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Microorganisms pumping iron: Anaerobic microbial iron oxidation and reduction

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Abstract

Iron (Fe) has long been a recognized physiological requirement for life, yet for many microorganisms that persist in water, soils and sediments, its role extends well beyond that of a nutritional necessity. Fe(II) can function as an electron source for iron-oxidizing microorganisms under both oxic and anoxic conditions and Fe(III) can function as a terminal electron acceptor under anoxic conditions for iron-reducing microorganisms. Given that iron is the fourth most abundant element in the Earth's crust, iron redox reactions have the potential to support substantial microbial populations in soil and sedimentary environments. As such, biological iron apportionment has been described as one of the most ancient forms of microbial metabolism on Earth, and as a conceivable extraterrestrial metabolism on other iron-mineral-rich planets such as Mars. Furthermore, the metabolic versatility of the microorganisms involved in these reactions has resulted in the development of biotechnological applications to remediate contaminated environments and harvest energy.

At pH values at or above a circumneutral pH (~pH 7), iron (Fe) exists primarily as insoluble, solid-phase minerals in the divalent ferrous (Fe(II)) or trivalent ferric (Fe(III)) oxidation states[1]. The solubility of Fe(III) increases with decreasing pH values[2]. Decreasing pH values also enhance the stability of Fe(II), and below pH 4.0, Fe(II) primarily exists as an aqueous species, even in the presence of oxygen. The biogeochemical role of Fe(II)-oxidizing microorganisms (FOM) in acidic environments has been well established (reviewed in Reference 3). At a circumneutral pH, microbial iron redox cycling can significantly affect the geochemistry of hydromorphic soils (that is, soils showing poor drainage) and sediments, leading to the degradation of organic matter, mineral dissolution and weathering, the formation of geologically significant minerals, and the mobilization or immobilization of various anions and cations, including contaminants.[1, 4, 5]

The redox transition between the Fe(II) and Fe(III) valence states has a fundamental role in modern environmental biogeochemistry and was probably an important biogeochemical process on early Earth. Before microbially mediated iron redox reactions were discovered, abiotic mechanisms were thought to dominate environmental iron redox chemistry. However, it is now accepted that microbial metabolism primarily controls iron redox chemistry in most environments. Microorganisms from both the Archaea and Bacteria domains are capable of metabolically exploiting the favourable redox potential between the Fe(III)/Fe(II) couple and various electron donors or acceptors. In this way, Fe(II) is used as an electron donor to provide reducing equivalents for the assimilation of carbon into biomass by lithotrophic FOM under both oxic and anoxic conditions, and Fe(III) can be used as a terminal electron acceptor under anaerobic conditions for lithotrophic and heterotrophic Fe(III)-reducing microorganisms (FRM) (Figure 1). This Review discusses the intricate biogeochemical role of microbially mediated iron oxidation and reduction in suboxic environments, detailing the metabolisms, the microorganisms and the mechanisms described so far.

Lithotrophic
A lithotrophic organism uses an inorganic substrate (usually of mineral origin) to obtain energy for growth.

Heterotrophic
A heterotrophic organism requires organic compounds as a carbon source.

Iron cycling: the microbial redox workout

In the anoxic zone of neoteric environments at pH >4.0, Fe(III) oxides are readily reduced and provide an important electron sink for both chemical and biological processes. It is now established that microbial reduction of Fe(III) oxide minerals by FRM primarily controls the Fe(III)-reductive process in non-sulphidogenic sedimentary environments[6] (Figure 1). Even in some sulphidogenic environments (for example, some marine sediments) where Fe(III) reduction is traditionally thought to result from an abiotic reaction with biogenic hydrogen sulphide (H_2S), direct enzymatic Fe(III) reduc-

tion by FRM has been shown to be substantial, and the activity of these organisms might account for as much as 90% of the oxidation of organic matter[7]. Organic carbon compounds are not the only electron donors that FRM are capable of utilizing. These microorganisms are also capable of utilizing inorganic electron donors such as hydrogen (H_2) (reviewed in Reference 6). Ammonium might have a role in Fe(III) reduction as an electron donor[8], however, direct pure-culture evidence is still required to support this observation. The activity of FRM results in the generation of aqueous Fe(II) ($Fe(II)_{aq}$), and solid-phase Fe(II)-bearing minerals ($Fe(II)_s$) including siderite, vivianite and geologically significant mixed-valence Fe(II)–Fe(III) minerals, such as magnetite and green rust.[9, 10] The ubiquity and phylogenetic diversity of FRM, combined with the elemental abundance of iron in the earth's crust, establishes the global significance of this metabolic process.[6]

Similarly, microbially mediated Fe(II) oxidation is also known to contribute to the dynamic iron biogeochemical cycle at circumneutral pH in both oxic and anoxic environments (Figure 1). The recent identification of FOM in various aquatic and sedimentary systems — both freshwater and marine — indicates that Fe(II) undergoes both biotic and abiotic oxidation in the environment. Abiotic oxidation of $Fe(II)_{aq}$ can be mediated by reaction with oxidized manganese (Mn(IV)) species or by the diffusion of $Fe(II)_{aq}$ into an oxic environment where it subsequently reacts with molecular oxygen (O_2). Disruption of sediments by macrophytes and macrofauna can induce particle mixing and aeration, resulting in the subsequent oxidation of both $Fe(II)_{aq}$ and $Fe(II)_s$ (References 11, 12). However, so far, microbially mediated oxidative processes in direct association with bioturbation have not been studied to any significant extent, with such studies being limited to the rhizosphere.[13, 14, 15] Microaerophilic FOM capable of competing with the abiotic oxidation kinetics between oxygen and Fe(II) have been shown to contribute to iron cycling in oxic environments, coupling this metabolism to growth.[16, 17, 18, 19]

Although the aerobic microbial oxidation of Fe(II) has been recognized for decades, the recent identification of anaerobic Fe(II) bio-oxidation closed a missing gap in the iron redox cycle.[20, 21] Recent evidence indicates that anaerobic Fe(II) oxidation can contribute to a dynamic anaerobic iron redox cycle[22, 23, 24], in addition to soil and sediment biogeochemistry, mineralogy, and heavy-metal and radionuclide immobilization.[4, 5, 24, 25] In anoxic environments, microbial Fe(II) oxidation has been demonstrated to be coupled to the reduction of nitrate, perchlorate and chlorate[21, 24, 26] (Figure 1). These FOM can oxidize $Fe(II)_s$ (References 4, 25), and Fe(II) associated with structural iron in minerals, such as the iron aluminum silicate almandine ($Fe_3Al_2(SiO_4)_3$) or staurolite ($(Fe,Mg,Zn)_2Al_{9\gamma}(Si,Al)_4O_{22}(OH)_2$) (References 4, 27). In zones of sufficient light penetration, Fe(III) can also be produced through the activity of Fe(II)-oxidizing phototrophic bacteria that utilize Fe(II) as a source of electrons to produce reducing equivalents for the assimilation of inorganic caβrbon (Figure 1). FOM are ubiquitous and have been identified in numerous environments. Anaerobic, biogenically formed

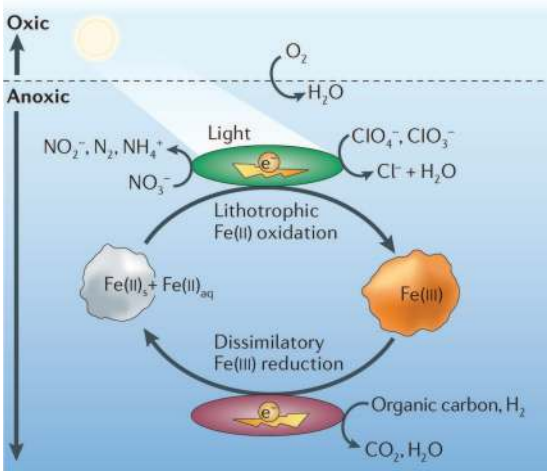


Figure 1. The microbially mediated iron redox cycle. Microorganisms have a significant role mediating iron oxidation and reduction reactions in soils and sedimentary environments. The reduction of Fe(III) oxides occurs in the absence of oxygen. The re-oxidation of the biogenic Fe(II) can occur through several biological mechanisms and is not simply limited to abiotic reactions with molecular oxygen. The regeneration of Fe(III) in the anoxic environment promotes a dynamic iron redox cycle.

Fe(III) oxides produced through the activity of these organisms potentially function as terminal electron acceptors for FRM, thereby perpetuating a dynamic microbially mediated iron redox cycle (Figure 1).

Microbial Fe(II) oxidation

The microbial oxidation of Fe(II) coupled to the reduction of oxygen in environments at acidic and circumneutral pH values has been recognized for more than a century (References 16, 28 and references therein). Yet, despite this historical knowledge, the geological significance of aerobic Fe(II) oxidation at circumneutral pH had been discounted based on the rapid rate of abiotic Fe(II) oxidation coupled to oxygenreduction[29]. It is now known that a broad diversity of microorganisms exist that are capable of aerobic, neutrophilic fe(II) oxidation and, although only three genera (Gallionella, Leptothrix and Marinobacter) have been described so far, several microaerophilic neutrophilic FOM were recently identified belonging to the α-, β-, and γ-proteobacteria[19, 30].

The environments that could potentially support aerobic, neutrophilic Fe(II) oxidation are stream sediments, groundwater iron seeps, wetland surface sediments, sediments associated with the rhizosphere, cave walls, irrigation ditches, subsurface bore holes, municipal and industrial water distribution systems, deep-ocean basalt and hydrothermal vents[30]. In these environments, microaerophilic FOM seem to successfully compete with the kinetics of abiotic Fe(II) oxidation. Although the quantitative significance of this microbial metabolic process in terms of accelerating Fe(II) oxidation rates is subject to interpretation, there is unequivocal evidence to demonstrate that FOM can conserve energy from this process and convert inorganic carbon, in the form of carbon dioxide (CO_2), into biomass[17].

Suboxic
An environment with a partial pressure of oxygen that is substantially lower than the atmospheric oxygen content.

Anoxic
An environment lacking oxygen.

Neoteric environments
Modern environments.

Electron sink
A compound that receives electrons as an endpoint of an oxidative reaction.

Bioturbation
The disturbance of sediment layers by biological activity.

Microaerophilic
An organism that is an obligate anaerobe but can survive in environments where the partial pressure of oxygen is substantially lower than in the atmosphere.

Phototrophic
A phototrophic organism obtains energy for growth from sunlight; carbon is derived from inorganic carbon (carbon dioxide) or organic carbon.

Neutrophilic Fe(II) oxidation
Microbial Fe(II) oxidation that occurs at circumneutral pH values (~pH 7).

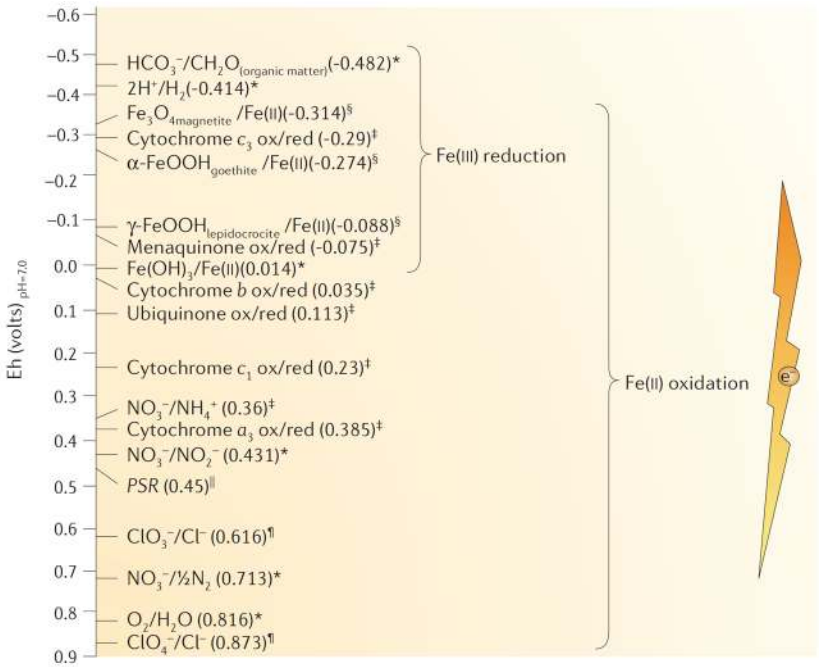


Figure 2. Potential electron donors and acceptors: a redox tower. Theoretical Eh (volts) values for reduction–oxidation couples that are significant to microbially mediated iron redox cycling, calculated at circumneutral pH, are shown. The redox tower is an effective way to visualize the potential electron donors and acceptors utilizable by microorganisms measuring the tendency of a compound to interact as an electron donor or acceptor. An electron donor will have a greater negative potential than the electron acceptor will. The directional flow of electrons (e-) is denoted by the orange (negative) to yellow (positive) bolt. Reduction potentials were obtained from the following sources: *Reference 149, ‡Reference 101, §Reference 150 and ‖Reference 151. ¶Calculated based on data collected from References 2 & 48.

Aerobic Fe(II) oxidation not only has a role in iron redox reactions at the oxic–anoxic interface, it also influences mineral weathering in the environment[19].

Before oxygen was available in the Fe(II)-rich Precambrian environment, anaerobic microbial oxidation of Fe(II) potentially provided an early respiratory metabolism, contributing to the precipitation of iron oxide minerals, including magnetite[4, 20, 31, 32]. The reduction potential of the Fe(III)/Fe(II) couple is sufficient to provide reducing power between bacterial photosystems or alternative terminal electron acceptors involved in respiratory processes (Figure 2) to sustain microbial growth. In the past decade the true extent of this metabolism has been realized and anaerobic Fe(II) oxidation mediated by members of both the Archaea and Bacteria domains has been described in anoxic environments at circumneutral pH[20, 21, 31].

Seeing the light — anaerobic, photoautotrophic oxidation of Fe(II). The discovery of photoautotrophic, anaerobic Fe(II) oxidation was the first demonstration of microbially mediated oxidation of Fe(II) in anoxic environments[20]. The FOM involved in this process oxidize Fe(II), utilizing light energy to fix CO_2 into biomass as shown below.

$$HCO_3^- + 4Fe(II) + 10H_2O \rightarrow CH_2O + 4Fe(OH)_3 + 7H^+$$

Although the phototrophic FOM in the Bacteria domain are phylogenetically diverse, including *Chlorobium ferrooxidans, Rhodovulum robiginosum, Rhodomicrobium vannielii, Thiodictyon* sp., *Rhodopseudomonas palustris* and *Rhodovulum* spp. (Figure 3), so far, an archaeon capable of this metabolism has not been identified.

Several purple and green anaerobic, anoxygenic, photosynthetic FOM have been isolated from freshwater and marine environments and described in pure culture[20, 32, 33, 34, 35, 36]. With the exception of *R. vannielii*, these phototrophic FOM can completely oxidize $Fe(II)_{aq}$ to Fe(III). Incomplete Fe(II) oxidation by *R. vannielii* has been attributed to encrustation of the bacterial cell with biogenic Fe(III) oxides, inhibiting further metabolic activity[20, 34]. The production of low-molecular-weight compounds that can solubilize biogenic Fe(III) has been suggested as a mechanism for preventing cell encrustation in cultures of other phototrophic FOM, including *R. robiginosum* and *C. ferrooxidans*[33, 37]. In contrast to *R. vannielii*[37], the concentrations of soluble Fe(II) and Fe(III) in spent culture media of *R. robiginosum* and *C. ferrooxidans* far exceeded the amount predicted by solubility constants and an uninoculated control[37]. However, a subsequent study failed to find significant evidence to support a direct role for organic compounds or chelators complexing iron when *R. robiginosum*, *C. ferrooxidans* and *Thiodictyon* sp. strain F4 were grown on $Fe(II)_{aq}$ or solid-phase ferrous sulphide (FeS)[38]. As such, it was proposed that Fe(III) was released from the active cell as an inorganic aqueous complex or colloidal aggregate[38]. Nonetheless, it remains to be explained why *R. vannielii* is subject to cell encrustation whereas the other phototrophic FOM are not affected. The production of compounds capable of solubilizing Fe(III) has profound implications not only for FOM cellular metabolism but also for the dissolution of solid-phase minerals and the release of soluble iron as a terminal electron acceptor or a micronutrient for other aquatic and terrestrial living organisms.

Phototrophic Fe(II) oxidation results in the formation of poorly crystalline Fe(III) oxides, which subsequently transform into the more crystalline Fe(III) oxide minerals goethite and lepidocrocite in the presence of metabolically active FOM[38]. However, the significance of phototrophic Fe(II) oxidation processes in natural terrestrial environments is limited by the maximum penetration of light to a depth of 200 μm in soil and sediments[39]. Furthermore, recent studies indicate that phototrophic FOM are unable to promote Fe(II) mineral dissolution and are limited by the mineral solubility[38]. Therefore, the impact of this microbial process on iron redox cycling and mineral weathering might be significant locally but of only minor importance in global iron biogeochemical cycling in terrestrial environments.

Working in the dark — anaerobic, nitrate-dependent Fe(II) oxidation. Anaerobic Fe(II) oxidation is not limited to environments exposed to light. At circumneutral pH, light-independent microbially mediated oxidation of both soluble and insoluble Fe(II) coupled to nitrate reduction has been demonstrated in various freshwater and saline environmental systems, including paddy soil, pond, stream, ditch, brackish lagoon, lake, wetland, aquifer, hydrother-

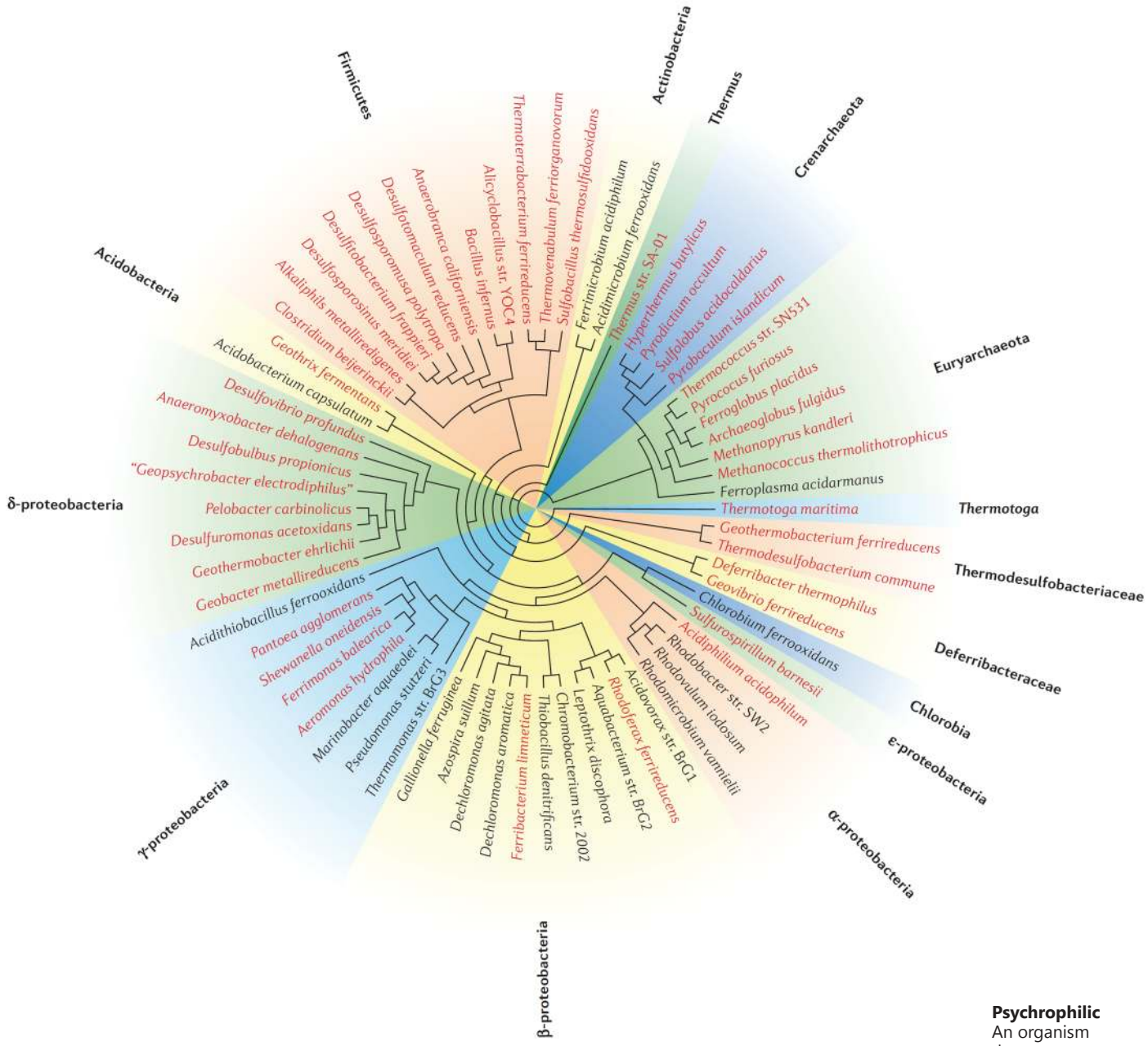


Figure 3. Phylogenetic affiliation of microorganisms contributing to iron redox cycling. Unrooted phylogenetic tree based on nearly complete 16S ribosomal DNA sequences from representative iron cycling prokaryotes. The names of iron reducers are given in red text, iron oxidizers are given in black text.

mal, and deep-sea sediments[4, 19, 21, 25, 27, 31, 40, 41, 42, 43]. These environmental systems support abundant nitrate-dependent Fe(II)-oxidizing microbial communities in the order of 1×10^3 to 5×10^8 cells g^{-1} sediment (Reference 44 and references therein), potentially contributing to iron redox cycling.

Various archaeal and bacterial genera (Figure 3), representing a range of optimal thermal growth conditions (from psychrophilic through mesophilic to hyperthermophilic), have been identified. The ubiquity and diversity of these anaerobic FOM suggests that metabolic, light-independent reactions such as nitrate-dependent Fe(II) oxidation have the potential to contribute to anoxic Fe(II) oxidation on a global scale, provided that adequate concentrations of a suitable electron acceptor are readily available. In environments where zones of nitrate reduction and Fe(III) reduction merge, this metabolism not only influences the iron cycle but can also influence the nitrogen cycle. However, the quantitative significance of this metabolism to global nitrogen cycling is currently unknown.

So far, FOM have been demonstrated to exploit the favourable thermodynamics between $Fe(OH_3)$/Fe(II) and nitrate reduction redox pairs ($NO_3^-/\frac{1}{2}N_2$, NO_3^-/NO_2^- and NO_3^-/NH_4^+)[21, 24, 26, 31], and between $Fe(OH_3)$/Fe(II) and perchlorate (ClO_4^-/Cl^-) and chlorate (ClO_3^-/Cl^-)[26] (Figure 2). Several phylo-

Psychrophilic
An organism that grows optimally in a cold environment (<15°C).

Mesophilic
An organism that grows optimally in a moderate environment (~25–45°C).

Hyperthermophilic
An organism that grows optimally in hot environments (>80°C).

Autotrophic
An autotrophic organism uses inorganic carbon (carbon dioxide) as a carbon source.

Chemolithoautotrophic
An organism that obtains energy from inorganic compounds and carbon from carbon dioxide.

gentically diverse mesophiles have been described as being capable of nitrate-dependent Fe(II) oxidation (Figure 3). However, in most cases growth was either not associated with this metabolism or was not demonstrated in the absence of an additional electron donor or organic carbon as an energy source at circumneutral pH[4, 21, 26, 27]. The oxidation of Fe(II), including $Fe(II)_s$, coupled to nitrate reduction is energetically favourable at neutral pH and should yield enough energy to support carbon fixation and microbial growth. However, so far autotrophic growth under nitrate-dependent Fe(II)-oxidizing conditions has only been demonstrated in two pure-culture isolates; a hyperthermophilic archaeon, Ferroglobus placidus[31], and a mesophilic β-proteobacterium, strain 2002 (Reference 45). Even in the case of a known Fe(II)-oxidizing chemolithoautotrophic bacterium, *Thiobacillus denitrificans*, energy conservation directly coupled to the nitrate-dependent oxidation of Fe(II) could not be demonstrated without an additional electron donor[46]. Nitrite (NO_2^-) and nitrogen gas (N_2) were thought to be the sole end products of nitrate reduction[4, 21, 31] until the recent demonstration of nitrate-dependent Fe(II) oxidation by *Geobacter metallireducens* resulting in the production of ammonium[24].

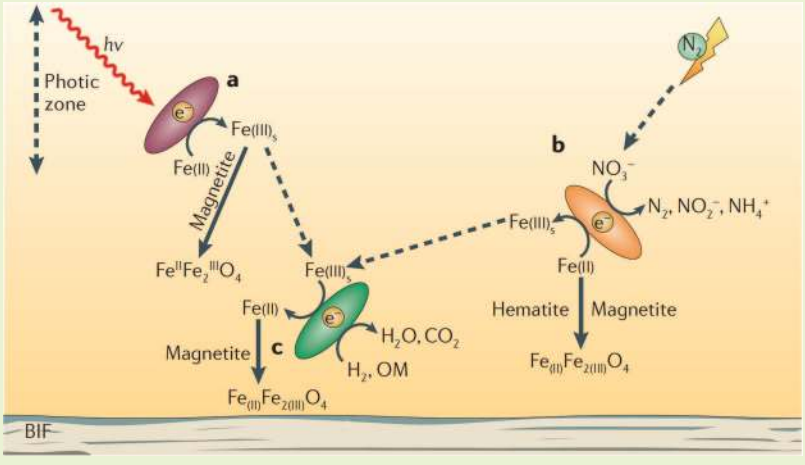
Box 1. Banded iron formations

The deposition of alternating iron-rich and silica-rich mineral layers in the late Fe(II)-rich Archean to early Proterozoic periods of the Earth's history resulted in the genesis of the Precambrian banded iron formations (BIFs)[125] (see figure). Early models suggested that BIFs were a consequence of abiotic reactions involving Fe(II) photo-oxidation[51, 126] and/or Fe(II) oxidation by the metabolic end product of oxygenic photosynthesis — oxygen (Reference 127). This led to the formation of the iron-rich laminae, containing hematite (Fe_2O_3) and the mixed-valence phase Fe(II)–Fe(III)-bearing mineral magnetite (Fe_3O_4).

In recent years, microbial reductive and oxidative respiratory metabolisms have been implicated in the deposition of iron-bearing minerals in the iron-rich laminae of BIFs[4, 20, 128, 129, 130]. Microaerophilic Fe(II) oxidizers, such as Gallionella ferruginea, might have metabolically oxidized Fe(II) coupled to the oxygen generated by oxygenic photosynthesis[129, 131]. However, this model assumes that oxygenic photosynthesis evolved enough oxygen to account for the precipitation of Fe(III)-rich minerals. There has been much debate concerning the evolution of oxygen in the early Proterozoic and therefore the role of oxygen in the deposition of BIFs[132, 133]. Other microbially mediated mechanisms influencing the precipitation of Fe(II)- and Fe(III)-rich minerals in anoxic environments have been proposed as plausible alternatives, including Fe(III) reduction, phototrophic Fe(II) oxidation, and nitrate-dependent Fe(II) oxidation (see figure).

The precipitation of magnetite associated with microbial Fe(III) reduction linked the deposition of BIFs to FRM[9, 53]. Yet the generation of Fe(III) as a terminal electron acceptor from available Fe(II) would have to precede metabolic Fe(III) reduction. In addition to photo-oxidation, the anoxic deposition of Fe(III) oxides by photoautotrophic[20, 130] and nitrate-dependent Fe(II)-oxidizing microorganisms[4] (FOM) during the late Archean to the early Proterozoic has been proposed (see figure). However, the bio-oxidation of Fe(II) not only results in the precipitation of Fe(III) oxides but also results in the direct precipitation of magnetite[4, 32, 36] and hematite[4], potentially linking FOM directly to the formation of BIFs. The availability of light in the Precambrian is inarguable, as such phototrophic Fe(II) oxidation has received much attention as a model for BIF deposition[130]. By contrast, light-independent Fe(II) oxidation coupled to nitrate reduction has received much less attention as a model; however, it does provide an additional mechanism leading to BIF formation in the Precambrian and, in contrast to known phototrophic FOM, extant nitrate-dependent FOM have been shown to produce significant quantities of extracellular magnetite and hematite[4, 32, 36]. Lightning discharge contributed to the fixation of nitrogen (N_2) into nitric oxide (NO (~10^{12} g per yr) further forming nitrous oxide (N_2O), the nitrite ion (NO_2^-) and the nitrate ion (NO_3^-) through abiotic disproportion reactions[134, 135]. Such oxidized nitrogen species could have functioned as an electron acceptor for early FOM, leading to BIFs in anoxic environments.

In modern environmental systems, complex microbial communities contribute to a dynamic anoxic iron redox cycle. Similarly complex communities consisting of phototrophic FOM, nitrate-dependent FOM and Fe(II)-reducing microorganisms (FRM) might have existed in the Precambrian era, each of which contributed to the formation of the BIFs rather these being the result of one individual metabolism. The precipitation of biomass with biogenic Fe(III) oxides would contribute to diagensis and mineralization of organic matter, resulting in heterotrophic reduction of Fe(III) by FRM and the formation of additional magnetite in BIFs[53, 130], which is further supported by the isotopically light carbon associated with BIFs[136].



The figure shows the models proposed for the microbial mediation of anoxic deposition of BIF. The deposition of Fe-rich laminae, hematite and magnetite, could be a result of anoxic microbial metabolisms. The direct oxidation of Fe(II) by photoautotrophic bacteria (a) and nitrate-dependent Fe(II) oxidizing bacteria (b) results in the formation of magnetite and hematite, and solid-phase Fe(III) oxides. The biogenic Fe(III) oxides are subsequently reduced by FRM (c) forming magnetite.

Growth of FOM that are capable of autotrophy or mixotrophy associated with perchlorate/chlorate-dependent Fe(II) oxidation has not yet been identified. However, perchlorate/chlorate-dependent Fe(II)-oxidizing metabolic activity is observed in stationary-phase cultures of *Dechloromonas agitata* and *Azospira suillum* strain PS (previously called *Dechlorosoma suillum*) containing acetate, perchlorate and Fe(II) (References 4, 47). Although perchlorate and chlorate are not naturally abundant compounds, their potential to serve as electron acceptors in environmental systems cannot be discounted[48]. The legal discharge of perchlorate into natural waters has led to widespread anthropogenic contamination throughout the United States. Given the ubiquity of perchlorate-reducing bacteria[48] and the ability of some of these microorganisms, specifically *A. suillum* and *D. agitata*, to oxidize Fe(II), anaerobic perchlorate/chlorate-dependent Fe(II) oxidation could affect iron biogeochemical cycling in environments exposed to contaminated waters. Further studies are needed to quantify the potential influence of perchlorate/chlorate-dependent Fe(II) oxidation.

Doing hard time: solid-phase microbial Fe(II) oxidation. In contrast to the reaction carried out by phototrophic FOM, $Fe(II)_s$ including surface-bound Fe(II) (References 24, 25), crystalline Fe(II) minerals (siderite, magnetite, pyrite, arsenopyrite and chromite)[4, 25], and structural Fe(II) in neosilicate (almandine and staurolite)[4] and phyllosilicate (nontronite)[27] are known to be subject to direct nitrate-dependent microbial oxidation. Although we know that nitrate-dependent FOM have a role in the oxidation of Fe(II) that is structurally incorporated into silicate minerals, and contribute to Fe(II) mineral dissolution, little is known about the mineral structure and stability of the residual material. The oxidative dissolution of $Fe(II)_s$ in anoxic environments presents an additional mechanism for rock weathering and the precipitation of Fe(III) oxide minerals in anoxic soils and sediments. So far, bio-oxidation products of $Fe(II)_{aq}$ and amorphous $Fe(II)_s$ have been characterized. Various biogenic Fe(III) oxide minerals, including 2-line ferrihydrite[40, 47], goethite[24], lepidocrocite (J. D.C. and K.W., unpublished data) and hematite[4], and mixed-phase Fe(II)–Fe(III) minerals, magnetite, maghemite and green rust[4] (J.D.C. and K.W., unpublished data), were identified as oxidation products. As a result of this biogenic formation of magnetite and hematite, nitrate-dependent Fe(II) oxidation has been implicated as having a direct role in the genesis of banded iron formations in Precambrian Earth[4, 31] (Box 1).

Function over form: physiology of anaerobic Fe(II) oxidation at circumneutral pH. Little is known about the biochemistry or genetic regulation of anaerobic Fe(II) oxidation at circumneutral pH and as such this can only be discussed in a general sense. The reduction potential of the possible Fe(III)/Fe(II) redox pairs ranges from –0.314V to +0.014V, indicating that electrons can readily be donated to the more electropositive type *b*, *c,* or *a* cytochrome components of an electron transport chain (Figure 2). In support of this, previous studies using the known FOM *D. agitata* and *A. suillum* demonstrated the involvement of *c*-type cytochrome(s) when grown under Fe(II)-oxidizing conditions with nitrate or chlorate, respectively[4, 26].

The demonstrated capability of some FOM to use CO_2 as the sole carbon source requires a CO_2-fixation pathway. In the case of the archaeon *Ferroglobus placidus* grown on CO_2, the reductive acetyl coenzyme A pathway is expressed, implicating its involvement in carbon assimilation[49]. Interestingly, genes associated with the reductive pentose phosphate cycle, RuBisCo, were identified in the finished genome sequence of *Dechloromonas aromatica*, an Fe(II)-oxidizing bacterium capable of utilizing nitrate, chlorate or perchlorate as alternative electron acceptors[45]; however, autotrophic growth associated with Fe(II) oxidation could not be demonstrated and the conditions under which these genes are expressed remain unidentified (J.D.C., unpublished data). By contrast, PCR amplification using degenerative RuBisCo primers of the genomic DNA from strain 2002, the mesophilic autotrophic nitrate-dependent FOM, did not yield a PCR product[45]. The CO_2-fixation pathway expressed in strain 2002 during growth under nitrate-dependent Fe(II)-oxidizing conditions is currently unknown. Nonetheless, the availability of genomic sequence information for the FOM *D. aromatica*, *Marinobacter aquaeolei, G. metallireducens*, *R. palustris*, and *T. denitrificans* provides the first opportunity to comprehensively investigate the functional genes and regulatory pathways associated with this metabolism. However, the genomes of other phylogenetically and physiologically distinct microorganisms such as *F. placidus*, strain 2002 and *A. suillum*, which also potentially have a key role in anaerobic nitrate-dependent Fe(II) oxidation, have yet to be sequenced.

The other half — microbial iron reduction

Antiquity of iron reduction. It has been proposed that life emerged on a hot (possibly as high as 140–150°C), Fe(II)-rich early Earth 3.8 billion years ago[50]. The abiotic photochemical generation of Fe(III) and H_2 would have provided an electron acceptor and energy source, respectively, for ancient life, as shown below (References 6, 51 and references therein).

$$2\,Fe(II) + 2\,H^+ \xrightarrow{h\nu} 2\,Fe(III) + H_2$$

As such, iron respiration has been proposed as one of the first forms of microbial metabolism to have evolved, preceding the development of oxygen, nitrate and sulphate respiration[52, 53]. In support of this, Fe(III) respiration has been identified in a diversity of extant microorganisms including those most closely related to the last common ancestor[52, 54]. Extracellular electron transfer to insoluble Fe(III) oxide minerals has been conserved in the hyperthermophilic Archaea[52, 54, 55, 56] and is widely distributed among the Bacteria[6, 53] (Figure 3), further suggesting that this is an early metabolism that has spread through the microbial domains throughout evolution.

Mixotrophy
A mixotrophic organism uses an inorganic chemical energy source and organic compounds as a carbon source.

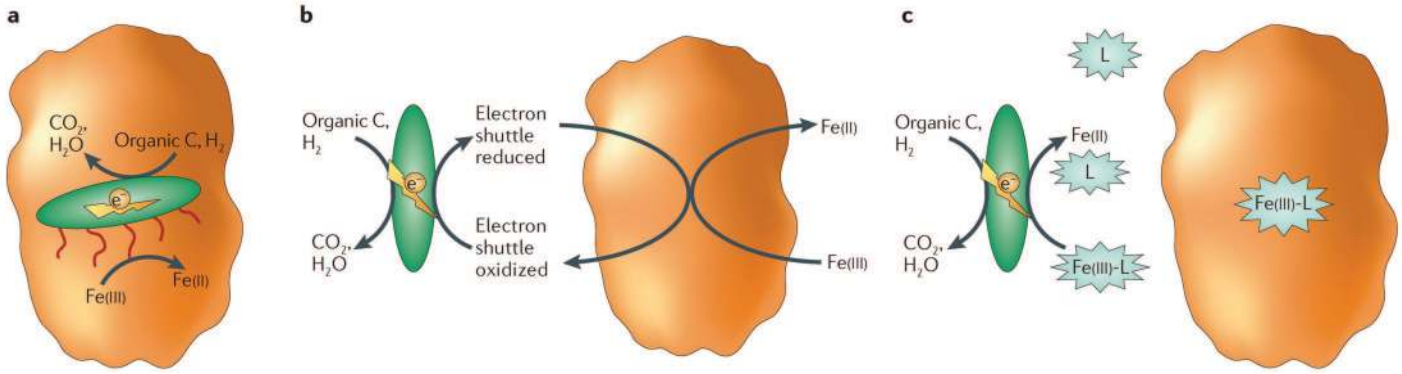


Figure 4. Microbial strategies mediating electron transfer to insoluble Fe(III) oxides. Three primary strategies have been proposed to facilitate the electron transfer between microorganisms and solid Fe(III) oxide surfaces. **a)** In *Geobacter* spp. direct contact with the oxide surface is required. The production of 'nanowires', conductive extracellular appendages, facilitates electron transfer by functioning as an electrical conduit to the Fe(III) oxide surface. **b)** An endogenously or exogenously produced electron shuttle mediates electron transfer to solid-phase Fe(III) oxides. **c)** The production of complexing ligands as in the case of *Geothrix* sp. aids in the dissolution of the solid-phase Fe(III) oxide providing a soluble Fe(III) form more readily available to the microorganism. Although these strategies have only been demonstrated for Fe(III)-reducing microorganisms, similar strategies might be used by Fe(II)-oxidizing microorganisms that are utilizing solid-phase Fe(II) electron donors. e^- = electrons; L = ligand

A hard rock chord: crystalline Fe(III) oxide reduction. Poorly crystalline Fe(III) oxide minerals, such as ferrihydrite, readily function as electron acceptors for FRM[6]. This is not surprising, given the relative energetic favourability of the reduction of amorphous ferric iron oxides (Figure 2). However, iron oxides predominantly exist in a crystalline phase or as a structural component of clays in modern soils and sediments. Although the thermodynamic favourability of crystalline Fe(III) oxide mineral reduction (goethite, hematite and magnetite) is decreased compared with amorphous Fe(III) oxides (Figure 2), previous studies have suggested that FRM are capable of living 'on the energetic edge,' utilizing structural[57] or crystalline solid-phase Fe(III) as an electron acceptor[58, 59]. There has been considerable debate regarding the direct environmental relevance of the laboratory studies that have examined the reduction of crystalline iron minerals under artificial organic and nutrient-rich conditions to optimize FRM growth[6, 60]. Glasauer and colleagues did not observe reduction of goethite and hematite in defined minimal media by *Shewanella putrefaciens* strain CN32, in direct contrast to previous reduction studies conducted with this organism in nutrient-rich media[10, 59]. However the inability of FRM to reduce crystalline Fe(III) oxide minerals under nutrient-limited conditions is not a universal observation. Under oligotrophic culture conditions, both a culture of *G. metallireducens* and a freshwater enrichment culture (1% vol:vol inoculum) reduced goethite beyond the amorphous Fe(III) oxide content (determined by 0.5 N HCl extraction)[24], indicating the possibility for microbial reduction of crystalline Fe(III) oxides in the environment.

Between a rock and a hard place: microbial strategies for reduction of an insoluble electron acceptor. The insoluble nature of Fe(III) oxide minerals at values >pH 4 creates a metabolic dilemma for microorganisms utilizing Fe(III) oxides as a respiratory terminal electron acceptor. Various mechanisms have been proposed as possible strategies that microorganisms might use to transfer electrons to extracellular Fe(III) oxide minerals. Direct contact between the microorganism and the solid-phase Fe(III) oxide mineral was shown to be a requirement for the reduction of insoluble Fe(III) oxides in *Geobacter* spp.[61] (Figure 4A). The molecular scale interaction(s) occurring between the cell surface and Fe(III) oxide is currently unknown.

The formation of flagella and pili had been proposed as the mechanism by which *Geobacter* spp. directly attached to the Fe(III) oxide surface[62]. Recent evidence, however, indicates that pili are not required for attachment of *Geobacter* spp. to the solid-phase Fe(III) oxide surface. Instead, the pili function as an electrical conduit for the transfer of electrons to insoluble Fe(III) oxides and, potentially, other solid-phase terminal electron acceptors[63]. The formation of these conductive cellular 'nanowires' expands the accessible spatial area available beyond the cell membrane, allowing the penetration of nanometer pore spaces in soils and sediments previously thought to be physically unavailable to the cell. The potential for these 'nanowires' to create a bridge between individual cells introduces the possibility of cell-to-cell communication[63], and for the cell attached to the Fe(III) oxide or other electron acceptor to function as an electron shuttle.

Conductive 'nanowires' were thought to be exclusive to *Geobacter* spp. as pili produced by other metal-reducing organisms tested, including *Shewanella oneidensis*, were not conductive[63]. However, recent evidence indicates that *S. oneidensis*, among other microorganisms, also produce conductive appendages under conditions in which the electron acceptor is limited[64]. The direct involvement of these appendages in electron transport or reduction of Fe(III) remains unproven and their functional role is currently unknown.

FRM might not necessarily need to establish direct contact with the solid-phase surface to reduce Fe(III) oxides, as other alternative active mechanisms could be used. Exogenous[65] and endogenously produced[66, 67, 68] soluble external electron shuttles can be exploited as mediators to complete the transfer of electrons to the solid-phase terminal electron acceptor (Figure 4B). The electron shuttle alleviates the need for the FRM to directly contact the Fe(III) oxide and functions in a combination of a microbially catalysed and abiotic process; the FRM oxidizes an electron donor coupled to the reduction of the soluble electron shuttle and the reduced electron shuttle diffuses and subsequently donates electrons to the solid-phase Fe(III) oxide abiotically[65] (Figure 4B). The abiotic regeneration of the oxidized electron acceptor restarts the cycle (Figure 4B).

Redox-reactive organic compounds common in soils and sediments, such as humic acids[65], plant exudates[69] and antibiotics[70], have been identified as electron shuttles. Although the significance of these compounds as electron shuttles for microbially mediated Fe(III) reduction in eutrophic environments is still unknown, their utility in oligotrophic environments might be limited owing to the low availability of suitable redox-reactive refractory organic substances. However, the endogenous production of an electron shuttle in two FRM genera, *Shewanella*[66, 68, 71, 72] and *Geothrix*[67], and the production of a chelating ligand by *Geothrix* sp. (Fig 4c), could further mitigate the reliance of these FRM on exogenous electron shuttles.

Although the energetic expense of producing and excreting redox-reactive compounds into the environment would not yield a competitive advantage in situations of low cell mass, it has been speculated that the release of electron shuttles in a biofilm community would facilitate electron transfer by cells distant from the substrate surface.[72] In addition, the recently identified ubiquitous biological re-oxidation of these diffusing electron shuttles, coupled to carbon assimilation in the presence of a suitable electron acceptor such as nitrate, offers the potential for a previously unidentified symbiotic relationship at the microbial level.[73, 74]

The iron maidens: diversity of FRM. A wide phylogenetic diversity of microorganisms capable of conserving energy coupled to growth by the dissimilatory reduction of Fe(III) have been identified throughout the Archaea and Bacteria (Figure 3) and across a range of chemical and physical conditions, demonstrating the ubiquity of this type of microbial metabolism. Among the isolated microorganisms, Fe(III)-reducing extremophiles including hyperthermophilic, thermophilic, psychrophilic, acidophilic and alkaliphilic Archaea and Bacteria have been described in pure culture[54, 75, 76, 77, 78, 79] (see Lovley et al.[6] for a review). One such isolate surviving in hydrothermal vents has pushed the upper temperature limit for life above 121°C (Reference 54).

In modern terrestrial and subsurface environments at circumneutral pH, microorganisms in the family Geobacteraceae are among the most common and most comprehensively studied FRM (Reference 6 and references therein). The Geobacteraceae are thought to have a significant role in dissimilatory Fe(III) reduction and the oxidation of organic matter in soils and sediments. Outside the δ-proteobacteria, the genus *Shewanella* in the γ-proteobacteria is another well-characterized group of FRM. Although *Shewanella* spp. have been isolated from diverse metal-reducing sediments[80, 81, 82], several studies focused on recovering 16S ribosomal DNA (rDNA) gene sequences representing these FRM from natural Fe(III)-reducing environments have not yielded a positive result[83, 84, 85]. As a result, these data indicated that these microorganisms might only have a minor role in the reduction of Fe(III) oxides in situ. However, this result might not be universal; the recent identification of 16S rDNA gene sequences closely related to *Shewanella* spp. in a minerotrophic wetland indicates the potential for this group of FRM to contribute to iron cycling in situ[86].

Recent evidence indicates that other organisms belonging to the β-proteobacteria might also have a role in the reduction of Fe(III) in sediments, although the extent of this role has yet to be quantified[24, 85, 87, 88] (J.D.C., unpublished data). Additionally, enzymatic Fe(III) reduction is not limited to the Proteobacteria. The potential of the poorly described Acidobacteria to contribute to Fe(III) reduction was demonstrated with the isolation of *Geothrix fermentans*[89] and supported by the identification of 16S rDNA gene sequences most similar to this microorganism in an analysis of subsurface sediments.[90] So far, the exact metabolic role of members of this phylum in the environment remains elusive because of the limited availability of pure cultures. However, given that Acidobacteria are ubiquitous in the environment,[91, 92] the contribution of these microorganisms to iron biogeochemical cycling in soils and sediments could be globally significant.

In addition to the FRM described above, which rely on energy conservation for growth from the dissimilatory reduction of Fe(III), various fermentative microorganisms have also been recognized to catalyse the enzymatic reduction of Fe(III) (References 93 & 94 and references therein). These fermentative microorganisms transfer only a minor fraction, approximately 5%, of the available reducing equivalents to Fe(III) (Reference 94), and Fe(III) is not required for growth. The diversion of reducing equivalents to Fe(III) might provide an energetic advantage, utilizing the oxidation of NAD(P)H coupled to Fe(III) reduction to yield ATP.[95] As such, Fe(III) reduction coupled to fermentative metabolism is considered to have a minor role in iron geochemical cycling relative to respiratory Fe(III) reduction.

In addition to the fermentative microorganisms that have been identified that reduce Fe(III), some sulphate-reducing[96, 97] and methanogenic[98] microorganisms are also capable of Fe(III) reduction without the demonstrated benefit of growth. Competition for available electron donors between microbial communities supporting these metabolisms has been implicated in the suppression of sulphate reduction and methanogenesis in sedimentary environments.[99, 100] However, the direct enzymatic reduction of Fe(III), coupled to the oxidation of hydrogen by sulphate-reducing and methanogenic microorganisms, would directly inhibit sulphate-reducing and methanogenic metabolisms at the

Eutrophic
Eutrophic waters are rich in minerals and organic nutrients.

Oligotrophic environment
An environment that is relatively low in nutrients and cannot support much plant life.

Thermophilic
An organism that grows optimally at temperatures ranging from 45–80°C.

Acidophilic
An organism that grows in an acid environment (<pH 6).

Alkaliphilic
An organism that grows in an alkaline environment (pH 9–pH 11).

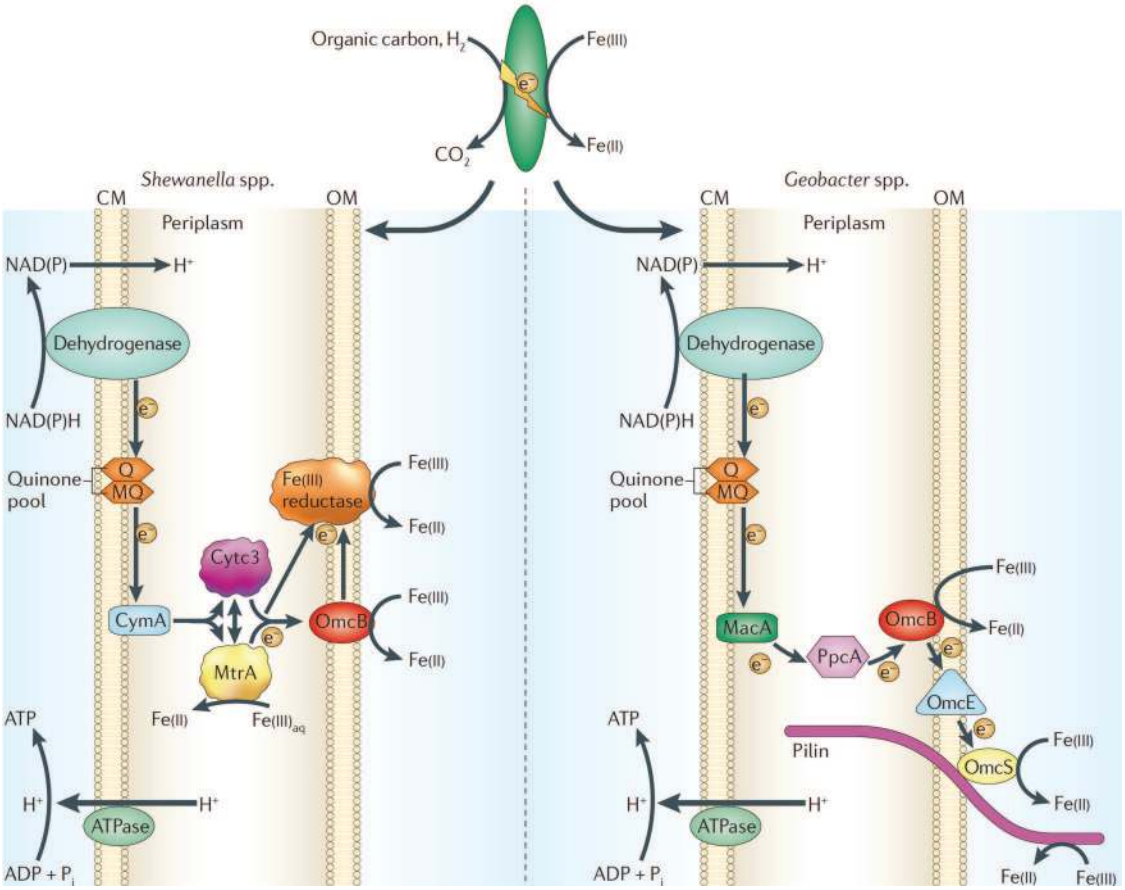


Figure 5. Physiological model of the biochemistry involved in microbial Fe(III) reduction by *Shewanella* and *Geobacter* spp. Full details of the model are given in the text. CM = cytoplasmic membrane; CymA = cytoplasmic-membrane-bound tetraheme *c*-type cytochrome; Cytc3 = periplasmic *c*-type cytochrome; e^- = electrons; MacA = cytoplasmic-membrane-bound cytochrome; MQ = menaquinone; MtrA = periplasmic decaheme *c*-type cytochrome; OM = outer membrane; Omc = outer-membrane-bound cytochromes B, E, and S partially exposed on the cell surface; PpcA = a triheme periplasmic *c*-type cytochrome; Q = quinone.

cellular level. The physiological advantage created by coupling hydrogen oxidization to Fe(III) reduction is unknown. In fact, this metabolism would scavenge reducing equivalents, impairing the ability of these microorganisms to grow with the transfer of electrons to sulphate or carbon dioxide. The true ecological implications of this alternative lifestyle still remain a mystery.

Go with the flow: electron transport to Fe(III) oxides. Iron(III) oxide minerals are insoluble and so are unable to diffuse inside microbial cells. Therefore electron transport to the solid-phase terminal electron acceptor cannot occur in the periplasm as it does for soluble electron acceptors such as nitrate[101] or fumarate in the case of *Shewanella frigidimarina*.[102] Instead, it would require the reduction of Fe(III) to occur outside the cell with a protein localized in the outer membrane, presumably a terminal iron reductase. Just as the strategies to utilize insoluble Fe(III) oxides as a terminal electron acceptor differ between the two model FRM, *Shewanella* spp. and *Geobacter* spp., the proteins involved in electron transport also differ. The only general similarity between the electron transport mechanisms involves the transfer of electrons from the dehydrogenase to a quinone pool consisting of ubiquinones and menaquinones in the cytoplasmic membrane to *c*-type cytochromes and finally to a reductase in the outer membrane[66, 103, 104, 105, 106]. Whereas a terminal iron reductase has so far eluded identification, significant developments have advanced our knowledge of Fe(III) reduction biochemistry in both model FRM.

The development of electron transport models is still in the formative stages as the number of *c*-type cytochromes directly implicated in Fe(III) reduction is far less than the number of *c*-type cytochromes that have been identified in completed genome sequences — the *S. oneidensis* and *G. sulfurreducens* genomes revealed 39 and 111 putative *c*-type cytochromes, respectively.[107, 108] The high number of *c*-type cytochromes in *G. sulfurreducens* has led to the suggestion of the existence of multiple electron-transport pathways.[109] The models depicted in Figure 5 illustrate the components known to be directly involved in electron transport to Fe(III) in both *Shewanella* spp. and *Geobacter* spp. so far.

In *Shewanella* spp. the electrons are transferred from the menaquinone to a tetraheme *c*-type cytochrome, CymA, located in the cytoplasmic membrane.[104, 110, 111] The electrons are then passed to electron carriers in the periplasm. Two *c*-type cytochromes in the periplasm have been identified so far as potential electron carriers necessary for Fe(III) reduction in *Shewanella* spp., MtrA and Cytc3 (References 112–114). Cytc3, a small tetraheme *c*-type cytochrome in the periplasm[115, 116] might function as an electron shuttle between electron carriers[117]. MtrA is a decaheme *c*-type cytochrome that is similarly thought to accept electrons from the cytoplasmic membrane electron carrier CymA, and transfer these electrons to an outer membrane protein (OMP)[113]. It has also been proposed that MtrA alternatively functions as a terminal reductase for soluble Fe(III) in the periplasm[114] (Figure 5). An outer membrane cytochrome partially exposed on the cell surface[113, 118], OmcB (formerly denoted as MtrC[113]), accepts electrons from periplasmic proteins and has the potential to reduce extracellular Fe(III) directly[118] (Figure 5). This is supported by studies involving a mutation in omcB that resulted in a decreased ability to reduce Fe(III). However, some Fe(III) reduction did still occur[119], indicating that Fe(III) reduction is not absolutely dependent on a functioning omcB (Figure 5).

Given the limited information available so far, the relationship between the periplasmic cytochromes and OMPs has not been firmly established in *Geobacter* spp.[120] A key periplasmic cytochrome, MacA, was identified in the cytoplasmic membrane and shown to have a central role in the transfer of electrons to Fe(III). MacA is predicted to function as an intermediate carrier similarly to MtrA in *Shewanella* spp.[120] and therefore might pass electrons to other periplasmic proteins such as PpcA, a tri-heme periplasmic *c*-type cytochrome involved in electron transport to OMPs[121] (Figure 5). One such OMP, OmcB, was determined to have a significant role in Fe(III) reduction[109, 122]. Disruptions in omcB by gene replacement impaired the ability of *G. sulfurreducens* to reduce Fe(III) by approximately 94–97% (Reference 122). However, the omcB-deficient mutant adapted to growth on soluble Fe(III) over time with similar reduction rates to the wild type, although growth was only approximately 60% of the wild type[109]. Interestingly, the adapted mutant was unable to reduce insoluble Fe(III) oxides, indicating that different electron transport mechanisms are used to reduce insoluble and soluble Fe(III) sources[109]. The recent identification of conductive 'nanowires' implicated the involvement of other OMPs in electron transport to Fe(III) oxides. Reguera et al.[63] have proposed that pili directly accept electrons from intermediary electron-transfer proteins located in the periplasm and/or outer membrane for transfer to the solid-phase Fe(III) oxide surface (Figure 5). In contrast to the appendages identified in *S. oneidensis*[64], redox-reactive structures have not been identified on the conductive pili observed in *Geobacter* spp.[63] The conductive nature of these pilin structures remains a mystery and introduces an exciting new twist to cell biology.

Box 2. Biotechnological applications

Heavy-metal and radionuclide immobilization

The end products of anaerobic microbial oxidation of Fe(II) can also affect the geochemistry of contaminants through the formation of various environmentally relevant Fe(III)-bearing minerals that can regulate contaminant solubility in natural environments,[5, 22, 43] such as ferric oxyhydroxide, goethite, hematite, iron hydrogen carbonate or maghemite.[4, 24, 40, 47] The precipitation of these biogenic Fe(III) oxides provides a mechanism for the immobilization of heavy metals and metalloids through co-precipitation or physical envelopment, and provides a reactive surface with an adsorptive affinity for anions (that is, PO_4^{3-}) and cations (that is, Zn(II), As(V), Co(II) and U(vi)) (References 5, 22). Heavy metals and radionuclides including U(VI) are rapidly removed from solution during anaerobic nitrate-dependent microbial Fe(II) oxidation in association with the biogenic Fe(III) oxides.[5] As such, the anaerobic formation of biogenic Fe(III)-oxide-containing minerals has been identified as a plausible bioremediative strategy for permanently immobilizing heavy metals and radionuclides.[5, 137]

Energy generation and contaminant immobilization or degradation

An electrode is another solid-phase electron acceptor used by Fe(II)-reducing microorganisms (FRM) that we can exploit to our energetic advantage. The harvest of electrical energy mediated by FRM in sediments and microbial fuel cells is a technological reality.[4, 138, 139, 140, 141, 142] The utilization of electrodes as an electron acceptor has not only been proposed to harvest energy from sediments but also to remediate environments contaminated with biodegradable organic compounds, such as mono- and polycyclic-aromatic hydrocarbons[141], potentially harvesting energy in the process. FRM are not only capable of generating electricity, previous studies have indicated an incredible metabolic versatility and these organisms have been demonstrated to transform various organic (that is, benzene, toluene, phenol and chlorinated compounds)[90, 143, 144, 145, 146] and heavy metal or radionuclide contaminants (that is, uranium, technetium and chromium).[55, 147, 148]

Concluding remarks

Over the past two decades, recognition of the microbial reduction and oxidation of the fourth most abundant element in the Earth's crust has identified a globally significant biogeochemical process. Although it is recognized that the microorganisms involved in these competing metabolisms are ubiquitous, the physiology of microbial Fe(III) reduction and Fe(II) oxidation remains an enigma, as a terminal Fe(III) reductase has yet to be identified and nothing apart from the implicated role of *c*-type cytochrome(s) is known of Fe(II) oxidation at circumneutral pH. Genome sequencing and the subsequent development of in silico physiological models can be used to predict microbial metabolism in particular environmental conditions[123, 124], and can potentially provide greater insight into microbially mediated iron oxidation and reduction reactions in environmental systems. These advances can also be applied to enhance and predict the behaviour of microorganisms exploited for their metabolism associated with biotechnological applications (Box 2). Although significant advances have been made, we are continuously learning of previously unknown microbially mediated iron redox reactions in diverse environments and searching for microorganisms responsible for catalysing these unique metabolisms. A topical example of this is the recently identified anaerobic oxidation of ammonium coupled to dissimilatory Fe(III) reduction[8] for which no organism has yet been identified. As these sorts of discoveries are made, the true ubiquity and diversity of the organisms involved in iron biogeochemistry will be uncovered. Because of the geological and geochemical importance of iron on a global scale, the activity of these organisms shapes the world we live in today.

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Dechloromonas aromatica
Geobacter metallireducens
Geothrix fermentans
Marinobacter aquaeolei
Rhodopseudomonas palustris
Shewanella frigidimarina
Shewanella oneidensis
Shewanella putrefaciens
Thiobacillus denitrificans

UniProtKB: http://ca.expasy.org/sprot
CymA
MtrA
Cytc3
PpcA

Further Information

John D. Coates' homepage: http://pmb.berkeley.edu/~coates

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