flocs (dominated by iron and silica oxides) was also observed in association with abundant diffuse venting in new lava flows, and around less extensive new venting on older basalts in the CoAxial Segment of the Juan de Fuca Ridge (Juniper et al., 1995). The formation of hydrothermal nontronite is similarly thought to occur by sorption of silica onto Fe-oxhydroxides (Köhler et al., 1994). The presence of filaments (resembling Leptothrix spp.) in clay deposits of seamounts in the East Pacific Rise (Alt, 1988), and a distinct microtube-like morphology of nontronite from white smoker chimneys in the Galapagos Rift and Mariana Trough (Köhler et al., 1994) implied that bacteria were directly involved in catalyzing mineral formation.

In addition to the hydrothermal deposits, in the past decade, a number of studies on bacterial communities have shown the presence of clay-like material commonly associated with freshwater biofilms. In the neutral pH waters of the Rio Solimões, Brazil (Konhauser et al., 1993), the alkaline, carbonate-saturated waters of the Speed River, Ontario, Canada (Konhauser et al., 1994a) and the Brahman River, Orissa, India (Konhauser et al., 1998), and metal-contaminated lake sediment in northern Ontario (Ferris et al., 1987), all bacterial populations, regardless of physiology, substrate type (i.e. sediment, plants, different rock types), and aqueous composition, consistently formed complex (Fe, Al)-silicates with variable composition and morphology (Fig. 10). These grains have several characteristic properties indicative of an authigenic origin. First, they were amorphous structures with sizes < 1 μm, although the majority of grains were < 100 nm. Second, the grains around lightly encrusted cells were commonly attached in a tangential orientation, while heavily encrusted cells had grains in a more random orientation. Third, the grains also had a composition dominated by iron, silicon and aluminum (in varying amounts) that differed both chemically and mineralogically from the materials carried in suspension.

Fig. 10. TEM image of a partially encrusted bacterial cell (stained), scraped off submerged freshwater algae in the Rio Solimões, Brazil. Several (Fe, Al)-silicate clay morphologies are precipitated on outer cell wall, including amorphous phases (a) and crystalline phases (c). Scale bar = 400 nm.
With the exception of potassium, no other metals were detected in association with the clay-like phases. Based on ternary plots of Fe, Si, and Al (on an atomic % basis) from all riverine samples analyzed (e.g. Fig. 11), most amorphous grains were similar in composition to chamosite [(Fe)$_{3}$,(Si$_{3}$Al)O$_{10}$(OH)$_{2}$], while other grains had compositions ranging from glauconite [K(Al$_{0.38}$Fe$_{1.28}$Mg$_{0.34}$)(Si$_{3.7}$Al$_{0.3}$)O$_{10}$(OH)$_{2}$] to muscovite [(Al)$_{2}$(Si$_{3}$Al)O$_{10}$(OH)$_{2}$·K] and illite [(Al)$_{2}$(Si$_{4-x}$Al$_{x}$)O$_{10}$(OH)$_{2}$·K] to those approaching the composition of the most weathered clay, kaolin [Al$_{2}$Si$_{2}$O$_{5}$(OH)$_{2}$].

The formation of these clayey materials appears to follow a sequence of reactions beginning with the electrostatic adsorption of cationic iron species to cellular material (Konhauser et al., 1993, 1994a, 1998). In some samples, bacterial cells initially showed the nucleation of small (~100 nm in diameter), dense, Fe-rich aggregates on the outer surfaces of the capsule. These nuclei presumably formed when ferric iron, which exhibits unstable aqueous chemical phases, was bound in sufficient amounts to precipitate as an insoluble hydroxide (Ferris et al., 1989a,b). Progressive mineralization, leading to the partial and complete encrustation of some bacterial cells, was also observed. These more ‘developed’

![Fig. 11. Distribution of Fe, Al and Si (on an atomic % basis) from epilithic bacterial cells collected from the Speed River, Canada. Amorphous (closed circle) and crystalline grains (open circle) are compared with several ideal clay minerals, including chamosite (slashed circle), kaolin and muscovite (closed star), illite (long rectangle to represent variable compositions) and glauconite (closed square). Taken after Konhauser et al. (1994b).](image-url)
Experimental studies using the bacterium *Bacillus subtilis* have also demonstrated the ability of bacteria to nucleate fine-grained, amorphous (Fe, Al)-silicates (Urrutia and Beveridge, 1994, 1995). Fe-pretreatment of bacterial cells resulted in Al adsorption onto the ferric hydroxide surface and it further enhanced silicate binding at pH 8.0 (Urrutia and Beveridge, 1994). This occurred presumably through a cationic bridging mechanism, whereby anionic silicate ions reacted with bound metal cations (Urrutia and Beveridge, 1993a). Growth of the precipitates then continued until complex structures were formed. As (Fe, Al)-silicates developed on bacteria, not only were several metals incorporated within the mineral fabric, including Pb, Cd, Zn, Ni and Cu (Urrutia and Beveridge, 1994), but these biominerals also retained the metals much more strongly than those bound directly to cell polymers (Urrutia and Beveridge, 1993b, 1994) and their inorganically formed counterparts (e.g. smectite) (Flemming et al., 1990).

2.5. Phosphates

The formation of phosphate minerals has frequently been observed in both ancient and modern sedimentary environments under high biological productivity. The organic matter not only serves as a source of phosphate to sediment pore waters through bacterial degradation (Gulbrandsen, 1969), but it also provides a substrate upon which phosphate preferentially nucleates (Southgate, 1986). For example, Briggs et al. (1997) have shown the mineralization of dinosaur tissue by phosphatized microbial mats that overgrew the carcass. In a study of Cambrian phosphorite profiles from Australia, Southgate (1986) observed phosphate cements (with goethite rims) occurring as irregular coatings on chamosolithic and epiphitic cyanobacterial filaments. Similarly, Riggs (1979) noted the association of bacteria with phosphate grains of Miocene deposits in Florida, while O'Brien et al. (1981) proposed that Eastern Australian continental margin phosphorites (some phosphatic nodules have up to 43.12% Fe₂O₃) originated through the slow bacterial assimilation of phosphorus from seawater. This is not surprising since phosphorus is an essential nutrient for bacteria (Fenchel and Blackburn, 1979), and organic phosphorus has been shown to be a major source of phosphorus for the production of phosphate minerals (Beveridge et al., 1983). In other studies, Jensen et al. (1992) and Jensen and Thamdrup (1993) described how the availability of iron hydroxide surfaces significantly decreased the concentration of dissolved phosphate in oxidized lake and marine sediments, respectively.

Direct evidence of bacterial iron phosphate formation comes from analysis of dark-coloured biofilms that grow on exposed rock outcrops on Ellesmere Island, in the Canadian Arctic (Konhauser et al., 1994b). TEM analysis of these epilithic biofilms, consisting of cyanobacteria and fungi symbiotically associated in a lichen, together with a consortium of free-living bacteria and algae, indicated that the microbial community was highly mineralized. The precipitates ranged from relatively large polyphosphate granules (approximately 250 nm in diameter) within their cytoplasmic membranes to smaller iron phosphate grains (generally less than 50 nm in diameter) associated with the periplasmic space and encompassing capsule (Fig. 12). Some cells were completely encrusted by the mineral grains. In EDS spectra, these aggregates exhibited Fe:P ratios compositionally similar to those of the mineral strengite [FePO₄ · 2H₂O], the expected stable solid phase predicted under the low pH conditions created by the production of organic acids in the biofilms (Stumm and Morgan, 1981). The formation of the iron phosphate involved a series of independent steps beginning with either the dissolution of the underlying rock (Fenchel and Blackburn, 1979) or release of cellularly bound phosphorus from degrading cells (Gächter and Meyer, 1993), transport of the ions along the rock surface in a continuous liquid-phase water film (Ugolini and Grier, 1969), and the concentration and immobilization of metals (e.g. iron) within the biofilm. Subsequent reactions between dissolved phosphate with the bound iron might be expected given the large surface area and high adsorptive affinity of ferricydrite for phosphate (Parfitt et al., 1975).

The ferrous phosphate, vivianite [Fe₆(PO₄)₃] has been detected in culture studies with magnetotactic bacteria. The needle-like, extracellular precipitates were formed as by-products after the metabolic release of ferrous iron into a phosphate-rich medium (Blakemore and Frankel, 1989). Similarly, when
Fe(III) was provided in a soluble form of Fe(III)-citrate to cells of *Geobacter metallireducens*, a white precipitate of vivianite was formed within 1 day after the completion of Fe(III)-reduction (Lovley and Phillips, 1988). Based on strong Fe and P peaks in EDS, stalks of *Gallionella* spp. grown in cultures
with excess $\text{PO}_4^{3-}$ may also contain vivianite (W.C. Ghiorse, pers. commun., 1997).

2.6. Carbonates

Roden and Lovley (1993) have recently shown the ability of some bacteria (in culture) to enzymatically reduce $\text{Fe}^{3+}$ directly to siderite ($\text{FeCO}_3$). When cells of *Desulfuromonas acetoxidans* were grown in a bicarbonate-rich media, less than 3% of the $\text{Fe(II)}$ produced from $\text{Fe(III)}$-reduction was present in solution, indicating that most of the $\text{Fe(II)}$ formed insoluble ferrous minerals. These findings correlate well with the production of fine-grained and rhombohedral siderite crystals (Fig. 13) by *Geobacter metallireducens* (Sparks et al., 1990; Mortimer and Coleman, 1997; Mortimer et al., 1997), in that the release of ferrous iron from bacterial $\text{Fe(III)}$-reduction, into a solution with excess bicarbonate, cause precipitation of siderite instead of magnetite.

The formation of siderite in marine sediments has previously been ascribed to the degradation of organic material by methanogenic bacteria in organic-rich marine mud (Gautier, 1982) and to $\text{Fe(III)}$-reduction in anoxic deep-sea sediment (Ellwood et al., 1988), hydrothermal sediments (Holm, 1987, 1989) and salt-marsh sediments (Coleman et al., 1993). In the latter study, the enrichment of *Desulfovibrio desulfuricans* within siderite concretions was a novel finding since these sulphate-reducing bacteria (SRBs) reduced $\text{Fe(III)}$ directly (reaction 3) instead of reducing $\text{Fe(III)}$ indirectly through the production of dissolved sulphide. The preference for $\text{Fe(III)}$ as an electron acceptor over sulphate may stem from the inability of *Desulfovibrio* to metabolize $\text{H}_2$ (the bacterium’s most important electron donor) with sulphate as an electron acceptor under the low concentrations of $\text{H}_2$ found in some aquatic sediments (Coleman et al., 1993). This is in agreement with other studies which have shown that microorganisms that use $\text{Fe(III)}$ as an electron acceptor for $\text{H}_2$ oxidation can metabolize $\text{H}_2$ concentrations lower than those that can be utilized by sulphate reducers in aquatic sediments and groundwater (see Lovley and Phillips, 1987; Lovley and Goodwin, 1988; Lovley et al., 1994; Lovley and Chapelle, 1995 for review). Therefore, the ability of *Desulfovibrio* to alternate electron acceptors and produce ferrous iron and hy-

![Fig. 13. Scanning electron micrograph showing typical rhombohedral siderite precipitated in cultures of *Geobacter metallireducens*. Reprinted with permission of the author and Geochimica et Cosmochimica Acta. Elsevier Sciences Ltd., Kidlington, UK (Mortimer and Coleman, 1997).](image-url)
droxyl ions, with seawater bicarbonate, resulted in siderite formation (reaction 4):

\[ \text{Fe}_3\text{O}_4 + \text{H}_2 + \text{H}_2\text{O} \rightarrow 2\text{Fe}^{2+} + 4\text{OH}^- \]  
(3)

\[ \text{Fe}^{2+} + \text{HCO}_3^- + \text{OH}^- \rightarrow \text{FeCO}_3 + \text{H}_2\text{O} \]  
(4)

Siderite is similarly precipitated in both freshwater mud and deeply buried marine sediments lacking sufficient dissolved sulphate to form sulphide minerals (Curtis et al., 1986).

Biofilm samples analyzed from an underground rock laboratory in Manitoba, Canada also showed the presence of siderite and hematite associated with bacterial cells (Brown et al., 1994; Sawicki et al., 1995). The bacterial communities were presumably capable of mediating both oxidation and reduction of iron in close proximity, and in the case of siderite formation, the Fe$^{2+}$ was probably bound to the anionic cellular surfaces where it reacted with bicarbonate derived from the fermentation of organic matter (Brown et al., 1994; Sawicki et al., 1995).

2.7. Sulphates

Ferric hydroxysulphate \([\text{Fe}_{10}\text{O}_{16}\text{(OH)}_{12}(\text{SO}_4)_2]\), schwertmannite \([\text{Fe}_8\text{O}_8(\text{OH})_8\text{SO}_4]\) and jarosite \([\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6]\) frequently occur as ochreous surface precipitates on stream beds receiving iron and sulphate-rich, acid mine drainage (AMD) (Ivarson, 1973; Lazaroff et al., 1982, 1985; Norstrom, 1982; Brady et al., 1986; Wichlacz and Unz, 1986; Bigham et al., 1990, 1994, 1996; Clarke et al., 1997). Generation of AMD results from the exposure and rapid oxidation of sulphide minerals associated with disused coal and metal mines. The general processes are outlined below (Kleinmann et al., 1981):

\[ \text{FeS}_2 + 3.5\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + 2\text{H}^+ + 2\text{SO}_4^{2-} \]  
(5)

\[ \text{Fe}^{2+} + 0.25\text{O}_2 + \text{H}^+ \rightarrow \text{Fe}^{3+} + 0.5\text{H}_2\text{O} \]  
(6)

\[ \text{FeS}_2 + 14\text{Fe}^{3+} + 8\text{H}_2\text{O} \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+ \]  
(7)

\[ \text{Fe}^{2+} + 2.5\text{H}_2\text{O} + 0.25\text{O}_2 \rightarrow \text{Fe(OH)}_3 + 2\text{H}^+ \]  
(8)

The first three reactions describe the initial abiotic or biotic oxidation of solid-phase sulphide (reaction 5), the subsequent microbial oxidation of Fe$^{2+}$ under acidic conditions by *Thiobacillus ferrooxidans* (reaction 6), and the accelerated oxidation of metal sulphides by dissolved Fe$^{3+}$ (reaction 7). When AMD comes in contact with fresh water at an off-site location, the oxidation and hydrolysis of Fe$^{2+}$ results in a voluminous yellow precipitate, characterized by its high reactivity in scavenging other ions from the effluent (Chapman et al., 1983). At low pH, ferric hydroxysulphate, schwertmannite and jarosite precipitate through anion bridging of ferric iron colloids (Parfitt and Smart, 1978; Lazaroff et al., 1982; Brady et al., 1986; Bigham et al., 1990, 1994, 1996), as predicted considering the high reactivity of ferrhydrate for counter-ions (Schwertmann and Fitzpatrick, 1992) and the high concentration of dissolved sulphate in the AMD sediment pore waters. At higher alkalinity, in the absence of appreciable sulphate (reaction 8), the neutralizing effects of relatively unpolluted stream water results in ferric hydroxide and goethite precipitation (Carlson and Schwertmann, 1980, 1981; Brady et al., 1986; Bigham et al., 1996).

Although bacteria are directly involved in the oxidation of sulphidic minerals and the generation of AMD, their involvement in the subsequent precipitation of amorphous iron and sulphur phases is less clear. In an experimental study with isolates of *Thiobacillus ferrooxidans*, Ivarson (1973) observed that bacterial oxidation of ferrous sulphate (in a liquid medium) accounted for the formation of jarosite, while growth on a solid medium of agar yielded ammoniojarosite \([\text{NH}_4\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6]\). Ivarson et al. (1979) further recognized a relationship between the widespread occurrence of jarosite in nature with the chemical activity of *Thiobacillus ferrooxidans*. These findings, in addition to further experimental study, prompted Lazaroff et al. (1982) to suggest that bacterial processes somehow catalyzed the production of the soluble precursors to ferric hydroxysulphates or jarosite by lowering the activation energy barriers to their formation. This work was followed by a mineralogical characterization of the solid residue generated during the microbiological treatment of AMD (Wichlacz and Unz, 1986). The authors observed bacteria on surfaces of the precipitated by-products, consisting of ferric hydroxysulphates, jarosite and magnetite, which formed during microbiological oxidation of ferrous iron in both natural and synthetic acid mine drainage effluent.
In West Glamorgan, Wales, analyses of sediment samples, collected from various depths (surface, 15 cm, 30 cm) in a coal mine drainage lagoon, indicated an abundance of mineralized bacterial cells (Clarke et al., 1997). In the surface samples, most cells appeared to have mineralized capsules, with the magnitude of capsule material varying from individual cells to the encapsulation of microcolonies. EDS analyses indicated that the exopolymers sequestered significant amounts of Fe, as well as detectable quantities of Zn, Ti, Mn and K. In the subsurface, a greater proportion of bacterial cells had granular, fine-grained Fe–S precipitates directly associated with them (Fig. 14A). These grains were similar in morphology to ferrihydrite prepared experimentally in the presence of a 250–1000 μg/ml dissolved sulphate solution (Brady et al., 1986). At greater depths (i.e. 30 cm) some cells were observed encrusted in a dense mineralized matrix in which individual precipitates appear to have coalesced (Fig. 14B). The Fe:S atomic weight ratio decreased from 3.5:1 at 15 cm to 1.9:1 at 30 cm, further suggesting the continued reactivity of the ferric iron phase to dissolved sulphate ions (i.e. more sulphate reacts with previously formed biomineral with depth).

2.8. Sulphides

In natural, anoxic environments, dissimilatory sulphate reduction is the major process by which dissolved sulphide is formed. This process is carried out by anaerobic bacteria that generate the energy required for growth by coupling the oxidation of simple organic molecules to the reduction of sulphate (Berner, 1978; Lovley and Goodwin, 1988). The sulphide generated subsequently reacts with solid-phase iron oxides or hydroxides within the sediment, locally precipitating iron monosulphide phases (mackinawite and amorphous FeS) and forming elemental sulphur (or other partially oxidized sulphur phases).
at the iron source (Canfield and Raiswell, 1991). These partially oxidized sulphur phases (or H₂S) then become the oxidants required to eventually convert FeS to pyrite (Berner, 1970), possibly with greigite (Fe₃S₄) as an intermediate step (Canfield and Raiswell, 1991). The direct precipitation of pyrite is unfavourable since the rate of pyrite nucleation is slow compared to pyrite formation via an FeS precursor (Schoonen and Barnes, 1991). Although most ferric iron is reduced by the reaction with dissolved sulphide, in some microenvironments, soluble ferrous iron is produced by Fe(III)-reducing bacteria, where it freely migrates into the zone of sulphate reduction to react with pore-water sulphide at the site of organic decay (Canfield, 1989).

In addition to being primarily responsible for the production of dissolved pore-water sulphide, bacteria can also serve as templates for mineral sulphide precipitation. In a metal-contaminated lake sediment in Sudbury, Ontario, Ferris et al. (1987) observed the formation of iron sulphides (mackinawite) directly on the outer surfaces of bacterial cells and their membranous debris. Most of the metal sulphides appeared as dense aggregates which ranged from 10 to 50 nm in diameter. Experimental evidence suggests that metals chemically complexed to bacteria are more reactive towards dissolved sulphide (Mohagheghi et al., 1984), and it is likely that the mackinawite in the Sudbury study formed to dissolved sulphide reacting with cellulary bound iron. Because there was less than 0.75 wt.% organic carbon associated with the lake sediment, the intensity of sulphate reduction was limited, and therefore, the lack of dissolved sulphide allowed for the persistence of the poorly crystalline, sulphide phase instead of forming pyrite (Ferris et al., 1987). Lauritzen and Bottrell (1994) observed μm-sized spherules of presumed bacterial origin coated with Fe–S minerals in sulphidic, thermal groundwater in Spitzbergen; their sulphur and carbon isotope studies showed that SRBs were active in these deep waters. More recently, Fortin and Beveridge (1997) observed with TEM the presence of intact remains of sulphate-reducing bacteria encrusted with iron sulphide minerals, possibly mackinawite and pyrite (SAED indicated poorly ordered mineral forms, precluding positive identification) from anoxic sediments within a sulphidic mine tailings impoundment, Kidd Creek, in Timmins, Ontario. Similar minerals were formed when a mixture of SRBs, isolated from the tailings, were grown in the laboratory under iron- and sulphate-rich conditions (Fortin and Beveridge, 1997). These findings correlate well with an ongoing study of marine sediment collected from the Humber Estuary, England, where extracellular iron monosulphide and pyrite (Fig. 15) were associated with bacterial cell surfaces at depths greater than 5 cm (Konhauser et al., 1996). The interesting feature in this work is that bacterial cells themselves may provide much of the reactive iron essential for sulphide formation, since the surface marine bacteria commonly had ferrihydrite deposits associated with them. The pyritization of organic material has also been documented in ancient sediments (Stürmer, 1985; Conway Morris, 1986; Underwood and Bottrell, 1994). Commonly the preservation of very degradable soft, organic parts can occur as a pyritized layer of bacteria which pseudomorphed the original structure (Canfield and Raiswell, 1991).

Fig. 15. TEM image of iron monosulphide and pyrite (arrow) on the capsular material of a lysed bacterial cell collected from 15 cm depth of sediment in the Humber Estuary, England. Magnified 73,000 times.
Bacterial sulphate reduction is likely not to be an important process in the formation of hydrothermal massive sulphides because these minerals are precipitated from solutions containing high concentrations of geothermally generated H₂S (Juniper and Tebo, 1995). However, it is possible for hydrothermal solutions containing Fe²⁺ and SO₄²⁻ to be fixed as pyrite by the action of sulphate-reducing bacteria (Bottrell and Morton, 1992). Whatever the sulphide source, bacteria are implicated in the formation of some hydrothermal metal sulphides by acting as sites for adsorption of metal ions, by providing a locally reducing environment that favours precipitation of some metals, and by providing a source of elemental sulphur for metal sulphide precipitation (Jonasson and Walker, 1987).

The formation of greigite (Fe₃S₄) proceeds by the controlled intracellular mineralization of magnetotactic bacteria (Bazylinski et al., 1993). Individual greigite particles appear to be membrane-bound, organized into chains (Fig. 16), and ferromagnetically ordered (Bazylinski et al., 1994), providing the bacteria with properties similar to magnetite-producing bacteria, although greigite is one-third as magnetic (Mann et al., 1990). Morphologies of greigite include cuboidal and rectangular prismatic crystals in the size range of 35 to 120 nm (Heywood et al., 1990; Bazylinski, 1995, 1996). In one magnetotactic bacterium pyrite crystals were observed along with greigite (Mann et al., 1990). Although pyrite may simply represent the slow transformation of greigite into a more stable phase under strongly reducing conditions at neutral pH, the lengthy conversion time seems to preclude this process during the cell's lifetime (Heywood et al., 1990; Bazylinski, 1995, 1996). Instead, greigite and pyrite may be biomineralized separately, indicating that the stoichiometry of the metal (Fe) and non-metal (S) can vary in some magnetotactic bacteria, resulting in different mineral assemblages (Bazylinski, 1995).

While magnetite-producing bacteria prefer the oxic zone, the greigite producers seem to prefer to be below the oxic–anoxic boundary where the hydrogen sulphide concentration is high, thus behaving as anaerobes (Bazylinski et al., 1990). Interestingly, one bacterium as described by Bazylinski et al. (1995) produced more magnetite in the oxic zone and greigite in the anoxic zone, further implying that local

Fig. 16. TEM image of a chain of cubo-octahedral greigite crystals within an unidentified, uncultured, rod-shaped bacterium collected from a sulphidic salt-marsh pool. Scale bar = 200 nm. Reprinted with permission of the author and ASM News, the American Society for Microbiology (Bazylinski, 1995).
oxygen and/or hydrogen sulphide concentrations regulated biomineralization in this microorganism.

3. Conclusions

From the preceding discussion it becomes apparent that bacterial biomineralization is no trivial affair. Because of their activity, either directly through metal adsorption and mineral precipitation onto their surfaces, or indirectly through the metabolic wastes they generate, bacteria shape and modify their immediate surroundings. Certainly in terms of the variety and quantity of iron minerals produced, bacteria also mediate geochemical cycles, from microenvironments to global scales. Several examples of this exist in both modern and ancient sedimentary environments. The ability of freshwater bacterial populations to bind, immobilize and retail Fe, Al, Si and other trace metals from solution has important implications for their transfer from the hydrosphere to the bottom sediment (Konhauser et al., 1993, 1994a, 1998). If only the microbe–water interface is considered (and not the planktonic populations), the large surface area of solid substratum on a river bed that is colonized by biofilms means that a substantial volume of water comes directly under microbial contact. It is thus not difficult to imagine how bacteria could effectively partition metals from solution into the sediment (Beveridge and Fyfe, 1985). The precipitation of ferrhydrite and ferric hydroxysulphate by AMD bacteria may aid in the remediation of acidic, mine effluent through incorporation of trace metals into their mineralized matrices (Chapman et al., 1983) and their potential resistance to microbial degradation (Clarke et al., 1997). Also, as a result of bacterial iron phosphate biomineralization in the Arctic, nutritional requirements of the microorganisms were met through a relatively closed recycling mechanism which maintained phosphorus within the biofilm, and thereby allowed a thriving microbial population to survive in an environment of low nutrient availability. The activity of primitive bacteria may even have played a significant role in the origin of banded iron formations (BIFs), the most abundant type of chemical sediment precipitated in the Early to Middle Precambrian (Klein and Beukes, 1992; Beukes and Klein, 1992). Based on observations of ferric hydroxide formation at modern hydrothermal sites (Konhauser and Ferris, 1996), it has been suggested that either direct or indirect bacterial oxidation of ferrous iron at the chemocline of a stratified Precambrian ocean may have been the means for mineral precipitation (Konhauser, 1998). The significance of iron biomineralization becomes self-evident when one takes into account that 90% of all iron mined today is derived from BIFs (Isley, 1995).

While the influence of bacteria in geochemistry is clear, one of the most important issues that remains unanswered is what benefit, if any, do microorganisms derive from the predominantly passive biomineralization processes? It has been established that bacteria have the ability to partially control their surface charge (Doyle, 1989), and therefore, should be able to inhibit biominerals from forming on their cellular surfaces, particularly in most aqueous environments with low metal concentration. The mere presence, therefore, of biominerals implies that biomineralization must either be advantageous or that the environmental conditions exceed the microorganism’s ability to compensate. While some Fe-hydroxides may concentrate needed trace metals and anions, or it may protect cells from O₂ toxicity (Ghiorse, 1984), what is the possible explanation, for example, of the (Fe, Al)-silicates? Is it the increased surface area for metal adsorption (Stotzky and Rem, 1966), or alternatively, a form of protection against metal toxicity (W.C. Ghiorse, pers. commun., 1997), a mechanism to retain sufficient water to safeguard some bacteria from excessive desiccation (Bushby and Marshall, 1977), or a mechanism to protect themselves from the detrimental effects of toxin-producing microorganisms (Habte and Barrion, 1984) and predation by grazing protozoans (Heynen et al., 1988)? Finding the answer to these questions will undoubtedly provide an invaluable insight into the way bacteria influence low-temperature geochemistry.

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