

Geomicrobiological Cycling of Iron

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INTRODUCTION

Iron is the most abundant element on Earth and the most frequently utilized transition metal in the biosphere. It is a component of many cellular compounds and is involved in numerous physiological functions. Hence, iron is an essential micronutrient for all eukaryotes and the majority of prokaryotes. Prokaryotes that need iron for biosynthesis require micromolar concentrations, levels that are often not available in neutral pH oxic environments. Therefore, prokaryotes have evolved specific acquisition molecules, called siderophores, to increase iron bioavailability. Acquisition of iron by siderophores is a complex process and is discussed in detail by Kraemer et al. (2005).

Here we focus on prokaryotes that generate energy for growth by oxidation or reduction of iron. In both processes single electron transfers are involved. Hence, for a significant extent of energy generation, turnover of iron in the millimolar rather than the micromolar range is necessary. Iron metabolizing organisms have therefore a strong influence on iron cycling in the environment. Microbial iron oxidation and reduction will be discussed, with emphasis on circumneutral pH environments that prevail on Earth. The active metabolic processes outlined above have to be distinguished from indirect biologically induced iron mineral formation in which prokaryotic cell surfaces simply act as passive templates ("passive iron biomineralization") (e.g., Konhauser 1997).

General aspects of the iron cycle

On our planet, iron is ubiquitous in the hydrosphere, lithosphere, biosphere and atmosphere, either as particulate ferric [Fe(III)] or ferrous [Fe(II)] iron-bearing minerals or as dissolved ions. Redox transformations of iron, as well as dissolution and precipitation and thus mobilization and redistribution, are caused by chemical and to a significant extent by microbial processes (Fig. 1). Microorganisms catalyze the oxidation of Fe(II) under oxic or anoxic conditions as well as the reduction of Fe(III) in anoxic habitats. Microbially influenced transformations of iron are often much faster than the respective chemical reactions. They take place in most soils and sediments, both in freshwater and marine environments, and play an important role in other (bio)geochemical cycles, in particular in the carbon cycle. Microbial iron cycling impacts the fate of both organic and inorganic pollutants, including those released from industrial and mining areas (Thamdrup 2000; Straub et al. 2001; Cornell and Schwertmann 2003).

Solubility and chemical transformation of Fe(II) and Fe(III) minerals

Different Fe(II), Fe(III) and mixed Fe(II)-Fe(III) minerals are found in the environment and many are used, produced or transformed by microbial activities (Table 1). Fe(III) minerals are characterized by low solubility at circumneutral pH and usually only very low, hardly

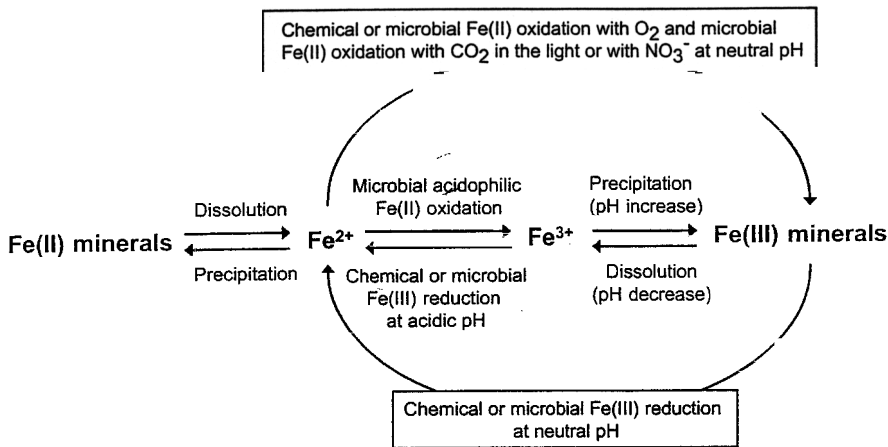


Figure 1. Microbial and chemical iron cycle.

detectable concentrations in the range of 10^{-9} M of Fe(III) are present in solution (Fig. 2). However, colloid formation or complexation by organic compounds can lead to elevated concentrations of dissolved Fe(III), even at neutral pH (Cornell and Schwertmann 2003; Kraemer 2004). At strongly alkaline or strongly acidic pH, ferric iron oxides can be dissolved because of their amphoteric character. Ferric iron oxides can be reduced chemically by a range of organic and inorganic reductants. However, the environmentally most important reducing agent for Fe(III) is hydrogen sulfide, which is a common end product of microbial sulfur and sulfate reduction (Thamdrup 2000; Cornell and Schwertmann 2003).

In contrast to Fe(III) minerals, some ferrous iron minerals, e.g., siderite or ferrous monosulfides, are considerably more soluble at neutral pH. This leads to concentrations of

Table 1. Names and formulas of some important iron minerals.

<i>Fe(III) oxides</i>	<i>Fe(III) oxyhydroxides and hydroxides¹</i>
	Goethite α -FeOOH
	Lepidocrocite γ -FeOOH
	Ferrihydrite ² $\text{Fe}_5\text{HO}_8 \cdot 4\text{H}_2\text{O}$
<i>Fe(II) minerals</i>	<i>Mixed Fe(II)-Fe(III) minerals</i>
	Magnetite Fe_3O_4
	Green rusts $\text{Fe}_x^{\text{III}}\text{Fe}_y^{\text{II}}(\text{OH})_{3x+2y-z}(\text{A}^-)_z$; $\text{A}^- = \text{Cl}^-; \frac{1}{2}\text{SO}_4^{2-}$
	Greigite Fe_3S_4

¹ For simplicity also commonly referred to as iron oxides.

² Ferrihydrite frequently is inadequately assigned as $\text{Fe}(\text{OH})_3$. However, if the identity of a poorly crystalline iron hydroxide is unknown, this formula can be used as approximation.

³ This term embraces a variety of minerals with slightly varying stoichiometries, i.e., $\text{Fe}_x\text{S}_{x \pm 1}$. Only troilite contains iron and sulfur in an exact 1:1 stoichiometry. Troilite rarely occurs on Earth, but is found in iron meteorites and lunar rocks (Lennie and Vaughan 1996).

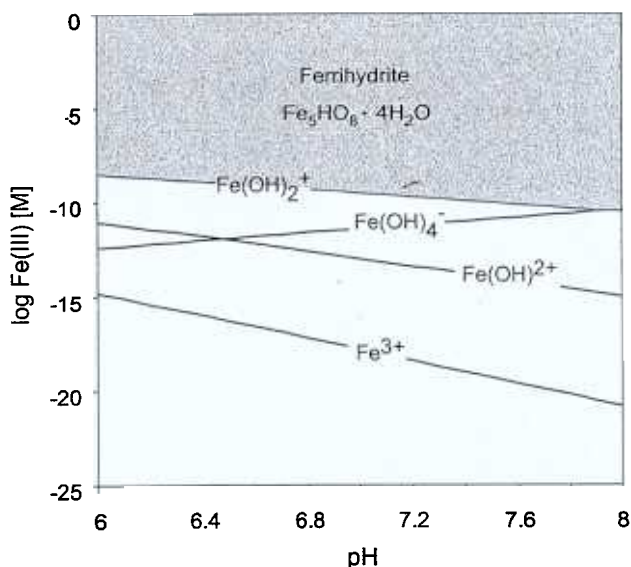


Figure 2. Dominance diagram showing the concentrations of different dissolved Fe(II) species in the presence of ferrihydrite at pH 6-8.

dissolved Fe(II) that can reach the μM range, even in the presence of bicarbonate or sulfide. However, Fe(II) is stable at neutral or alkaline pH only in anoxic environments and is oxidized to Fe(III) minerals by molecular oxygen. At acidic pH, Fe(II) can persist, even in oxic habitats (Stumm and Morgan 1996; Cornell and Schwertmann 2003). Under anoxic conditions, Mn(IV), nitrate, nitrite and nitrous oxide were shown in laboratory studies to oxidize Fe(II) chemically. In anoxic natural habitats, however, Mn(IV) is the only relevant oxidant of Fe(II) (Buresh and Moraghan 1976; Moraghan and Buresh 1977; Myers and Nealson 1988).

Surface area and reactivity of ferric iron oxides

The rates of chemical and microbial transformations of iron minerals depend on the number of available reactive surface sites, e.g., on the number of reactive surface-OH functional groups in case of ferric hydroxides (Roden 2003). The mineral surface area in turn inversely depends on the crystal size of the ferric iron oxides. Different iron minerals and samples of the same iron mineral with different crystal sizes vary significantly in surface area and therefore in stability and reactivity. This influences dissolution kinetics, transformation reactions and adsorption of organic and inorganic compounds. Values for surface areas can be determined experimentally by different methods, although these may produce slightly varying results. Surface areas determined by the Brunauer-Emmett-Teller method (BET) as extent of N_2 -adsorption to an outgassed sample of the respective mineral span from a few m^2/g (e.g., 8–16 m^2/g for highly crystalline goethite) to a few hundreds of m^2/g (e.g., 100–400 m^2/g for poorly crystalline ferrihydrite) (Cornell and Schwertmann 2003).

Ferrihydrite

Ferrihydrite is widespread in many natural environments. It is frequently used in laboratory studies with Fe(III)-reducing microorganisms and was observed as a product in cultures of Fe(II) oxidizers (Fig. 3). Ferrihydrite is a high-surface area iron oxide that consists of nanometer-sized crystals. Although it has been reported to be hexagonal, its structure remains a matter of debate (Mancaeu and Drits 1993; Jambor and Dutriziac 1998; Janney et al. 2000, 2001). It is a material that exhibits considerable disorder, but it is not amorphous (for more details see Gilbert and Banfield 2005). The crystallinity of the different ferrihydrite species depends on the conditions

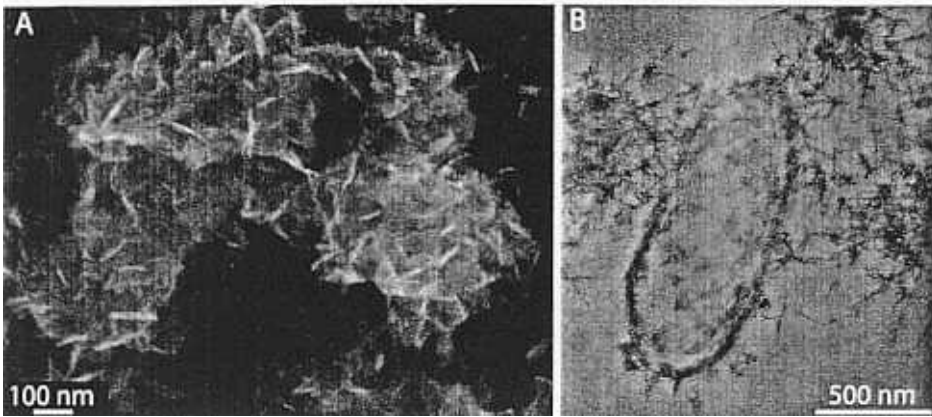


Figure 3. Scanning (A) and transmission (B) electron micrographs of ferrihydrite produced by the anoxygenic phototrophic Fe(II)-oxidizing bacterium '*Rhodobacter ferrooxidans*' strain SW2. Note that particles of the biologically produced ferrihydrite are of nm-size and thus much smaller than microbial cells of typical size.

during synthesis, e.g., formation rate and the presence of organic and inorganic compounds (Cornell and Schwertmann 2003). The small, nanometer-sized crystals of ferrihydrite often aggregate to form colloids with sizes in the μm -range (Fig. 3).

Forms of iron present in the environment

In the environment, iron is rarely present as pure, well crystalline mineral phase but rather is found:

- in association with or covered by natural organic matter (e.g., humic substances, biofilm exopolysaccharides)
- in particles to which anions such as phosphate (PO_4^{3-}) and arsenate (AsO_4^{3-}) or positively charged metal ions (e.g., Fe^{2+} , Cu^{2+} , Mn^{2+}) have adsorbed
- as minerals that are mixed or co-precipitated with other minerals (e.g., clays)
- in minerals in which other cations, e.g., Al, Cr, Mn, partially substitute for iron
- as nano-sized mineral particles or as aggregates of nano-sized particles (colloids)
- complexed (e.g., by organic acids) and thus dissolved.

Such complex natural systems provide a huge variety of microenvironments, and thus microniches, for microorganisms with different physico-chemical requirements. In fact, it is hard, if not impossible, to simulate this complexity in the laboratory. This difficulty might be one explanation for the poor growth of many iron-metabolizing bacteria in the laboratory.

Role of iron for microbial energy metabolism

Different physiological groups of prokaryotes can use iron as a substrate for energy generation (Fig. 1, Table 2). In the following two sections we will discuss such Fe(II)-oxidizing and Fe(III)-reducing microorganisms in more detail, focusing on electron transfer between cells and iron minerals. Intracellular electron transfer in Fe(III)-reducing bacteria via redox active proteins such as cytochromes was recently reviewed by Lovley et al. (2004). The rapid growth in availability of genomic information will significantly improve our understanding of the electron transport chains of iron cycling microorganisms (e.g., Nelson and Methé 2005). The third section focuses on microbial iron cycling catalyzed by the cooperation of these two

Table 2. Physiological groups of prokaryotes that catalyze iron redox transformations.

Habitat	Electron donor	Electron acceptor	pH	Microbial metabolism	Representative strains
Oxic	Fe(II)	O ₂	acidic		<i>Thiobacillus ferrooxidans</i> <i>Sulfobacillus acidophilus</i>
	Fe(II)	O ₂	neutral	Fe(II) oxidation	<i>Gallionella ferruginea</i> <i>Leptothrix ochracea</i>
Anoxic	Fe(II)	NO ₃ ⁻	neutral	NO ₃ ⁻ -dependent Fe(II) oxidation	<i>Acidovorax</i> sp. strain BrG1 <i>Azospira oryzae</i> strain PS
	Fe(II)	CO ₂	neutral	Phototrophic Fe(II) oxidation	<i>Rhodobacter ferrooxidans</i> strain SW2 <i>Rhodovulum iodolum</i>
	Organic or inorganic compounds	Fe(III)	acidic	Fe(III) reduction	<i>Acidiphilium cryptum</i> sp. JF-5 <i>Thiobacillus thiooxidans</i>
	Organic or inorganic compounds	Fe(III)	neutral	Fe(III) reduction	<i>Geobacter metallireducens</i> <i>Shewanella oneidensis</i>

physiological groups. Finally, some environmental implications are described and tasks for future investigations defined.

MICROBIAL OXIDATION OF Fe(II)

Competition between chemical and microbial oxidation of Fe(II)

The chemical oxidation of Fe(II) with oxygen depends mainly on the pH and the concentration of oxygen (Fig. 4). At pH values above 5, the Fe(II) oxidation rate has a first-order dependence on Fe(II) and O₂ concentrations and a second-order dependence on the OH⁻ concentration. Thus, an increase of one pH unit increases the rate of Fe(II) oxidation 100-fold. Therefore in O₂-saturated water at neutral pH, Fe(II) is readily oxidized to Fe(III) with a half-life in the order of several minutes (Stumm and Morgan 1996). Aerobic, neutrophilic Fe(II)-oxidizing microorganisms compete successfully with this fast chemical process. However, some of them thrive only in microoxic niches with low oxygen concentrations and hence a slower chemical oxidation of Fe(II) by oxygen (Emerson 2000). In contrast, under acidic conditions Fe(II) persists for long periods of time, even in the presence of atmospheric O₂ levels.

Under anoxic conditions, only manganese oxides and nitrite have been shown to oxidize freely dissolved Fe(II) chemically (Myers and Nealson 1988; Moraghan and Buresh 1977). However, neither nitrate nor sulfate react chemically with Fe(II) at appreciable rates at low temperature. Therefore, anaerobic Fe(II)-oxidizing bacteria are the most important catalysts/oxidants for the generation of Fe(III) in anoxic habitats.

Aerobic acidophilic Fe(II)-oxidizing microorganisms

Due to the stability of ferrous iron at acidic pH even in the presence of O₂, aerobic acidophilic Fe(II)-oxidizing microorganisms can readily compete with chemical oxidation. However, at acidic pH the redox couple Fe³⁺/Fe²⁺ relevant for the redox reaction catalyzed by these bacteria has a redox potential of +770 mV. Therefore, at pH 2 only ~33 kJ/mol iron is produced during the oxidation with O₂, since the relevant redox potential of the redox couple O₂/H₂O is +1106 mV. This difference is just big enough for the synthesis of 1 mol ATP. Under

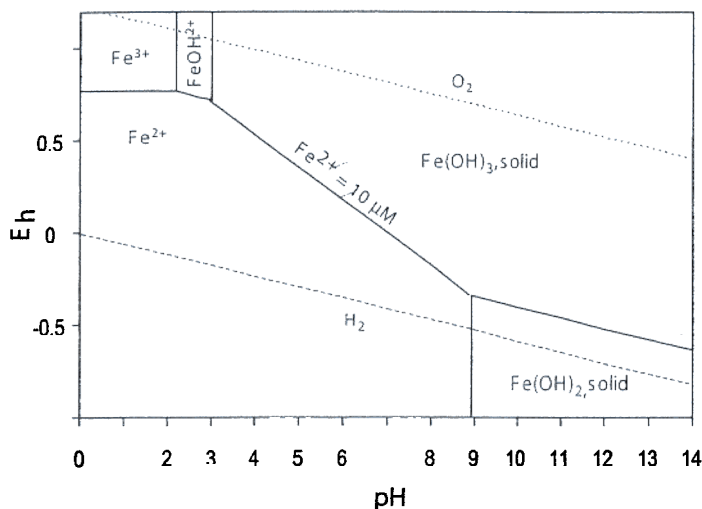


Figure 4. Eh-pH diagram for Fe(II), Fe(III), O₂ and H₂ calculated with a Fe²⁺ concentration of 10 μM. For simplicity Fe(OH)₃ is used as approximation for the Fe(III) precipitates that are formed.

such conditions, ~90 mol Fe(II) has to be oxidized to fix 1 mol of CO₂ as biomass (Ehrlich 2002). This relationship explains the huge amount of iron that is oxidized by aerobic acidophilic microorganisms, for instance in acid mine drainage (Baker and Banfield 2003; Druschel et al. 2004). Note that at pH values above 2, Fe(III) starts to precipitate and the oxidized product is removed, leading to a lowering of the redox potential of the Fe(III)/Fe(II) couple to less positive values. Since the redox potential of the O₂/H₂O couple is less pH-dependent (59 mV change per pH unit) than the Fe(III)/Fe(II) couple (177 mV change per pH unit), growth at less acidic pH values is more favorable for aerobic acidophilic Fe(II) oxidizers.

A number of lineages of acidophilic iron-oxidizing organisms have been described to date. These were reviewed comprehensively by Nordstrom and Southam (1997) and more recently by Blake and Johnson (2000) and Baker and Banfield (2003). Furthermore, aspects of the population biology of acidophilic microbial communities sustained by iron oxidation are reviewed by Whitaker and Banfield (2005).

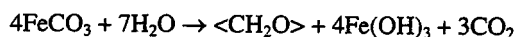
Aerobic neutrophilic Fe(II)-oxidizing microorganisms

This physiological group of microorganisms uses O₂ as electron acceptor for enzymatic oxidation of Fe(II) at neutral pH. To gain energy for growth they have to compete with the chemical oxidation of Fe(II) by O₂. Initially, research on oxygen-dependent, neutrophilic Fe(II) oxidizers focused on species of the genera *Gallionella* and *Leptothrix*. Organisms of these two groups were already recognized in the 19th century to grow in oxic iron-rich environments. *Gallionella ferruginea*, a bean-shaped autotrophic bacterium, typically produces twisted stalks that are encrusted with ferric iron minerals (Hanert 1981). *Gallionella* spp. are very good examples of gradient organisms: growth is observed only under conditions that are neither strongly reducing nor highly oxidizing. The heterotrophic bacterium *Leptothrix ochracea* forms tubular sheaths which are also covered with ferric iron minerals (Emerson and Revsbech 1994). It has been suggested that the deposition of iron oxide minerals on the stalks or sheaths avoids encrustation of Fe(II)-metabolizing cells. Encrustation of living cells might impair both substrate uptake and metabolite release, and may even cause cell death (Hanert 1981; Hallberg and Ferris 2004).

A range of novel microaerophilic Fe(II)-oxidizing bacteria were isolated with gradient culture techniques using gradients of Fe(II) and O₂ to mimic natural environments. Representatives of the α-, β- and γ-subgroup of *Proteobacteria* were isolated from groundwater, deep sea sediments and freshwater wetland samples (Emerson and Moyer 1997; Edwards et al. 2003; Sobolev and Roden 2004). More details on aerobic bacterial Fe(II) oxidation at neutral pH are given by Emerson (2000).

Anaerobic Fe(II)-oxidizing phototrophic bacteria

About a decade ago anoxygenic phototrophic bacteria were discovered which grow in the light with ferrous iron as sole electron donor (Widdel et al. 1993). Experimental results were in good agreement with the following equation, assuming <CH₂O> as the approximate formula of cell mass:



In the meantime, seven cultures of anoxygenic Fe(II)-oxidizing phototrophic bacteria have been established (Table 3). They include representatives of the three major phylogenetic lineages of anoxygenic phototrophs, and furthermore include freshwater and marine species. All known anoxygenic phototrophs oxidized Fe(II) optimally only within the narrow pH-range of 6.5 to 7. This allows them to use Fe(II) as electron donor since the standard redox potential for Fe²⁺/Fe³⁺ (+770 mV at pH 1) is shifted at neutral pH to less positive values (around 0 mV) due to the low solubility of Fe(III) (Fig. 4; Widdel et al. 1993; Stumm and Morgan 1996). Therefore, Fe(II) can donate electrons to the photosystems of purple or green bacteria, with midpoint potentials around +450 mV or +300 mV, respectively (Clayton and Sistrom 1978).

Fe(II)-oxidizing phototrophic bacteria can oxidize dissolved Fe(II). In addition, they grow with relatively soluble Fe(II) minerals such as siderite or ferrous monosulfide (Kappler and Newman 2004). In contrast, they were unable to utilize less soluble Fe(II) minerals, e.g., pyrite (FeS₂) or magnetite (Fe₃O₄). These results indicate that the phototrophs studied so far may depend on the supply of dissolved Fe(II).

Geological records indicate that oceans contained considerable amounts of dissolved ferrous iron and hardly any molecular oxygen in the beginning of the Precambrian. It is therefore intriguing how massive iron mineral deposits, known as banded iron formations (BIFs), were generated at that time. This is even more puzzling, given doubt that the

Table 3. Ferrous iron-oxidizing phototrophic bacteria from different phylogenetic groups.

Phylogenetic group	Species	Strain	Source	Ref.
Purple sulfur bacteria	<i>Thiodictyon</i> sp. ^a	F4	Freshwater marsh	(1)
Purple non-sulfur bacteria	<i>'Rhodobacter ferrooxidans'</i>	SW2	Freshwater ditch	(2)
	<i>Rhodomicrobium vannielii</i>	BS-1	Freshwater	(3)
	<i>Rhodopseudomonas palustris</i>	TIE-1	Iron-rich freshwater mat	(4)
	<i>Rhodovulum iodosum</i>	N1	Marine sediment	(5)
	<i>Rhodovulum robiginosum</i>	N2	Marine sediment	(5)
Green bacteria	<i>Chlorobium ferrooxidans</i> ^b			

^a Mixed culture, highly enriched in *Thiodictyon* sp.

^b Defined co-culture with chemoheterotrophic *'Geospirillum'* sp.

References: (1) Croal et al. 2004; (2) Ehrenreich and Widdel 1994; (3) Widdel et al. 1993; (4) Jiao et al. 2005; (5) Straub et al. 1999; (6) Heising et al. 1999

photochemical oxidation of Fe(II) by UV light (Cairns-Smith 1978; Francois 1986; Anbar and Holland 1992) plays a major role in complex environments such as seawater. Until recently, BIFs were mainly considered the product of chemical or microbial oxidation of dissolved Fe(II) with O₂ that was released by cyanobacteria during early oxygenic photosynthesis (Fig. 5) (e.g., Konhauser et al. 2002). Today, the anaerobic oxidation of Fe(II) by anoxygenic phototrophs is regarded as an alternative or additional explanation for the generation of BIFs (Fig. 5) (Widdel et al. 1993; Konhauser et al. 2002). Interestingly enough, in the literature it was speculated that anoxygenic Fe(II)-oxidizing phototrophs participated in the generation of BIFs even before such organisms had been isolated (Hartman 1984). A recent study considering rates of anoxygenic phototrophic Fe(II) oxidation under light regimes representative of ocean water at depths of a few hundred meters suggest that, even in the presence of cyanobacteria, anoxygenic phototrophs living beneath a wind-mixed surface layer provide the most likely explanation for BIF deposition in a stratified ancient ocean (Kappler et al. 2005).

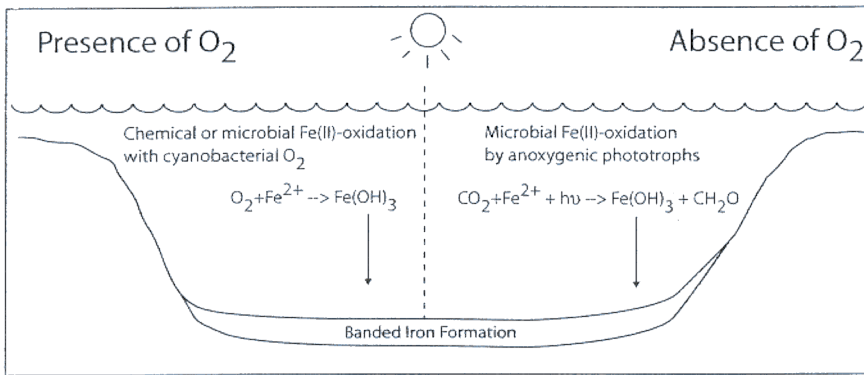


Figure 5. Proposed mechanisms for the deposition of Precambrian banded iron formations in the presence or absence of molecular oxygen: oxidation of Fe(II) either indirectly by cyanobacterially produced O₂ or directly by anoxygenic photosynthetic Fe(II)-oxidizing microorganisms.

Anaerobic Fe(II)-oxidizing nitrate-reducing bacteria

Furthermore, it was discovered that microorganisms are capable of coupling oxidation of ferrous iron to dissimilatory reduction of nitrate (Hafenbradl et al. 1996; Straub et al. 1996). At pH 7, all redox pairs of the nitrate reduction pathway can accept electrons from ferrous iron because their redox potentials are more positive than that of the redox couple Fe(III)/Fe(II) (Tables 4 and 5). The first observations of this metabolism were made with a lithotrophic enrichment culture that was transferred successively several times in medium that contained ferrous iron as sole electron donor (Straub et al. 1996). In this culture, ferrous iron oxidation coupled to nitrate reduction definitely supported cell growth; no oxidation of Fe(II) occurred in the presence of heat-inactivated cells or when nitrate was omitted. This type of metabolism is likely to be more abundant than ferrous iron oxidation by anoxygenic phototrophs since it is not restricted to habitats that are exposed to light. Furthermore, most-probable-number studies combined with molecular techniques indicated that the ability to oxidize ferrous iron with nitrate as electron acceptor is widespread among bacteria: members of the α -, β -, γ - and δ - subgroup of the *Proteobacteria* as well as gram-positive bacteria are probably able to oxidize ferrous iron (Straub and Buchholz-Cleven 1998; Straub et al. 2004). For these reasons, it was not surprising that enrichments of ferrous iron-oxidizing nitrate reducers were successfully established with a variety of marine, brackish or freshwater sediment samples. However, continuous cultivation

with ferrous iron as sole electron donor turned out to be impossible for most of these enrichments. After a few transfers, ferrous iron was oxidized only in the presence (of low concentrations) of an organic substrate, e.g., 0.5 mM acetate. Accordingly, most Fe(II)-oxidizing-nitrate reducers isolated so far need an organic co-substrate for growth, i.e. grow only mixotrophically with ferrous iron (Straub et al. 1996; Benz et al. 1998; Straub and Buchholz-Cleven 1998; Lack et al. 2002; Straub et al. 2004). For many of these strains that need an additional organic substrate, it is questioned whether ferrous iron oxidation is beneficial and supports cell growth or whether iron is just oxidized in a rather unspecific side reaction. Experiments with *Azospira oryzae* strain PS (formerly known as *Dechlorosoma suillum*) undoubtedly showed that oxidation of ferrous iron initiated only after the organic (co-) substrate was completely oxidized (Chaudhuri et al. 2001). However, at least for some mixotrophically ferrous iron-oxidizing strains, i.e. *Acidovorax* sp. strain BrG1, *Aquabacterium* sp. strain BrG2 and *Thermomonas* sp. strain BrG3, the situation was more complex

because the oxidation of ferrous iron seemed to be regulated. Only if electrons from the organic substrate exceeded those from ferrous iron by a factor of ten or if the concentration of nitrate was limited, ferrous iron oxidation ceased completely (Straub et al. 2004).

Recently, some strains were isolated from the deep sea that oxidized Fe(II) with nitrate in the absence of an additional organic substrate. Unfortunately, it is not clear whether these strains can actually grow with ferrous iron as the sole electron donor for several successive generations (Edwards et al. 2003).

Mechanisms of microbial Fe(II) oxidation

The mechanism of microbial Fe(II) oxidation has been studied best with the acidophilic Fe(II) oxidizer *Thiobacillus ferrooxidans*. According to a present model, Fe(II) is oxidized to Fe(III) at the outer membrane of the cell (Blake and Johnson 2000). The electron is then transferred to a copper-containing protein (rusticyanin) which in turn transfers it to a periplasmic *c*-type cytochrome. From such cytochromes, electrons are finally passed on to O₂ via cytochrome oxidase to form water. The exact pathway of the electron transfer from ferrous iron to oxygen is still not completely understood, and slightly varying models are described in the literature. However, there is general agreement that the initial step, i.e. the oxidation of ferrous iron, occurs outside the cell (Blake and Johnson 2000).

In addition, it was shown for neutrophilic aerobic Fe(II)-oxidizing *Leptothrix* spp. that the oxidation of Fe(II) is catalyzed by Fe(II)-oxidizing compounds that are actively secreted by

Table 4. Redox potentials of redox pairs relevant for microbial nitrate reduction at pH 7.0 and 25 °C (Thauer et al. 1977).

Redox pair	E_0' [mV]
NO ₃ ⁻ /NO ₂ ⁻	+430
NO ₂ ⁻ /NO	+350
NO/N ₂ O	+1180
N ₂ O/N ₂	+1350

Table 5. Redox potentials¹ of some redox pairs relevant for microbial reduction of iron oxides at pH 7.0 and 25 °C (Thamdrup 2000).

Redox pair	E_0' [mV]
Fe ₃ HO ₈ ·4H ₂ O (ferrihydrite)/Fe ²⁺	+2
γ-FeOOH (lepidocrocite)/Fe ²⁺	-88
α-FeOOH (goethite)/Fe ²⁺	-274
α-Fe ₂ O ₃ (hematite)/Fe ²⁺	-287
Fe ₃ O ₄ (magnetite)/Fe ²⁺	-314

¹ Slightly varying data can be found in the literature because redox potentials strongly depend on pH, temperature, concentrations of reactants, crystal size of the iron oxide and thermodynamic data chosen for calculations.

the cell (De Vrind-de Jong et al. 1990); an Fe(II)-oxidizing protein with a molecular weight of 150 kDa was identified from spent culture medium of strain *Leptothrix discophora* (Corstjens et al. 1992).

For anaerobic Fe(II) oxidation, it is unknown where in the cell or at the cell surface Fe(II) is oxidized, and it is not understood how the bacteria deal with the poor solubility of the product. In particular, it is unclear how Fe(II)-oxidizing microorganisms either avoid encrustation with ferric iron minerals (such as the phototrophic Fe(II)-oxidizer '*Rhodobacter ferrooxidans*' strain SW2) or overcome encrustation such as the nitrate-reducing Fe(II)-oxidizing strain BoFeN1 (Fig. 6). A microenvironment of lowered pH values in vicinity of the cells was observed around colonies of phototrophic Fe(II) oxidizers ('*Rhodobacter ferrooxidans*' strain SW2) fixed in semi-solid agarose (Kappler and Newman 2004). Such an acidification could explain why these microorganisms do not become encrusted with ferric iron minerals during oxidation of Fe(II) (Fig. 6).

With the aerobic Fe(II)-oxidizing strain TW2, deposition of Fe(III) minerals was observed not at the cell surface but at a certain distance from the cells. It was suggested that Fe(III) was released in a ligand-bound dissolved form. The dissolved Fe(III)-ligand complex is thought to

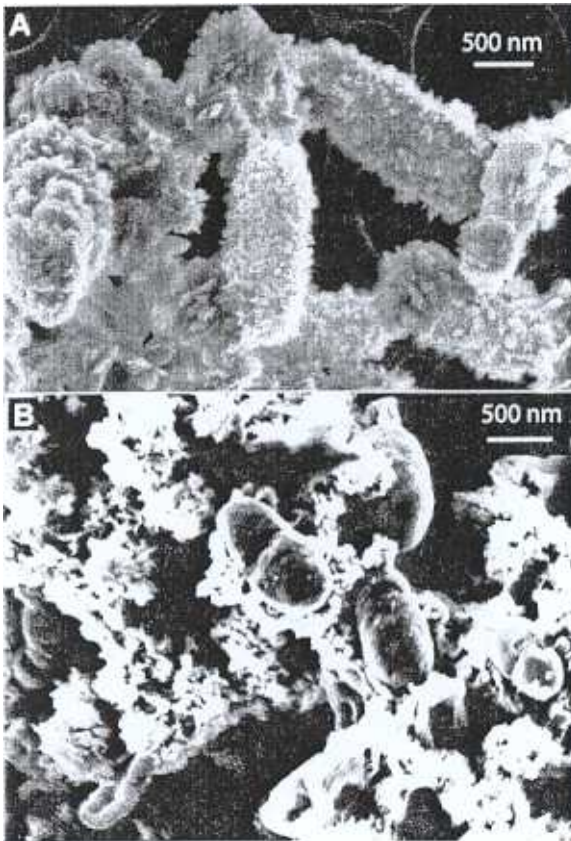


Figure 6. Scanning electron micrographs showing (A) cells of the nitrate-reducing Fe(II)-oxidizing strain BoFeN1 highly encrusted with Fe(III) minerals and (B) anoxygenic photosynthetic Fe(II)-oxidizing microorganisms that are associated but not encrusted with Fe(III) minerals.

diffuse away from the cells. Destabilization of the Fe(III)-ligand complex would finally lead to hydrolysis and precipitation of Fe(III) minerals distant from the metabolically active cells (Roden et al. 2004). The nature of the Fe(III)-ligand and the trigger necessary for destabilizing the dissolved Fe(III)-ligand complex are unknown so far. However, this hypothesis is supported by energetic calculations. The estimated biomass yield for growth was 0.15 mol cell-C per mol oxidized Fe(II), and hence approximately 7.5× more than experimentally observed in gradient cultures. This suggests that a substantial amount of energy is available for synthesis of other cellular components, including Fe(III)-binding ligands.

Formation of Fe(III) minerals by microbial Fe(II) oxidation

Microbial oxidation of Fe(II) and precipitation of Fe(III) minerals might be better understood by comparing observations from microbial cultures to results from chemical Fe(II) oxidation experiments (e.g., Cornell et al. 1989). Mono- and dinuclear dissolved species of ferrous iron such as $[\text{FeOH}]^{2+}$ and $[\text{Fe}_2(\text{OH})_2]^{4+}$ are formed initially during abiotic oxidation of Fe(II). Subsequently, these dissolved species transform into polymeric Fe(III) colloids before they precipitate as poorly crystalline ferrihydrite particles with a size of ~2–5 nm in diameter. Depending on the reaction conditions, the initial precipitation might be followed by further transformations of ferrihydrite. Either “solid-state conversion” to hematite (Fe_2O_3) by internal rearrangement of iron and oxygen atoms is induced or dissolution to low-molecular weight polynuclear iron species occurs which then transform to better crystalline iron oxides such as goethite (“dissolution-reprecipitation mechanism”) (Hansel et al. 2003; Schwertmann and Cornell 2003).

Transformation of ferrihydrite to goethite via dissolution-reprecipitation could be facilitated in particular by enhanced proton activities close to cell surfaces. Lowered pH values and transformation of ferrihydrite to goethite were indeed observed in the vicinity of anoxygenic phototrophic Fe(II)-oxidizing bacteria (Kappler and Newman 2004). The formation of crystalline iron oxides during microbial Fe(II) oxidation might accelerate the speed of Fe(II) oxidation by an autocatalytic mechanism. Excess dissolved Fe(II) has a high affinity for surface-OH groups of iron oxides. These surface OH-groups are electron-donor ligands that increase the electron density of the adsorbed ferrous iron. An increased electron density stabilizes +3 charged iron better than +2 charged iron. Therefore, adsorption of Fe(II) on iron oxide surfaces increases the rate of Fe(II) oxidation (Wehrli et al. 1989; Elsner et al. 2003). An electron transfer from surface-adsorbed Fe(II) through the underlying iron oxide to the cell (where electrons could be accepted by outer membrane compounds) would abolish the need for the Fe(II)-oxidizing microbe to be in direct contact with the dissolved Fe(II). The first evidence for such an electron transfer between adsorbed Fe(II) and Fe(III) from the underlying ferric iron oxide was recently reported by Williams and Scherer (2004).

Formation of a variety of different iron minerals by different Fe(II)-oxidizing microorganisms indicates that, apart from medium composition, concentration of possible co-substrates and incubation conditions, the mechanism of Fe(II) oxidation, metabolic rates and the presence of nucleation sites influence (and maybe even control) the mineralogy of the Fe(III) minerals produced. As an example, in a recent report polysaccharide strands were suggested to be extruded to act as a template-for formation of akaganeite pseudo-single crystals (Chan et al. 2003)

MICROBIAL DISSIMILATORY REDUCTION OF Fe(III)

Microbial reduction of ferric iron was known as a phenomenon for many decades before its (bio)geochemical relevance was recognized. It was presumed that microorganisms cause reduction of Fe(III) only indirectly, e.g., by lowering the redox potential or the pH. In addition,

only few bacteria were known that transferred just few electrons to Fe(III) during fermentative growth (for details see Lovley 1991). This perspective changed notably with the discovery of bacteria that respire ferric iron and thereby reduce substantial amounts of it (Balashova and Zavarzin 1979; Lovley and Phillips 1988; Myers and Nealson 1988). Today, it is generally accepted that dissimilatory ferric iron-reducing prokaryotes, i.e. organisms that gain energy by coupling the oxidation of organic or inorganic electron donors to the reduction of ferric iron, have a strong influence on the geochemistry of many environments (e.g., Lovley 1997; Thamdrup 2000).

Acidophilic Fe(III)-reducing microorganisms

The ability to reduce Fe(III) to Fe(II) under acidophilic conditions seems to be widespread among acidophilic microorganisms, but the degree of Fe(III) reduction varies significantly (Johnson and McGinness 1991). Chemolithotrophic and heterotrophic prokaryotes (bacteria and archaea) are able to couple the reduction of Fe(III) to the conservation of energy. Interestingly enough, acidophilic iron reduction does not require strict anoxia in some strains and proceeds most rapidly even under microoxic conditions (Johnson et al. 1993). Studies with *Acidiphilium* sp. strain SJH showed that this bacterium is able to reduce a variety of different Fe(III) forms, with the highest reduction rates observed for dissolved Fe(III) (Bridge and Johnson 2000). Barely soluble poorly crystalline iron oxides (e.g., ferrihydrite) were reduced faster than better crystalline iron oxides (e.g., goethite). Apparently, *Acidiphilium* sp. strain SJH causes dissolution of ferric iron indirectly since direct contact between bacterial cells and solid ferric iron was not necessary for ferric iron reduction to occur. The strain appears to produce an extracellular compound that accelerates Fe(III) dissolution but not reduction. The nature of this extracellular compound and further details of the dissolution process are still unknown (Bridge and Johnson 2000).

Microbial reduction of Fe(III) at neutral pH

In the past decade, numerous strains of dissimilatory ferric iron-reducing bacteria and archaea have been isolated from a vast range of habitats. A comprehensive list of Fe(III)-reducing microorganisms was recently published by Lovley et al. (2004). The widespread occurrence of Fe(III)-reducing prokaryotes correlates with the ubiquitous presence of ferric iron. Many sediments and soils may contain ferric iron minerals in the range of 50-200 mmol per kg dry matter. Ferric iron is therefore often the dominant electron acceptor although it is barely soluble at neutral pH. According to experimental observations, Fe(III)-reducing microorganisms developed three different strategies to cope with the difficulty of transferring electrons from the cell to the surface of a barely soluble electron acceptor (Fig. 7) (reviewed by Hernandez and Newman 2001; Lovley et al. 2004):

- A. Physical contact between cell surface/cell surface compounds and ferric iron allows direct delivery of electrons.
- B. Iron chelators increase the solubility of Fe(III) and hence alleviate Fe(III)-reduction.
- C. Electron-shuttling compounds transfer electrons from the cell to Fe(III) without the necessity of physical contact between cells and ferric iron.

Considering the complexity of natural environments and the wealth of microbial capabilities, it is not surprising that different organisms as well as single organisms developed different strategies in order to reduce diverse Fe(III) compounds under varying conditions. For example, some evidence indicates that *Shewanella algae* and *Geothrix fermentans* produce and release both Fe(III)-chelators and electron shuttles (Nevin and Lovley 2002a; Lovley et al. 2004). Furthermore, evidence in *Geobacter* spp. indicates that different cellular compounds are involved in reduction of dissolved Fe(III)-citrate and barely soluble ferrihydrite (Straub

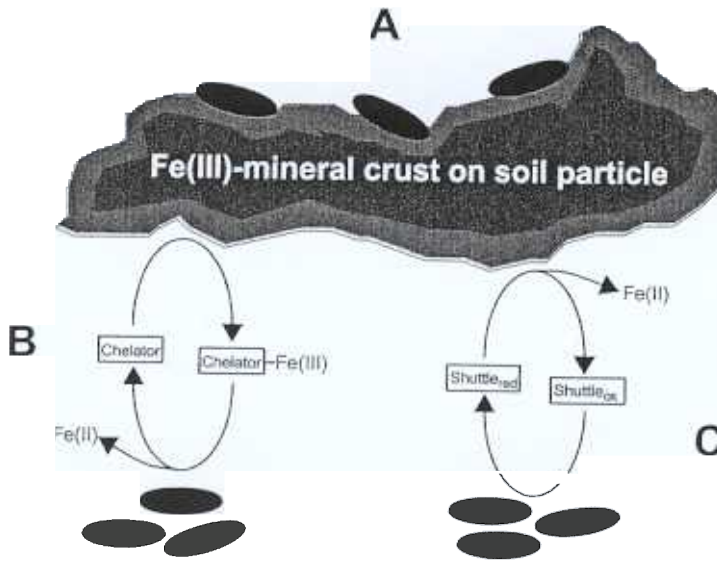


Figure 7. Schematic illustration of different microbial strategies to transfer electrons to ferric iron: (A) Physical contact between cell surface and ferric iron allows direct delivery of electrons; (B) Iron chelators increase solubility of ferric iron; (C) Electron-shuttling molecules transfer electrons to ferric iron. Note: single crystals of ferric iron oxides might be smaller than bacteria (see Fig. 3). However, in nature iron oxides may form crusts on soil particles as depicted here.

and Schink 2004a, Leang et al. 2005). As the methods to study microbial mechanisms of Fe(III) reduction are pivotal, they will be discussed in the next section.

Methods to study mechanisms of microbial Fe(III) reduction

Physiological studies of microbial ferric iron reduction at neutral pH are rather difficult. Low solubility of ferric iron is the most prominent obstacle. It impedes the monitoring of cell growth by means of optical density and the separation of cells from iron minerals by simple centrifugation. To circumvent this difficulty, iron chelators (e.g., citrate, EDTA) were applied in many studies to keep iron in solution. However, chelators change the redox potential of ferric iron, may enter the periplasm and can react unspecifically with electron-releasing cellular compounds (reviewed by Straub et al. 2001). In addition, there is growing awareness that culturing microorganisms in rich medium (in particular with other electron acceptors than ferric iron) may cause production of cell compounds which will not be produced under iron-reducing conditions in natural habitats (Glasauer et al. 2003). Caution in the interpretation of results is also necessary when supernatants were prepared either by filtration or centrifugation. Cells of *Geobacter* spp. were shown to artificially release compounds (e.g., cytochromes) by filtration with 0.2 μm filters as well as by centrifugation (Straub and Schink 2003). In other studies, semi-permeable membranes were used to separate cells and iron oxides physically in order to determine whether prokaryotic cells produce Fe(III)-chelators or electron-shuttling molecules. However, it was recently shown that Fe(III)-chelators and electron-shuttling molecules were unable to diffuse freely through dialysis membranes with the largest pore size available (Nevin and Lovley 2000). Therefore, results from studies with semi-permeable membranes need critical assessment, in particular when positive controls with known electron-shuttling molecules are lacking. To minimize artifacts that might be induced by centrifugation or filtration, further methods were developed to study production of Fe(III)-chelators or electron-shuttling

molecules *in vivo*. In a simple one, ferric iron is entrapped in medium solidified with 1% agar (Straub and Schink 2003). Technically more elaborate is the use of iron containing microporous alginate (Nevin and Lovley 2000) or iron-containing glass beads (Lies et al. 2005).

Microbial mechanisms of Fe(III) reduction at neutral pH

For Fe(III) reduction, species of the genus *Geobacter* appear to require physical contact to ferric iron oxides (Nevin and Lovley 2000; Lovley et al. 2004). The latest study with *Geobacter sulfurreducens* showed that pili (a special type of cell appendages) were produced during growth with poorly soluble Fe(III), but not with dissolved Fe(III)-citrate as electron acceptor. In addition, experiments with a pilus-deficient mutant implied that those pili were not just required for attachment of cells to ferric iron, and conducting-probe atomic force microscopy indicated that the pili were highly conductive. Together these results suggest that *Geobacter sulfurreducens* attaches and delivers electrons to the surface of ferric iron oxides via pili (Reguera et al. 2005).

Initially it was thought that such a physical contact between Fe(III)-reducing prokaryotes and ferric iron minerals is mandatory for the delivery of electrons from the cells to the minerals. Today, it is generally accepted that Fe(III)-reducing microorganisms also use Fe(III)-chelators or electron-shuttling molecules to reduce barely soluble ferric iron oxides (e.g., Hernandez and Newman 2001; Rosso et al. 2003; Lovley et al. 2004). Diffusible chelators and shuttling molecules help to bridge spatial distance between cells and ferric iron oxides (Fig. 7). This is of particular importance since microorganisms and ferric iron oxides are not evenly distributed in natural environments.

Plant root exudates and plant debris can release organic acids which are known to chelate Fe(III), e.g., oxalate or citrate. Accordingly, highly elevated levels of dissolved Fe(III) in the range of 20 to 50 μM were reported for soils in laboratory incubations with rice (Ratering and Schnell 2000). In comparison to the nM range of dissolved Fe(III) at neutral pH (Fig. 2), significantly elevated levels of dissolved, presumably chelated Fe(III) in the range of 4 to 16 μM were reported furthermore for freshwater sediment and groundwater samples (Nevin and Lovley 2002b).

Plant debris is also the source for phenolic compounds and humic substances which can act as electron-shuttling molecules (e.g., Lovley et al. 1996, 1998). The oxidized form of an electron-shuttling molecule is used as the electron acceptor by the metabolically active cell. The electrons are then transferred from the reduced shuttling molecule in a chemical reaction to ferric iron. It is important that this chemical reaction regenerates the oxidized form of the shuttling molecule (Fig. 7). Prokaryotes that reduce ferric iron oxides only via electron-shuttling molecules are not ferric iron-reducing bacteria in a strict sense as their electron acceptor is the shuttling molecule rather than ferric iron. In that respect it is worth mentioning that sulfur-reducing bacteria also can benefit from indirect reduction of ferric iron oxides via sulfur cycling, with sulfide as reductant of ferric iron (Straub and Schink 2004b). The impact of prokaryotes that reduce ferric iron oxides only indirectly with the help of naturally occurring electron shuttles on the total Fe(III) reduction in anoxic environments has not yet been evaluated.

Finally, it is useful to discuss what advantages, if any are available to iron-reducing microorganisms that specifically produce and excrete Fe(III)-chelating or electron-shuttling molecules. For a single bacterium, production and release of such specialized molecules might be too expensive, in particular if such molecules are lost or degraded before the costs of biosynthesis have been compensated. However, in bacterial communities (e.g., biofilms, cell aggregates) such expenses might be balanced: each cell contributes just few chelator or shuttle molecules and the whole community benefits from the accessibility of ferric iron as electron

acceptor. To date, no Fe(III)-chelating or electron-shuttling compound that was specifically produced and excreted in ferric iron-reducing cultures *in vivo* has been identified. However, some evidence indicates that *Shewanella algae* and *Geothrix fermentans* produce and release both Fe(III)-chelators and electron shuttles (Nevin and Lovley 2002a; Lovley et al. 2004). Furthermore, it was recently demonstrated that some antibiotics, e.g., phenazine-1-carboxamide (PCN), bleo-mycin and pyocyanine, function as electron shuttles between bacteria and Fe(III) minerals (Hernandez et al. 2004). These redox-active antibiotics, exemplified in Figure 8 by PCN, structurally resemble humic substances with regard to aromaticity and redox-active functional groups. *Pseudomonas chlororaphis* can transfer electrons to ferric iron oxides only due to the production and reduction of PCN. In addition to the PCN-producing strain, *Shewanella oneidensis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Vibrio cholerae* were able to reduce PCN and thus indirectly reduce Fe(III) minerals (Hernandez et al. 2004). So far it is unknown whether the antibiotic-producing and/or antibiotic-reducing strains actually gain energy through this indirect Fe(III) reduction. It might just as well be a new microbial mechanism to acquire iron for assimilatory processes (compare to Kraemer et al. 2005). Interestingly enough, appreciable concentrations of phenazines in the range of 27 to 43 ng per g root (with soil) were found in the rhizosphere of wheat plants (Thomashow et al. 1990).

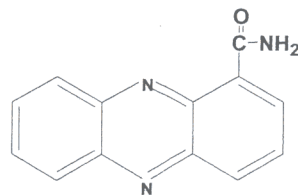


Figure 8. Chemical structure of phenazine-1-carboxamide, PCN, a redox-active antibiotic produced by *Pseudomonas chlororaphis* that functions as electron shuttle between different microorganisms and ferrihydrite.

MICROBIAL IRON CYCLING

Many reactions relevant to geochemistry are driven and/or accelerated by the activity of prokaryotes. Examples are manifold and include carbon mineralization, nitrogen fixation and sulfate reduction as well as iron transformations. In particular, prokaryotes that gain energy through oxidation of Fe(II) or reduction of Fe(III) have a strong influence on the global iron cycle (for details see Kraemer et al. 2005). For example, in most acidic aerobic environments, Fe(II) would persist if not oxidized by acidophilic Fe(II) oxidizers.

Microbial iron cycling under acidic conditions

Understanding microbial cycling of iron at acidic pH has implications for the leaching of ores and the development of (bio)remediation techniques for acid mine drainage. Evidence for *in vivo* iron cycling was obtained from mining sites and was investigated in more detail in the laboratory (Johnson et al. 1993). Mixed cultures of acidophilic Fe(II)-oxidizing and Fe(III)-reducing microorganisms cycled iron between the oxidation states +II and +III when the concentration of dissolved oxygen fluctuated and sufficient electron donor for Fe(III) reducers was supplied. Similarly, iron cycling could also be demonstrated in pure cultures since some acidophiles, e.g., *Thiobacillus ferrooxidans* and *Sulfobacillus acidophilus* catalyze both Fe(II) oxidation and Fe(III) reduction under appropriate conditions (reviewed by Johnson et al. 1993; Blake and Johnson 2000).

Microbial iron cycling at neutral pH

Ferric iron is the dominant electron acceptor for the mineralization of carbon particularly in anoxic freshwater habitats (Thamdrup 2000). Therefore, processes that regenerate Fe(III) minerals that are again available for Fe(III)-reducing prokaryotes have become of significant interest. Since microbial Fe(III) reduction and Fe(II) oxidation were recognized, microbial

cycling of iron seemed plausible and is hypothesized for many environments. For example, it was estimated that in marine coastal sediments each iron atom cycled approximately 100 to 300 times before being buried in the sediment (Canfield et al. 1993). However, the natural complexity of habitats aggravates direct measurements of microbial iron transformation reactions and thus microbial iron cycling *in vivo* has not yet been clearly demonstrated.

Prerequisites for microbial iron cycling at neutral pH

Microbial iron cycling needs iron plus appropriate supplementary substrates, i.e., electron donors for Fe(III) reduction and electron acceptors for Fe(II) oxidation (Fig. 9). Furthermore, the nature of the iron minerals formed is crucial for an efficient cycling since not all iron minerals are equally good substrates. For instance, the redox potential of an iron redox couple determines whether it is available as electron donor or acceptor in terms of energetics (Table 5). At pH 7, molecular oxygen and all redox pairs of the nitrate reduction pathway (Fig. 4) can accept electrons from ferrous iron, independently from the Fe(III) mineral produced. The situation is more complex with ferric iron oxides as electron acceptor. The oxidation of acetate ($\text{CO}_2/\text{acetate}$, $E_0' = -290$ mV) is energetically favorable just with iron oxides such as lepidocrocite or ferrihydrite. On the other hand, for the reduction of goethite, hematite or magnetite, electron donors with a lower redox potential are necessary, e.g., molecular hydrogen ($2\text{H}^+/\text{H}_2$, $E_0' = -414$ mV) or formate ($\text{CO}_2/\text{formate}$, $E_0' = -432$ mV). Hence, theoretically acetate can fuel microbial cycling of iron only if ferrihydrite or lepidocrocite is the product of microbial Fe(II) oxidation. Furthermore, it is essential that supplementary electron donors and acceptors can diffuse since ferric iron is barely soluble and thus rather immobile in natural environments. The solubility of Fe(III) in equilibrium with ferrihydrite is in the range of 10^{-9} M (Fig. 2). The solubilities of goethite and hematite are even lower and the Fe(III) concentrations in the presence of these minerals is in the range from 10^{-10} M to 10^{-13} M (Kraemer 2004). In natural environments, the concentration of dissolved Fe(II) is controlled by adsorption or precipitation and is therefore insignificant in comparison to solid Fe(II). Dissolved Fe(II) adsorbs to soil particles, cell surfaces and also to the surface of ferric iron oxides (e.g., Liu et al. 2001; Cornell and Schwertmann 2003); in model calculations for a coastal sediment, adsorbed Fe(II) exceeded the concentration of freely dissolved Fe(II) 30-fold (Van Cappellen and Wang 1996; Thamdrup 2000).

Oxygen-dependent microbial cycling of iron

The product of microbial aerobic Fe(II) oxidation is often identified as poorly crystalline ferrihydrite, a ferric iron oxide that is a favorable electron acceptor for ferric iron-reducing prokaryotes. However, traces of oxygen may repress iron respiration in facultatively anaerobic Fe(III) reducers and can even inhibit the activity of strictly anaerobic ferric iron-reducing

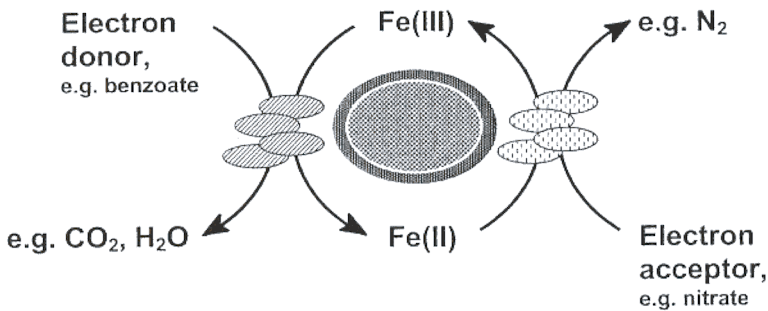


Figure 9. Schematic illustration of microbial iron cycling.

microorganisms, as shown e.g., for *Geobacter* spp. (Straub and Schink 2004a). Hence, oxygen-dependent microbial cycling of iron (most likely) always depends on a transition between oxic and anoxic conditions. In natural environments, such transitions are supported by temporary oxygen release by roots, bioturbation by burrowing and boring animals and mixing of sediments by waves or storm events.

Of particular interest for oxygen-dependent iron cycling are microaerophilic Fe(II) oxidizers since they thrive in oxic-anoxic transition zones, allowing for microscale microbial redox cycling. Such oxic-anoxic transition zones are characterized by the simultaneous presence of ferrous iron which was produced during anaerobic Fe(III) reduction and of low concentrations of oxygen which reached this zone via diffusion from overlying oxic zones (Sobolev and Roden 2002).

Oxygen-independent microbial cycling of iron

The identification of ferrihydrite as the primary product of anaerobic Fe(II) oxidation by phototrophs (Straub et al. 1999; Kappler and Newman 2004) or nitrate-reducing bacteria (Straub et al. 1996, 1998) indicated the possibility of anaerobic iron cycling. Biologically produced ferrihydrite has been shown to be an excellent electron acceptor for Fe(III)-reducing bacteria, which reduced it completely to the ferrous state (Straub et al. 1998, 2004). Similar to ferric iron, nitrate is used as electron acceptor only in anoxic zones after oxygen is depleted. In contrast to iron, nitrate is soluble at pH 7. Finally, it was feasible to show an anaerobic iron cycling in laboratory co-culture experiments (Straub et al. 2004). For these experiments, Fe(II)-oxidizing nitrate reducers were chosen that were unable to oxidize benzoate. As a counterpart, an Fe(III) reducer was selected that utilized benzoate with Fe(III) but not with nitrate as the electron acceptor. Only in experiments that were inoculated with Fe(II) oxidizers plus Fe(III) reducers was benzoate completely oxidized with nitrate in the presence of iron (Fig. 9). Although the transient iron phases in such co-cultures were not analyzed, stoichiometric considerations suggest that iron cycled 6 times between the oxidation states +II and +III in these experiments (Straub et al. 2004). Clearly, the relevance of anaerobic nitrate-dependent iron cycling for the complex flow of electrons in anoxic environments still needs to be determined.

Microbial anaerobic iron cycling is possible with the participation of anoxygenic Fe(II)-oxidizing phototrophs. Light-dependent, anaerobic cycling of iron may occur in top layers of shallow sediments that are reached by light or in (iron rich) microbial mats.

ENVIRONMENTAL IMPLICATIONS

Microbial reduction of Fe(III) and oxidation of Fe(II) may have left geological imprints during Earth's history, and continues to significantly affect modern environments. Due to the considerable amount of iron in soils and sediments, Fe(III) usually represents the most abundant electron acceptor in anoxic soils and freshwater sediments; only in marine sediments is this dominance counterbalanced by the high sulfate concentration of seawater (Thamdrup 2000). Carbon cycling, mobility of micronutrients and in particular the degradation, transformation and (im)mobilization of organic and inorganic pollutants are closely linked in many environments to the microbial iron cycle.

Degradation of organic compounds coupled to dissimilatory Fe(III) reduction

In pristine environments, Fe(III)-reducing microorganisms typically couple the reduction of Fe(III) to the oxidation of H₂ or other fermentation products such as simple fatty acids or ethanol. Some ferric iron-reducing strains have in addition the ability to oxidize aromatic, organic pollutants such as benzene, toluene, ethylbenzene, phenol, *p*-cresol and *o*-xylene (e.g., Lovley et al. 1989; Lovley and Lonergan 1990; Lovley and Anderson 2000; Jahn et al.

2005). If at contaminated sites ferric iron oxides are available for dissimilatory iron-reducing bacteria and other essential nutrients for microbial growth (e.g., nitrogen, phosphorous, sulfur) are present, microbial Fe(III) reduction has the potential to significantly contribute to the degradation of aromatic pollutants in a process termed 'natural attenuation'.

Iron minerals as adsorbents

Many ferric iron mineral surfaces are positively charged at neutral pH due to their high points of net zero charge (ZPC). The pH ZPCs are ~7.9 for ferrihydrite, ~8.5 for hematite and ~9.0–9.4 for goethite (Cornell und Schwertmann 2003). Such iron oxides therefore constitute good adsorbents for negatively charged compounds like phosphate (PO_4^{3-}), bicarbonate (HCO_3^-) and oxyanions of toxic metal ions such as arsenate (AsO_4^{3-}), arsenite (AsO_3^{3-}) or chromate (CrO_4^{2-}). Furthermore, negatively charged natural organic matter (humic substances) also binds strongly to ferric iron mineral surfaces (Stumm and Morgan 1996; Cornell and Schwertmann 2003). Anions were shown to adsorb to ferrihydrite surfaces via replacement of surface hydroxyl groups, leading to tight bonds of almost covalent character. For weak organic acids also an outer-sphere adsorption via weak electrostatic interactions was observed. Cations usually adsorb to iron oxides via hydroxyl-bridged inner-sphere complexes at the oxide surface. A comprehensive overview on adsorption processes on iron oxides is given by Cornell and Schwertmann (2003).

Transformation of iron minerals and pH changes in the environment both influence the adsorption of cations and anions to ferric iron oxides. In some cases this has dramatic consequences, as in the well-documented example of arsenic: Arsenite and arsenate both strongly bind to ferric iron oxides (Dixit and Hering 2003). There is evidence from extended X-ray absorption fine structure (EXAFS) studies for inner sphere complexation but the nature of the surface complexes is still controversial (e.g., Waychunas et al. 1993; Shermann and Randall 2003). The microbially induced reductive dissolution of arsenic-loaded iron oxides is thought to play a key role in As-release into the groundwater, which leads to enormous drinking water contaminations observed in countries such as Bangladesh and India (Cummings et al. 1999; Smedley and Kinniburgh 2002; Islam et al. 2004; Harvey et al. 2005). In addition, arsenate can be released into the groundwater when high-surface area ferrihydrite transforms into hematite or goethite with significantly lower surface areas (Ford 2002).

Immobilization of toxic metal ions by microbial Fe(II) oxidation and Fe(III) reduction

Reductive dissolution of metal-loaded iron oxides releases adsorbed metal ions into the environment. In contrast, Fe(II) oxidation can lead to the immobilization of toxic metal ions. Either co-precipitation during Fe(II) oxidation (Gunkel 1986; Richmond et al. 2004) or adsorption to synthetic or natural iron oxides potentially provides an applicable biotechnological method to remove toxic metal ions such as arsenic efficiently from drinking water. Natural removal of arsenic by iron oxides was observed when ferrihydrite was precipitated together with arsenic from arsenic- and iron-rich hydrothermal fluids (Pichler and Veizer 1999).

In addition to mobilization of adsorbed compounds by dissolution of Fe(III) minerals or immobilization of pollutants by adsorption to or co-precipitation with biogenic iron minerals, iron-metabolizing microorganisms can also have a more direct effect on the fate of pollutants. For example, Fe(III)-reducing microorganisms were shown to convert toxic metal ions from more soluble forms (e.g., Cr(VI) and U(VI)) to less soluble forms that are likely to be immobilized in the subsurface (e.g., Cr(III) and U(IV)) (e.g., Lovley 1993; Lovley and Phillips 1992).

Formation of reactive iron minerals

During microbial Fe(III) reduction, different minerals are formed depending on the chemical composition of the medium, on the substrate concentrations and on the incubation

conditions (e.g., Roden and Zachara 1996; Lovley 1997; Fredrickson et al. 1998; Urrutia et al. 1999; Benner et al. 2002; Zachara et al. 2002; Hansel et al. 2003; Kukkadapu et al. 2004). In particular, the presence of different counter ions leads to the precipitation of different Fe(II) minerals, e.g., iron mono- or disulfides ('FeS' or FeS₂), ferrous iron phosphate (vivianite), carbonate (siderite) or magnetite (Cornell and Schwertmann 2003). Also transformation of ferrihydrite to the more crystalline iron oxides hematite and goethite was observed during Fe(III) reduction (Hansel et al. 2003). Knowing the products of microbial Fe(III) reduction is quite important since the Fe(II)-species formed as the result of microbial Fe(III) reduction (either Fe(II) minerals or mineral-adsorbed and thus activated Fe(II)-species) can be efficient reductants in contrast to free aqueous Fe(II). They were shown to reduce organic contaminants such as nitroaromatic and chlorinated organic compounds (Hofstetter 1999) but also to reduce inorganic compounds such as U(VI) and Cr(VI) (Buerge and Hug 1999; Liger et al. 1999; Lovley and Anderson 2000; Jeon et al. 2005). Because different Fe(II)-species show different reactivities with respect to reductive pollutant transformation, understanding the mechanisms and conditions leading to different Fe(II)-species is necessary (Haderlein and Pecher 1999; Pecher et al. 2002; Elsner et al. 2003).

SOME TASKS FOR FUTURE INVESTIGATIONS

Prokaryotes that gain energy from iron redox transformations have a strong influence on the geochemistry of pristine or polluted environments. This was recognized only in the recent past and still needs to be studied in more detail. In particular the influence of the microbial cycle of iron on the global cycles of other elements, such as carbon, nitrogen or sulfur is not completely understood. The majority of ferrous iron-oxidizing and ferric iron-reducing prokaryotes were isolated during the last decade. Therefore, it is not surprising that our knowledge of these prokaryotes and their iron metabolism is still in its infancy. One major task in microbial physiology is to explain how prokaryotes transfer electrons from or to iron minerals. In this context, the combination of physiology with molecular genetics to track the activity of certain proteins is very promising as recently summarized by Croal et al. (2004) and reviewed by Newman and Gralnick (2005). Anticipated genomic data from isolates (as described by Nelson and Methé 2005) and natural communities (as discussed by Whitaker and Banfield 2005) will assist in the identification of targets. Furthermore, the identification of Fe(III)-chelating and electron-shuttling molecules intentionally produced and released by prokaryotes is key to understand the biological and ecological importance of these postulated mechanisms. The advancement of different microscopic methods, e.g., cryo transmission electron or environmental scanning electron microscopy, will help to describe intimate interactions between microorganisms and iron minerals. Finally, consequences of microbial iron transformations for the fate of organic and inorganic pollutants have to be explored in more detail to better understand the process of natural attenuation and to foster remediation of polluted sites. An interdisciplinary approach as pursued in the emerging field of geomicrobiology which comprises such diverse fields as microbial physiology, molecular genetics, geochemistry and mineralogy will certainly help to answer many open questions.

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