



Dissimilatory Metal Reduction: from Early Life to Bioremediation

Diverse bacteria and archaea use a novel form of respiration—oxidizing hydrogen or organic compounds with the reduction of metals

Derek R. Lovley

It is well known that metals, and iron in particular, are key components of proteins involved in transferring electrons to terminal acceptors such as oxygen and sulfate. However, it has only recently been recognized that metals can serve as terminal electron acceptors to support the anaerobic growth of microorganisms. Here again, iron is the most important metal, reflecting the considerable abundance of insoluble Fe(III) oxides in the Earth's crust, but other metals and metalloids such as manganese, uranium, chromium, technetium, cobalt, selenium, and arsenic can also serve as electron acceptors. Microbial reduction of Fe(III) and the oxidized forms of other metals influences not only the biogeochemical cycles of these metals, but also the fate of organic matter and nutrients in a variety of environments.

The use of metals as terminal electron acceptors is called “dissimilatory metal reduction,” distinguishing it from the reduction of metals associated with metal uptake into cells. A phylogenetically diverse group of bacteria (Fig. 1) and archaea are known to conserve energy to support growth by oxidizing hydrogen or organic compounds with the reduction of Fe(III), and novel Fe(III)-reducing microorganisms are continually being discovered. However, not all dissimilatory metal reduction is linked to energy conservation. For example, our studies demonstrate that sulfate-reducing and methanogenic microorganisms oxidize hydrogen with the reduction of Fe(III). Although these microorganisms do not appear capable of growing with Fe(III) serving as the sole terminal electron acceptor, they may preferentially reduce Fe(III) over sulfate or carbon dioxide.

Dissimilatory metal reduction is relevant to many issues, ranging from the beginnings of life on Earth to environmental remediation, but the study of this process is in its infancy. Of all of the major forms of anaerobic respiration, it has received the least attention. However, this field seems poised to develop rapidly, particularly as the Department of Energy and the National Science Foundation are now supporting numerous research groups that are investigating both geochemical and biochemical aspects of dissimilatory metal reduction.

A Model for Early Microbial Respiration

Geological and microbiological evidence suggests that Fe(III) reduction was a very early form of respiration on Earth. Geochemists have proposed that high levels of ultraviolet radiation produced abundant Fe(III) oxides and H₂ on the anoxic, prebiotic Earth (Fig. 2). Other geological sources of hydrogen were also likely. Although the oxidation of hydrogen coupled with the reduction of Fe(III) is energetically favorable, catalysts are required for this reaction at temperatures at which life can exist. Thus, developing a means for catalyzing this process would have provided a life form with an excellent strategy for extracting energy from the early Earth environment. Michael Russell from the University of Glasgow and colleagues have proposed that the initial step in the evolution of life was the formation of inorganic iron-sulfur membranes capable of oxidizing hydrogen coupled to Fe(III) reduction. Then as organic-based life evolved from this inorganic proto-life, these earliest forms of life are likely to have gained energy from the same reaction.

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Microbiological evidence supports this scenario. The physiological properties of the last common ancestor(s) of modern organisms are typically inferred from the physiology of the most deeply branching extant microorganisms in the 16S rRNA-based tree of life. All of these “deep branchers” are hyperthermophilic *Archaea* and *Bacteria*. All of the hyperthermophiles that have been tested can oxidize hydrogen with the reduction of Fe(III), making this process one of the most highly conserved features of hyperthermophiles. Thus, the last common ancestor(s) most likely had the ability to oxidize hydrogen with the reduction of Fe(III), in agreement with geological considerations.

Modern Biogeochemical Cycles

In addition to hydrogen, modern dissimilatory Fe(III)-reducing microorganisms can oxidize a variety of organic compounds to carbon dioxide, with Fe(III) oxides serving as the sole electron acceptor. For the most part, such microorganisms are not competitive with fermentative microorganisms for substrates such as sugars and amino acids, but Fe(III)-reducing microorganisms can oxidize typical products of fermentative microorganisms, of which acetate is by far the most important in sedimentary environments. Furthermore, Fe(III) reducers can also oxidize aromatic compounds and long-chain fatty acids.

Thus, through cooperative activity, fermentative and Fe(III)-reducing microorganisms can oxidize complex organic matter in anoxic sedimentary environments to carbon dioxide (Fig. 3). Mn(IV) can substitute for Fe(III) in this process. Oxidation of organic matter coupled to the reduction of Fe(III) and Mn(IV) is a major degradative process in freshwater and marine aquatic sediments, submerged soils (as in flooded rice paddies and wetlands), and aquifers. It may also be important in hot (i.e., 80–100°C) environments, since recent studies in our laboratory show that some hyperthermophiles can also oxidize acetate, aromatic compounds, and long-chain fatty acids with Fe(III) as the electron acceptor.

The production of soluble Fe(II) as the result of microbial Fe(III) oxide reduction in aquifers has important implications for water quality. When Fe(II)-rich groundwaters are pumped to the surface and contact oxygen, Fe(II) is reoxidized to insoluble Fe(III) oxides, which can clog wells, discolor water, and stain just about everything they contact. Removing this dissolved Fe(II) is expen-

sive. High Fe(II) levels are a prevalent groundwater problem worldwide.

Fe(III) and Mn(IV) oxides tenaciously bind many trace metals and phosphorous. Thus, microbial Fe(III) and Mn(IV) reduction releases trace metals and phosphorous into pore waters of aquatic sediments and groundwaters. This process influences the nutrient status of soils, and can affect primary productivity in freshwater environments because phosphorous levels often determine whether algal blooms develop.

Dissimilatory metal-reducing microorganisms can influence the biogeochemical cycles of some trace metals by using these metals as electron acceptors. For example, many dissimilatory metal-reducing microorganisms can reduce uranium. U(VI), which is soluble in natural waters, is reduced to U(IV), which precipitates as the mineral uraninite. Reductive precipitation of uranium in marine sediments is an important global sink for uranium entering the ocean, controlling uranium levels in seawater. Reductive precipitation of uranium in the subsurface is a common source of uranium ores. Several dissimilatory metal-reducing microorganisms can use gold as an electron acceptor, reducing soluble oxidized gold, Au(III), to the insoluble metallic form Au(0). This mechanism may explain how some gold deposits have been formed.

Under appropriate conditions, one of the end products of microbial reduction of Fe(III) oxide is the magnetic mineral magnetite. Large accumulations of magnetite in Precambrian iron deposits as well as in the deep, hot subsurface and around hydrocarbon deposits could be geological signatures of the activity of Fe(III)-reducing microorganisms.

Bioremediation of Organic and Metal Contaminants

Much recent research on dissimilatory metal-reducing microorganisms has focused on their role in bioremediating contaminated subsurface environments. For example, when subsurface environments are contaminated with organic compounds, such as petroleum or landfill leachate, anoxic conditions typically develop as microorganisms consume the small amount of oxygen typically available in groundwater. With the development of anoxic conditions, Fe(III) is generally the most abundant potential electron acceptor for organic matter oxidation in the

subsurface. According to geochemical studies, significant amounts of organic contaminants, such as aromatic hydrocarbons, can be oxidized within subsurface environments with Fe(III) serving as the electron acceptor.

Pure cultures of dissimilatory Fe(III)-reducing microorganisms, such as *Geobacter metallireducens*, can oxidize a variety of aromatic contaminants. In fact, *G. metallireducens* was the first organism of any kind found to anaerobically oxidize an aromatic hydrocarbon. Studies with contaminated sediments and enrichment cultures have demonstrated that Fe(III)-reducing microorganisms can even oxidize unsubstituted aromatic hydrocarbons, such as benzene and naphthalene, that were thought to be resistant to anaerobic degradation.

Although Fe(III)-reducing microorganisms can degrade organic contaminants within polluted aquifers, this process can be slow. One of the key limiting factors is the rate at which Fe(III)-reducing microorganisms can access the insoluble Fe(III) oxides in the subsurface. One strategy for stimulating the activity of Fe(III) reducers in aquifer sediments is to add humic acids or other quinone-containing compounds, to which Fe(III)-reducing microorganisms can transfer electrons.

The resulting hydroquinones react spontaneously with Fe(III) oxides, reducing Fe(III) to Fe(II) and regenerating the quinones to undergo additional cycles of reduction and oxidation. This electron shuttling via extracellular quinones can greatly accelerate the rate and extent of the anaerobic microbial bioremediation of benzene and additional otherwise refractory contaminants such as methyl-tert-butyl ether (MTBE), vinyl chloride, and dichloroethene.

Besides controlling the spread of organic contaminants in the subsurface, dissimilatory metal-reducing microorganisms may also prevent the migration of metal contaminants in groundwater. Consider the radioactive metal uranium, which is a major contaminant in many areas where it has

been mined and processed. In many instances, the contaminated groundwater contains dissolved oxygen and uranium in its soluble form, U(VI). When a simple organic compound such as acetate is added to the subsurface, microorganisms metabolize it, quickly consuming available dissolved oxygen and nitrate. Then dissimilatory metal-reducing microorganisms begin to metabolize the remaining acetate, oxidizing it to carbon dioxide while reducing available metals.

Even in uranium-contaminated subsurface environments, Fe(III) is generally the most abundant metal electron acceptor. However, while reducing Fe(III), the microbial Fe(III) reducers can also convert U(VI) to U(IV), which precipitates from the groundwater and is immobilized

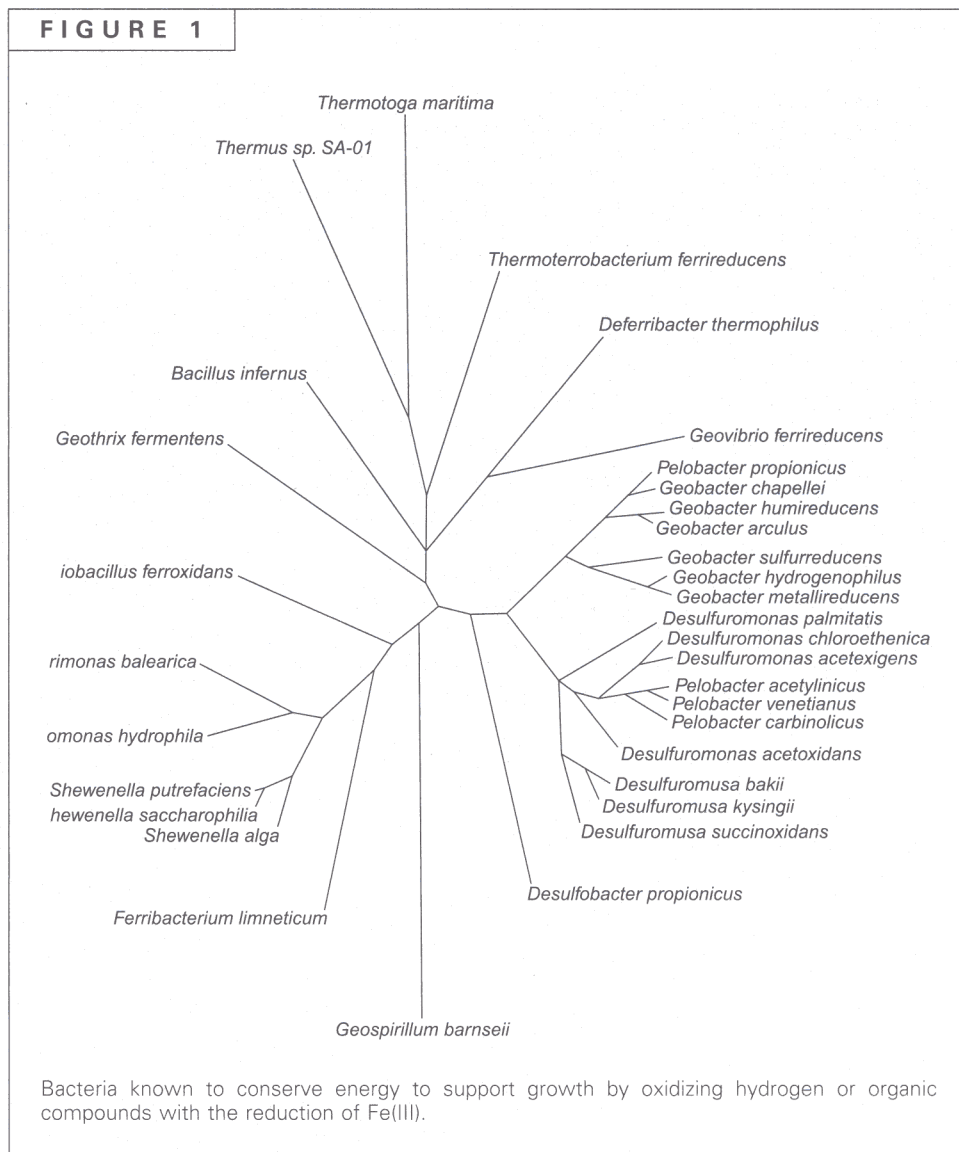
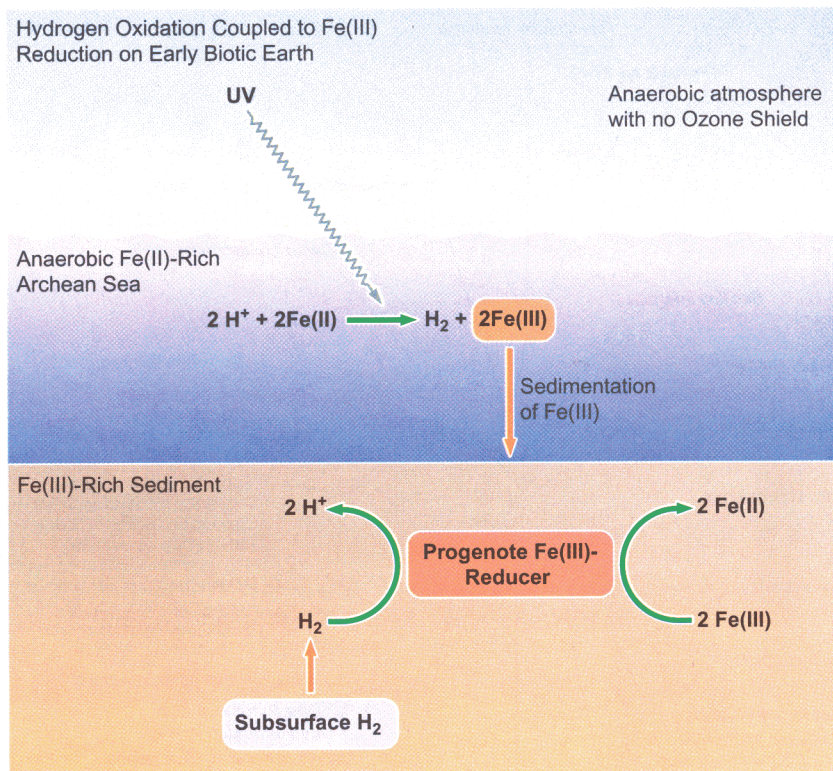




FIGURE 2



Model for microbial metabolism on early Earth.

in the subsurface (Fig. 4). Laboratory studies and preliminary field experiments suggest that this mechanism may be effective for stopping further spread of subsurface uranium contamination and for concentrating uranium in a discrete zone for eventual retrieval. Similar approaches are being considered for immobilizing other radioactive metal contaminants such as technetium, cobalt, and highly toxic chromium, as well as the metalloid selenium.

Microorganisms Involved in Dissimilatory Metal Reduction

Many different Fe(III)-reducing microorganisms have been recovered from a diverse range of aquatic sediments, submerged soils, and other subsurface environments. The most intensively studied Fe(III)-reducing microorganisms are *Shewanella* species, which are in the gamma subclass of the *Proteobacteria*. One feature that makes this genus attractive for study is that cells can be grown to high densities using oxygen as

an electron acceptor and then placed under anoxic conditions to study reduction of Fe(III).

The complete genome of *Shewanella oneidensis* (formerly *S. putrefaciens*) is now available (www.tigr.org), and *Shewanella* species are amenable to genetic studies. However, investigators who have used unbiased molecular techniques to analyze microbial communities have consistently found that *Shewanella* are not significant components of communities in a wide variety of environments in which Fe(III) reduction is important. One reason for their relative scarcity is that their preferred organic electron donors, such as lactate, are not important intermediates for anaerobic metabolism in sedimentary environments. Another reason is that, as detailed below, *Shewanella* species appear to transfer electrons to Fe(III) oxides in ways that are not well suited for low-energy, nutrient-poor sedimentary environments.

Microorganisms in the family *Geobacteraceae* represent another group of well-studied Fe(III)-reducing microorganisms. This family is within the delta subclass of the *Proteobacteria* and includes the genera *Geobacter*, *Desulfuromonas*, *Desulfuromusa*, and *Pellobacter*. Although these organisms were previously classified as strict anaerobes, recent evidence suggests that they readily tolerate oxygen exposure. Oxidation of organic matter coupled to Fe(III) reduction has primarily been studied in *Geobacter* species. For instance, *Geobacter metallireducens* (formerly strain GS-15) was the first microorganism found to completely oxidize organic compounds to carbon dioxide with Fe(III) or other metals serving as the electron acceptor.

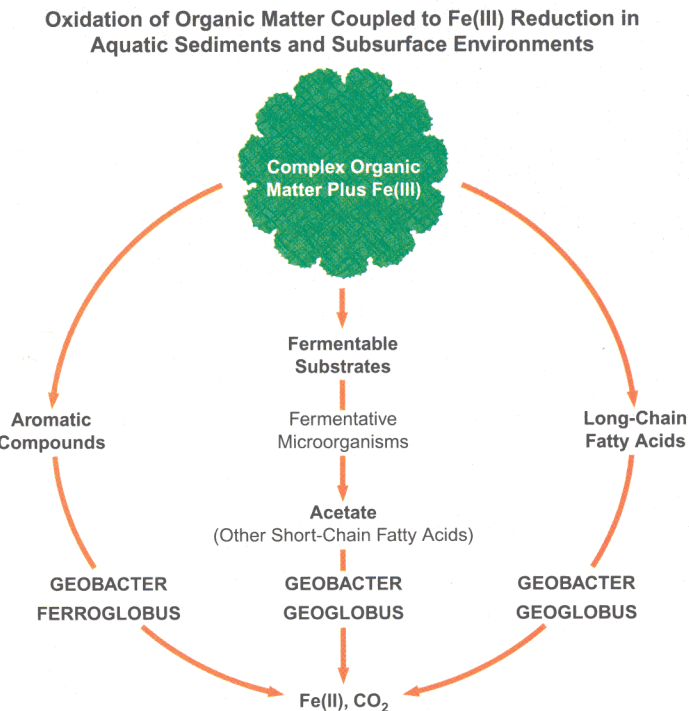
One of the most environmentally relevant organic electron donors that *G. metallireducens* and other *Geobacter* species oxidize is acetate, a key intermediate in the anaerobic metabolism of organic matter in sedimentary environments. Furthermore, *G. metallireducens* provides a pure culture model for the oxidation of aromatic contaminants coupled to the reduction of Fe(III) in subsurface environments, as it can also oxidize a variety of aromatic compounds, including important contaminants such as phenol, p-cresol,

and even the aromatic hydrocarbon toluene, with Fe(III) as the electron acceptor.

Interest in *Geobacteraceae* has increased as molecular analyses have indicated that *Geobacteraceae* are significantly enriched in a variety of sedimentary environments in which dissimilatory metal reduction is an important process. This was first observed in a petroleum-contaminated aquifer in which Fe(III)-reducing microorganisms were removing benzene and other aromatic hydrocarbon contaminants from the groundwater. Similar enrichments of *Geobacteraceae* were observed in other aquifers in which the introduction of petroleum or other organic compounds led to development of Fe(III)-reducing conditions. For instance, *Geobacteraceae* accounted for more than 40% of the microbial community when organic compounds were added to promote U(VI) reduction in uranium-contaminated aquifer sediments. Studies by Kenneth Nealson and colleagues at California Institute of Technology on aquatic sediments, and by H.W. van Verseveld at the University of Amsterdam on groundwater contaminated with landfill leachate, have similarly found a predominance of *Geobacteraceae* under conditions in which Fe(III) reduction is important.

The availability of pure cultures closely related to the *Geobacteraceae* that live in subsurface environments provides a rare opportunity in environmental microbiology to study an environmentally relevant organism under defined laboratory conditions. Thus, the *Geobacteraceae* provide an excellent opportunity not only to learn more about the factors controlling the rate and extent of microbial metal reduction in the environment, but also to elucidate other physiological properties that might help microorganisms colonize anoxic environments.

The genomic sequence of one member of the *Geobacteraceae*, *G. sulfurreducens*, is available (www.tigr.org), and researchers at the Joint Genome Institute (www.jgi.doe.gov) have completed draft genomic sequences of *G. metallireducens* and *Desulfuromonas acetoxidans*. A genetic system for *G. sulfurreducens* has been developed, and whole-genome DNA microar-



Mesophilic Environments-*Geobacter* species
Hot (>80°C) Environments-*Geoglobus* and *Ferroglobus* Species

Oxidation of organic matter coupled to Fe(III) reduction in aquatic sediments and subsurface environments.

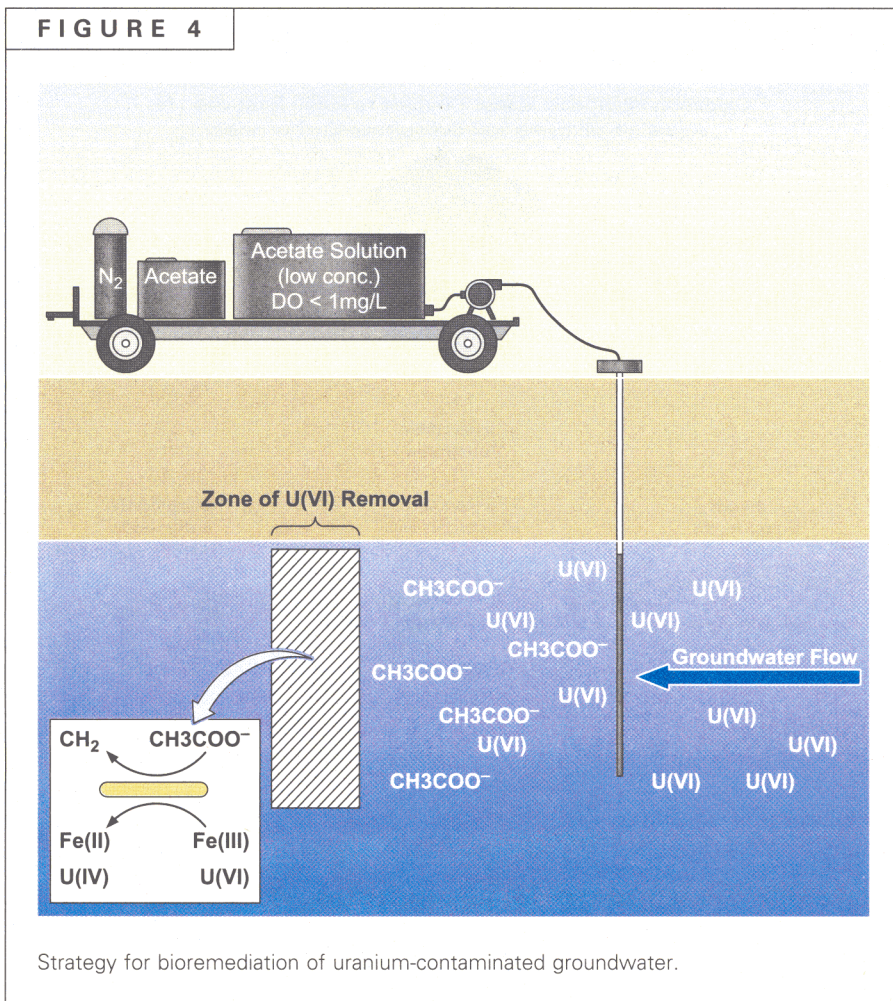
rays are being constructed. Coupled with proteomics investigations and more traditional biochemical studies, these approaches are expected to lead to a much better understanding of *Geobacter* physiology in the near future.

A diverse collection of other mesophilic Fe(III)-reducing microorganisms has been recovered from a variety of environments, but less information is available on their distribution and physiology. For example, in some instances molecular analysis has detected microorganisms closely related to *Geothrix fermentans* in the Fe(III)-reducing zone of aquifer sediments, but always orders of magnitude less than the *Geobacteraceae*.

Of the thermophilic and hyperthermophilic Fe(III) reducers, the archaea *Geoglobus ahangari* and *Ferroglobus placidus* are of special interest. These are the first hyperthermophiles documented to anaerobically oxidize acetate with any electron acceptor. With Fe(III) as the electron acceptor, *F. placidus* can also oxidize



FIGURE 4



aromatic compounds, and *G. abhangari* can oxidize long-chain fatty acids. The discovery of these forms of metabolism in hyperthermophiles greatly expands the known metabolic diversity of *Archaea* and provides evidence that organic matter may be anaerobically oxidized in hot ecosystems in a manner similar to that previously described for more temperate environments.

Mechanisms for Metal Reduction

The biochemical mechanisms for Fe(III) reduction are beginning to be understood. Thomas DiChristina and collaborators at the Georgia Institute of Technology showed that the export of key electron transport components, such as *c*-type cytochromes, to the outer membrane is essential for Fe(III) reduction in *S. oneidensis*. Other studies, most notably by Charles Myers and collaborators at the Medical College of Wisconsin in Milwaukee, Wis., demonstrated that *c*-type cytochromes are essential intermediates

in electron transfer to metals in this organism. However, the terminal metal reductases are not yet identified. Genetic studies in our laboratory demonstrated that *c*-type cytochromes are involved in Fe(III) and U(VI) reduction in *G. sulfurreducens*, and earlier biochemical studies suggested that the *c*₃ cytochrome is the U(VI) reductase in *Desulfovibrio vulgaris*.

Some Fe(III)-reducing microorganisms reduce Fe(III) without directly contacting the Fe(III) oxide—a finding that overturns a longstanding assumption that direct contact is necessary (Fig. 5). After determining that *S. oneidensis* releases quinones into the culture medium during growth, Dianne Newman, now at California Institute of Technology in Pasadena, Calif., and Roberto Kolter of Harvard University in Cambridge, Mass., suggested that quinones might serve as an electron shuttle between *S. oneidensis* and Fe(III) oxide.

Meanwhile, by sequestering Fe(III) oxides within microporous alginate beads, which only admit molecules that are smaller than 12 kDa, my colleagues and I confirmed that *Shewanella alga*—and also *Geothrix fermentans*—reduce Fe(III) oxides without directly contacting them. Although our preliminary evidence suggests that quinones serve as the electron shuttles, the precise nature of the shuttle molecules remains to be determined. In addition to producing electron shuttling compounds, both *S. alga* and *G. fermentans* solubilize Fe(III) during growth on insoluble Fe(III) oxide, presumably by releasing one or more Fe(III)-chelating compounds—representing another mechanism for Fe(III)-reducing microorganisms to overcome the need for direct contact with Fe(III) oxides.

In contrast, *Geobacter metallireducens* does not release electron shuttling compounds or solubilize Fe(III) from Fe(III) oxide and must contact Fe(III) oxide to reduce it. *G. metallireducens* is highly adapted for this mode of Fe(III) oxide reduction. When growing on soluble electron acceptors, including chelated Fe(III), it is nonmotile. However, when only insoluble Fe(III) or Mn(IV) oxides are available as electron acceptors, *G. metallireducens* produces flagella and is

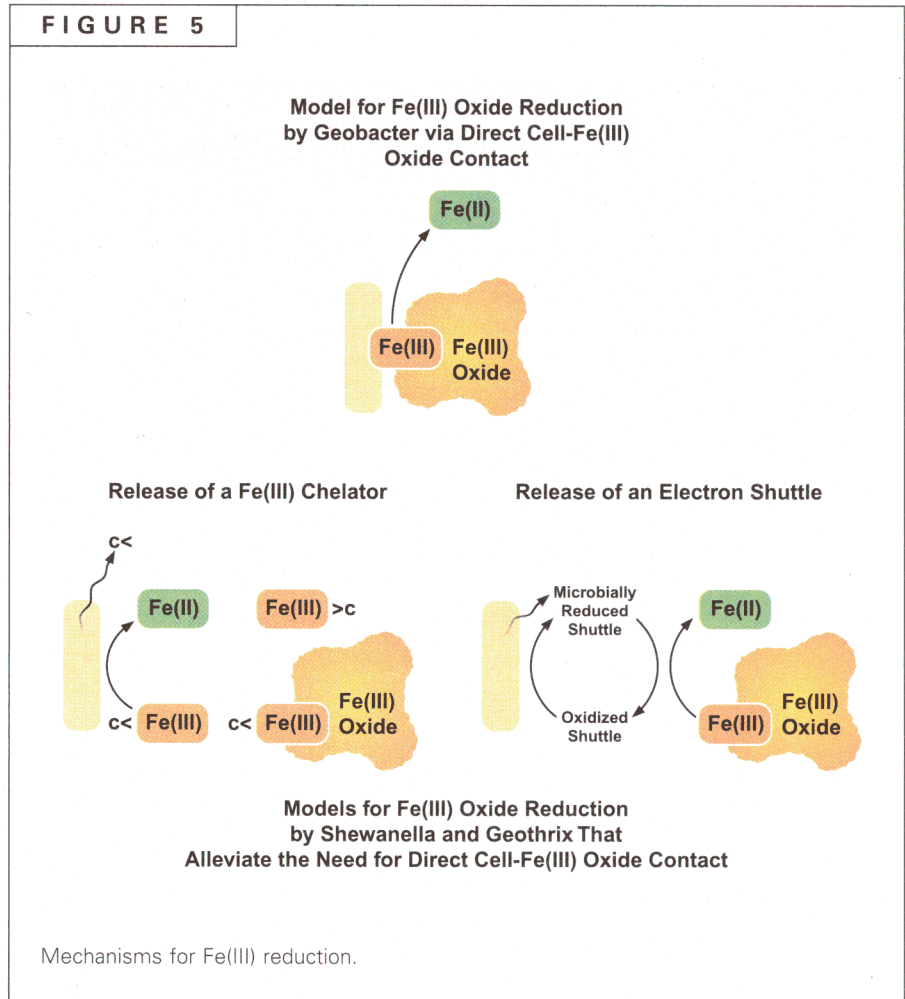
chemotactic to Fe(II) and Mn(II), following the gradient of these metals that emanate from Fe(III) and Mn(IV) oxides under anoxic conditions. Pili are also specifically produced during growth on the oxides and are necessary for Fe(III) oxide reduction, presumably serving as a means of attachment to the oxides. *G. metallireducens*' strategy for the reduction of Fe(III) and Mn(IV) oxides is considered to be a more energetically effective mechanism than the production of electron shuttles and Fe(III) chelators under the conditions typically found in subsurface environments, which may explain the predominance of *Geobacteraceae* in such environments.

Harvesting Electricity with Metal Reducers

The fact that we are still only beginning to realize the unique capabilities of dissimilatory metal-reducing microorganisms is clear from our recent finding that *Geobacteraceae* are useful for harvesting energy from the environment in the form of electricity. Lenny Tender of the Naval Research Laboratory, Washington, D.C., and Clare Reimers of Oregon State University in Corvallis found that placing a graphite electrode in anaerobic marine sediments and connecting it to similar electrode in the overlying water yielded electrical current. We found, in studies supported by the Office of Naval Research, that the surface of the electrodes in the sediments were highly enriched in *Geobacteraceae* and that members of this family could produce electricity and grow in pure culture with organic compounds as the electron donor and a graphite electrode as the sole electron

acceptor. Although the immediate application of this technology is for the deployment of electronic monitoring devices in the ocean, with further optimization *Geobacteraceae* living on electrodes have significant potential for harvesting energy from a variety of organic wastes and in bioremediation.

FIGURE 5



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