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DISSIMILATORY METAL REDUCTION[1]

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ABSTRACT

Microorganisms can enzymatically reduce a variety of metals in metabolic processes that are not related to metal assimilation. Some microorganisms can conserve energy to support growth by coupling the oxidation of simple organic acids and alcohols, H_2, or aromatic compounds to the reduction of Fe(III) or Mn(IV). This dissimilatory Fe(III) and Mn(IV) reduction influences the organic as well as the inorganic geochemistry of anaerobic aquatic sediments and ground water. Microorganisms that use U(VI) as a terminal electron acceptor play an important role in uranium geochemistry and may be a useful tool for removing uranium from contaminated environments. Se(VI) serves as a terminal electron acceptor to support anaerobic growth of some microorganisms. Reduction of Se(VI) to Se(0) is an important mechanism for the precipitation of selenium from contaminated waters. Enzymatic reduction of Cr(VI) to the less mobile and less toxic Cr(III), and reduction of soluble Hg(II) to volatile Hg(0) may affect the fate of these compounds in the environment and might be used as a remediation strategy. Microorganisms can also enzymatically reduce other metals such as technetium, vanadium, molybdenum, gold, silver, and copper, but reduction of these metals has not been studied extensively.

INTRODUCTION

Microorganisms that use metals as terminal electron acceptors, or reduce metals as a detoxification mechanism, have an important influence on the geochemistry of aquatic sediments, submerged soils, and the terrestrial subsurface. Furthermore, it is becoming increasingly apparent that microbial metal reduction may be manipulated to aid in the remediation of environments and waste streams contaminated with metals and certain organics. The purpose of this review is to give a brief overview of the physiology and ecology of microorganisms that reduce environmentally significant metals for non-assimilatory purposes.

Fe(III) REDUCTION

Microbial reduction of Fe(III) to Fe(II) has been studied not only because of its influence on iron geochemistry but also because Fe(III) is one of the most abundant potential electron acceptors for organic matter decomposition in many aquatic sediments and subsurface environments (71). Until recently, much of the Fe(III) reduction in sedimentary environments was generally considered to result from nonenzymatic reactions. However, we now know that in the anaerobic, nonsulfidogenic environments in which Fe(III) reduction

is most important, dissimilatory Fe(III) reducers enzymatically catalyze nearly all of the Fe(III) reduction (71, 85).

Fe(III)-Reducing Microorganisms

In most sedimentary environments such as aquatic sediments, submerged soils, and aquifers, the oxidation of organic matter coupled to the reduction of Fe(III) requires the cooperative activity of several metabolic types of dissimilatory Fe(III) reducers (71) (Figure 1). For example, a great diversity of microorganisms that can metabolize sugars or amino acids with the reduction of Fe(III) have been described (32, 33, 36, 70). However, in all the cases examined, Fe(III) reduction is a trivial side reaction in the metabolism of these organisms (71). The primary products of the metabolism of the fermentative Fe(III)-reducing microorganisms are typical fermentation acids, alcohols, and H_2. Microorganisms that can completely oxidize sugars and amino acids to carbon dioxide with Fe(III) as the sole electron acceptor are unknown. Numerous attempts to isolate or enrich for such organisms have been unsuccessful (72). Even if microorganisms that completely oxidize glucose to carbon dioxide with Fe(III) as the sole electron acceptor exist, their

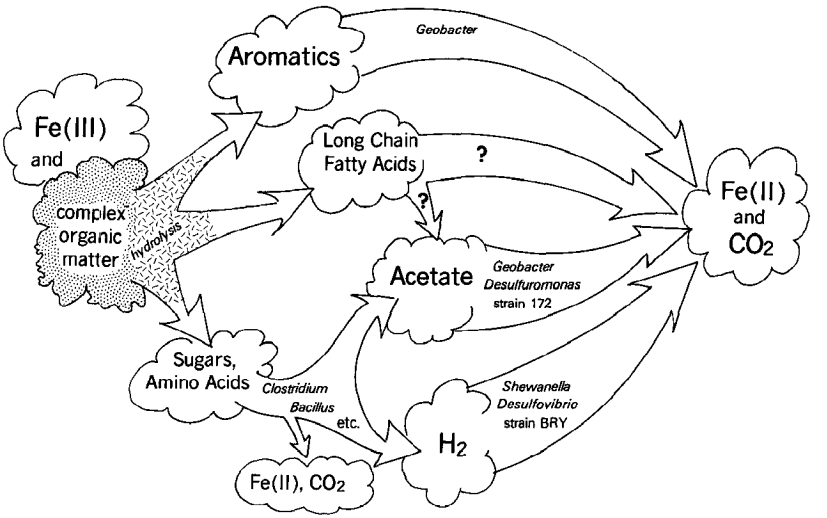

Figure 1 Model for oxidation of organic matter in sediments coupled to dissimilatory Fe(III) reduction showing examples of the microorganisms in pure culture known to catalyze the various reactions. Fermentation of sugars and amino acids has been simplified to designate the production of only the two major fermentation products, acetate and H_2. However, other short chain fatty acids are produced in lesser amounts. These include propionate and formate, which may be directly oxidized to carbon dioxide by organisms such as *Geobacter metallireducens* (propionate) or *Shewanella putrefaciens* (formate), as well as lactate, which is oxidized to acetate and carbon dioxide by organisms such as *S. putrefaciens*.

metabolism does not appear to be important in Fe(III)-reducing sediments as glucose is fermented to fatty acids rather than oxidized directly to carbon dioxide (80). Thermodynamic considerations support this idea, suggesting that microorganisms attempting to completely oxidize glucose to carbon dioxide with Fe(III) would be at a competitive disadvantage with microorganisms that convert glucose to fermentation products (80). These findings have led to the hypothesis that, in Fe(III)-reducing environments, fermentative microorganisms metabolize sugars and amino acids with relatively little Fe(III) reduction during this initial step.

Most of the electron transfer to Fe(III) during the metabolism of sugars and amino acids results from the oxidation of the fermentation products (71). Acetate is considered to be the most important fermentation product in Fe(III)-reducing sedimentary environments (80). The freshwater isolate, *Geobacter metallireducens* (formerly strain GS-15) was the first known acetate-oxidizing Fe(III) reducer (76, 79, 86). *G. metallireducens* conserves energy to support growth by the reaction:

$$\text{acetate}^- + 8\ \text{Fe(III)} + 4\ H_2O \rightarrow 2\ HCO_3^- + 8\ \text{Fe(II)} + 9\ H^+.$$

G. metallireducens oxidizes various other volatile fatty acids and simple alcohols.

G. metallireducens is in the delta proteobacteria (76). Its closest known relative is *Desulfuromonas acetoxidans*. This organism was previously known primarily for its unique ability to couple the oxidation of acetate to the reduction of S^0. However, *D. acetoxidans* can also oxidize acetate with Fe(III) as the electron acceptor (116). This finding demonstrates that some marine organisms can also effectively couple the oxidation of organic compounds to Fe(III) reduction.

Another acetate-oxidizing Fe(III) reducer, strain 172, was recovered from deep subsurface sediments of an Atlantic Coastal Plain aquifer (75). Although a detailed characterization of 172 is not yet available, the preliminary evidence suggests that it is not a *Geobacter* or *Desulfuromonas* species.

Shewanella putrefaciens (formerly *Alteromonas putrefaciens*) can also conserve energy to support growth by coupling the oxidation of organic compounds to the reduction of Fe(III) (74, 84, 96). Formate is oxidized to carbon dioxide whereas lactate and pyruvate are incompletely oxidized to carbon dioxide and acetate:

$$\text{formate}^- + 2\ \text{Fe(III)} + H_2O \rightarrow HCO_3^- + 2\ \text{Fe(II)} + 2\ H^+;$$

$$\text{lactate}^- + 4\ \text{Fe(III)} + 2\ H_2O \rightarrow \text{acetate}^- + HCO_3^- + 4\ \text{Fe(II)} + 5\ H^+;$$

$$\text{pyruvate}^- + 2\ \text{Fe(III)} + 2\ H_2O \rightarrow \text{acetate}^- + HCO_3^- + 2\ \text{Fe(II)} + 3\ H^+.$$

Formate oxidation coupled to Fe(III) reduction is a potentially important process in sediments if formate replaces H_2 as an important fermentation product in Fe(III)-reducing environments (84). However, the metabolism of lactate and pyruvate by *S. putrefaciens* is expected to be a minor pathway for carbon and electron flow in Fe(III)-reducing environments (71, 84).

The estuarine microorganism, strain BrY (22), and several *Desulfovibrio* species (D. R. Lovley, E. J. P. Phillips, J. C. Woodward & E. E. Roden, submitted) also incompletely oxidize lactate to acetate and carbon dioxide with Fe(III) as the electron acceptor. Like *S. putrefaciens,* BrY conserves energy to support growth from Fe(III) reduction. In contrast, there is no net growth during Fe(III) reduction by the *Desulfovibrio* species, but this metabolism may provide some energy.

Aquaspirillum magnetotacticum grows slowly and reduces Fe(III) in anaerobic medium that contains succinate as the sole electron donor (42). The authors of this study concluded that it is not clear whether *A. magnetotacticum* conserves energy from electron transport to Fe(III), and no data on succinate metabolism were provided.

Although the heterotrophic Fe(III)-reducing microorganisms discussed above grow best at circumneutral pH, numerous acidophilic heterotrophic bacteria reduce Fe(III) while growing in complex medium containing glucose or glycerol as well as tryptone (54). One isolate studied in detail reduces Fe(III) under both aerobic and anaerobic conditions. Evidence that energy is conserved from Fe(III) reduction is the finding that there is more cell growth as more Fe(III) is provided.

Several microorganisms can couple the oxidation of H_2 to the reduction of Fe(III). A *Pseudomonas* sp. (3), *S. putrefaciens* (84), and BrY (22) conserve energy to support growth via the reaction:

$$H_2 + 2\ \text{Fe(III)} \rightarrow 2\ H^+ + 2\ \text{Fe(II)}.$$

Data (71) did not substantiate claims that another organism could grow by coupling the oxidation of H_2 to the reduction of Fe(III) (55).

Several *Desulfovibrio* species also oxidize H_2 with the reduction of Fe(III) at rates comparable to those observed with other Fe(III) reducers, but no net cell growth occurs (D. R. Lovley, E. J. P. Phillips, J. C. Woodward & E. E. Roden, submitted). The minimum threshold for H_2 uptake in *D. desulfuricans* is lower with Fe(III) serving as the electron acceptor than with sulfate, suggesting that under conditions of limiting electron donor availability, *Desulfovibrio* species will preferentially reduce Fe(III).

A wide variety of monoaromatic compounds can be completely oxidized to carbon dioxide with Fe(III) serving as the sole electron acceptor (68, 85). *G. metallireducens* is the only aromatic-oxidizing, Fe(III)-reducing microor-

ganism as yet available in pure culture (73, 77). Aromatics oxidized by *G. metallireducens* include prevalent contaminants such as toluene, *p*-cresol, and phenol. The metabolism of *G. metallireducens* serves as a model for the oxidation of aromatic contaminants coupled to Fe(III) reduction frequently observed in polluted aquifers (73, 77).

Long-chain fatty acids are likely another important component of organic matter that is metabolized in sediments. Enrichment cultures that can oxidize long-chain fatty acids have previously been established (71), but no isolates have been purified and the pathways for oxidation of long-chain fatty acids in Fe(III)-reducing environments have not been elucidated.

In acidic environments, elemental sulfur can serve as an electron donor for Fe(III) reduction. *Thiobacillus ferrooxidans, Thiobacillus thiooxidans,* and the thermophile, *Sulfolobus acidocaldarius,* reduce Fe(III) by the following reaction (19):

$$S^0 + 6\ Fe(III) + 4\ H_2O \rightarrow HSO^-_4 + 6\ Fe(II) + 7\ H^+.$$

Initial studies indicated that neither *T. thiooxidans* nor *T. ferrooxidans* (58, 118, 130) could conserve energy to support growth from this reaction. However, subsequent studies have demonstrated that Fe(III) reduction provides energy to support amino acid transport (111) and growth (110) in *T. ferrooxidans*. Microorganisms that oxidize sulfur with the reduction of Fe(III) at circumneutral pH have not been reported.

In addition, *S. acidocaldarius* provides evidence for the existence of thermophilic Fe(III) reducers. The recovery of large quantities of ultrafine-grained magnetite from depths as great as 6.7 km below the land surface presumably indicates the presence of Fe(III)-reducing life in such environments (37).

Electron Transport to Fe(III)

Investigations into electron transport to Fe(III) in dissimilatory Fe(III) reducers are important not only for a better understanding of the mechanisms for Fe(III) reduction but also because of the potential evolutionary significance of Fe(III) reduction. The geological evidence suggests that microbial Fe(III) reduction may have evolved before other respiratory processes, such as sulfate reduction, nitrate reduction, and oxygen reduction, which can also completely oxidize multicarbon organic compounds back to carbon dioxide (71).

Much of the early work on electron transport to Fe(III) conducted with organisms grown under aerobic conditions was recently reviewed (71). The relevance of these studies to anaerobic dissimilatory Fe(III) reduction has been questioned because aerobically grown cells produce Fe(III) reductases that are not linked to energy conservation (71).

The three strains of *S. putrefaciens* that grow on nonfermentable substrates under anaerobic conditions with Fe(III) as the sole electron acceptor (28, 84, 96) must produce Fe(III) reductases that are linked to energy conservation. When cell suspensions of anaerobically grown *S. putrefaciens* are provided with Fe(III), the pH of the external medium drops, suggesting that electron transport to Fe(III) is associated with proton translocation (98). Growth under anaerobic conditions stimulates the production of *c*-type cytochromes in the outer membrane of *S. putrefaciens* (95). If these cytochromes can reduce Fe(III), this suggests a mechanism by which *S. putrefaciens* might transfer electrons to insoluble Fe(III) oxides (95). Although the Fe(III) reductase in *S. putrefaciens* has yet to be identified, it appears to be a different enzyme than the nitrate reductase (28, 98).

G. metallireducens contains a membrane-bound Fe(III) reductase also distinct from the nitrate reductase (39), as well as several membrane-bound and soluble *c*-type cytochromes (76, 99; J. E. Champine & S. Goodwin, submitted), some of which appear to be involved in electron transport to Fe(III) and other metals (76). *c*-Type cytochromes may also serve as electron carriers during Fe(III) reduction in the closely related *D. acetoxidans* (116). Menaquinone and ferredoxin are other likely components of the electron transport chain to Fe(III) in *G. metallireducens* (76; J. E. Champine & S. Goodwin, submitted). *G. metallireducens* derives electrons for Fe(III) reduction by oxidizing acetate via the citric acid cycle (24).

Thiobacillus ferrooxidans contains several enzymes that can reduce soluble Fe(III) under acidic conditions and could be involved in dissimilatory Fe(III) reduction with reduced sulfur compounds as the electron donor. A periplasmic enzyme purified to an electrophoretically homogeneous state (127) catalyzes the oxidation of sulfide to sulfite with Fe(III) as the electron acceptor (125). Another Fe(III) reductase activity located in the plasma membrane oxidizes sulfite to sulfate (126).

The first enzyme capable of reducing insoluble Fe(III) oxide at circumneutral pH was purified from the dissimilatory Fe(III) reducer, *Desulfovibrio vulgaris* (D. R. Lovley, P. K. Widman & J. C. Woodward, submitted). Attempts to find the U(VI) reductase in this organism (see below) indicated that the c_3 cytochrome was an active metal reductase. When a combination of hydrogenase and H_2 was used as a source for electrons for c_3, c_3 reduced a poorly crystalline Fe(III) oxide-producing soluble Fe(II) and the magnetic mineral magnetite. Electron transport via cytochrome c_3 may account for the ability of *D. vulgaris* and other *Desulfovibrio* species to reduce Fe(III) oxides.

Environmental Significance and Ecology of Fe(III) Reduction

Dissimilatory Fe(III) reduction has a greater overall environmental impact than microbial reduction of any other metal. Microbial Fe(III) reduction has

been directly shown or implicated as an important process in the following phenomena: organic matter decomposition in a variety of freshwater, estuarine, and marine sediments; the decomposition of aromatic hydrocarbons in contaminated aquifers; the generation of undesirably high concentrations of dissolved iron in deep pristine aquifers; the oxidation of organic matter coupled to Fe(III) that resulted in the accumulation of magnetite in the Banded Iron Formations; the accumulation of magnetite around hydrocarbon seeps and formation of ultrafine-grained magnetite in aquatic sediments; the formation of other ferrous iron minerals such as siderite and vivianite; the control of the extent of methane formation in shallow freshwater environments; the release of phosphate and trace metals into water supplies; soil gleying; and corrosion of steel. A recent review examined the literature on this subject (71).

Although several microorganisms can now serve as models for the enzymatically catalyzed reduction of Fe(III), it is not clear that any of the organisms currently available in pure culture are the dominant Fe(III) reducers in sedimentary environments. For example, a study on the mechanisms for siderite ($FeCO_3$) concretion formation in salt marsh sediments found that organisms related to *G. metallireducens* or *S. putrefaciens* were not abundant in the zone of Fe(III) reduction (25a). The concretions were enriched with *Desulfovibrio*-type organisms that are now known to be active dissimilatory Fe(III) reducers (see above), though at the time they were not generally regarded as such. Further circumstantial evidence for the potential role of sulfate-reducing organisms in dissimilatory Fe(III) reduction is that sulfate reducers are abundant in the zones of deep aquifers of the Atlantic Coastal Plain in which Fe(III) reduction is the terminal electron-accepting process (25, 94). Sulfate reducers living for such long periods in these subsurface environments, in which no detectable sulfate reduction occurs, probably gain energy for survival from electron transport to Fe(III). Such findings emphasize the need for more intensive study of community structure of Fe(III)-reducing environments.

Mn(IV) REDUCTION

Mn(IV)-Reducing Microorganisms

Microbial reduction of Mn(IV) to Mn(II) greatly parallels dissimilatory Fe(III) reduction. Most of the microorganisms that reduce Mn(IV) reduce Fe(III) and vice versa. Furthermore, the fact that Fe(II) rapidly reduces Mn(IV) means that, in most environments, any microorganism that can enzymatically reduce Fe(III) will also indirectly reduce Mn(IV) (78, 97). As with Fe(III), much of the early work on dissimilatory Mn(IV) reduction was primarily conducted with microorganisms that only used Mn(IV) as a minor electron acceptor

during metabolism. This early literature was reviewed previously (31, 32, 36).

In general, Fe(III)/Mn(IV) reducers oxidize the same electron donors with Mn(IV) as they do with Fe(III). The exception is the H_2-oxidizing, Fe(III)-reducing *Pseudomonas* sp., which cannot oxidize H_2 with Mn(IV) (3). All of the other well-studied microorganisms that can conserve energy from dissimilatory Fe(III) reduction such as *G. metallireducens* (79), *S. putrefaciens* (84, 96), strain BrY (22), and *D. acetoxidans* (116) can also grow with Mn(IV) as the sole electron acceptor. However, in most instances, studies on electron donor metabolism with Mn(IV) as the electron acceptor have not been as extensive as studies with Fe(III). For example, although *G. metallireducens* can reduce Mn(IV) when benzoate is provided as an electron donor (D. R. Lovley, unpublished data) the oxidation of aromatic compounds coupled to Mn(IV) reduction in this or other organisms has not been studied in detail. It seems likely, however, that organic matter could be oxidized to carbon dioxide with the reduction of Mn(IV) in a manner similar to that proposed in Figure 1 for Fe(III) reduction.

Electron Transport to Mn(IV)

Electron transport systems to Mn(IV) have been investigated in several *Bacillus* species that reduce Mn(IV) (27, 31, 36). However, the available evidence suggests that this Mn(IV) reduction is a minor side reaction that does not conserve energy to support growth for these organisms, and thus this metabolism may have little relevance to the bulk of dissimilatory Mn(IV) reduction in sedimentary environments (71).

Almost nothing is known about electron transport to Mn(IV) in organisms that conserve energy to support growth from Mn(IV) reduction. Growth of *G. metallireducens* (79), *S. putrefaciens* (84, 96), strain BrY (22), and *D. acetoxidans* (116) on nonfermentable substrates with Mn(IV) as the electron acceptor implies energy conservation through electron transport to a Mn(IV) reductase. Evidence of the potential for energy conservation as the result of electron transport to Mn(IV) was the finding that cell suspensions of *S. putrefaciens* translocated protons to the external medium when provided with lactate and Mn(IV) (98). The *c*-type cytochrome(s) in *D. acetoxidans* are oxidized by Mn(IV), suggesting that they are involved in electron transport to Mn(IV) (116).

Environmental Significance of Mn(IV) Reduction

The environmental significance of enzymatic mechanisms for Mn(IV) reduction is less clear than for Fe(III) reduction. This is because Mn(IV) is more easily reduced through nonenzymatic mechanisms than is Fe(III) (71). Most notably, no practical method has been devised for differentiating between

enzymatic reduction of Mn(IV) and nonenzymatic reduction of Mn(IV) by Fe(II). However, the distinction in this case may not be meaningful because the enzymatic reduction of Fe(III) oxide followed by nonenzymatic reduction of Mn(IV) by Fe(II) results in the same end products, Mn(II) and Fe(III) oxide, as does direct enzymatic reduction of Mn(IV) (78).

In analogy to Fe(III) reduction, microbial Mn(IV) reduction has the potential to be important in the formation of reduced manganese minerals, the release of dissolved manganese into sediment pore and ground waters, and the release of trace metals bound to Mn(IV) oxides (71). Mn(IV) reduction may also serve to oxidize organic matter in aquatic sediments, deep pristine aquifers, and shallow aquifers contaminated with organics, but quantitative data are scarce (71).

U(VI) REDUCTION

Microbial reduction of soluble U(VI) to insoluble U(IV) is an important process in the global uranium cycle and may also be a useful technique for removing uranium from contaminated environments (40, 81–83). Early evidence that microorganisms might reduce U(VI) to U(IV) was the finding that cell-free extracts of *Vellionella atypica* (formerly *Micrococcus lactilyticus*) reduce U(VI) along with a variety of other metals (141). However, there is no evidence that this metal reduction is an enzymatic reaction or has any physiological significance. Subsequent studies have demonstrated that whole cells of *V. atypica* do not reduce U(VI) (D. R. Lovley, unpublished data).

U(VI)-Reducing Microorganisms

G. metallireducens was the first organism found to use U(VI) as a terminal electron acceptor. *G. metallireducens* grows by carrying out the reaction:

$$\text{acetate}^- + 4\ \text{U(VI)} + 4\ H_2O \rightarrow 2HCO_3^- + 4\ \text{U(IV)} + 9\ H^+.$$

S. putrefaciens can also grow with U(VI) as the sole electron acceptor and H_2 as the electron donor:

$$H_2 + \text{U(VI)} \rightarrow 2\ H^+ + \text{U(IV)}.$$

Both organisms will grow in high-concentration (8 mM) dissolved uranium.

Several *Desulfovibrio* species can also enzymatically reduce U(VI) with either H_2 or lactate (82) (D. R. Lovley, unpublished data). However, attempts to grow *D. desulfuricans* with U(VI) as the sole electron acceptor have been unsuccessful (82).

Electron Transport to U(VI)

The enzymatic mechanisms for U(VI) reduction by *G. metallireducens* and *S. putrefaciens* are ill defined. The fact that these organisms conserve energy to support growth by oxidizing nonfermentable substrates with U(VI) as the sole electron acceptor suggests that electron transport-linked phosphorylation must be involved. Electron transport to or through *c*-type cytochrome(s) is likely based on the observation that U(VI) oxidizes the *c*-type cytochromes in whole-cell suspensions of *G. metallireducens* (76).

A U(VI) reductase has been isolated from the U(VI) reducer, *D. vulgaris* (D. R. Lovley, P. K. Widman & J. C. Woodward, submitted). The soluble fraction of *D. vulgaris* rapidly reduces U(VI) with H_2 as the electron donor. If cytochrome c_3 is removed from the soluble fraction of *D. vulgaris,* then all capacity for U(VI) reduction is lost. If cytochrome c_3 is added back, then the capacity for U(VI) reduction is restored. U(VI) rapidly oxidizes previously reduced cytochrome c_3. U(VI) is rapidly reduced when c_3 is combined with hydrogenase, the physiological electron donor for c_3, and H_2.

Environmental Significance of U(VI) Reduction

The environmental significance of microbial U(VI) reduction is that U(VI) is highly soluble in most natural waters, whereas U(IV) is highly insoluble (65). The reduction of U(VI) to U(IV) in anaerobic marine sediments is the most globally significant sink for dissolved uranium (1, 59, 136). The reductive precipitation of uranium from ground water is considered to be the mechanism for the formation of some sandstone or roll-type uranium ores (50, 53, 65). The earlier geochemical literature (50, 53, 65) suggested that U(VI) reduction in anaerobic environments results from nonenzymatic reduction of U(VI) reduction by sulfide or H_2. However, neither are effective U(VI) reductants at the temperatures and pH typical of most aquatic sediments or ground waters, and sterilization of anaerobic sediments inhibits U(VI) reduction (83). These findings suggest that U(VI)-reducing enzymes are responsible for U(VI) reduction in these environments.

Microbial U(VI) reduction may be used to remove uranium from contaminated waters and soils. In most natural surface and ground waters, U(VI) is in the form of uranyl-carbonate complexes (65). Furthermore, uranyl-carbonate complexes are the typical dissolved uranium form that results from various human activities utilizing uranium (81). Studies with *G. metallireducens* (40) and *D. desulfuricans* (82) demonstrated that U(VI)-reducing microorganisms can reduce the U(VI) in U(VI)-carbonate complexes. The U(IV) precipitates as uraninite (UO_2), all of which is extracellular. Thus, microbial uranium reduction can potentially take uranium that is dispersed in a large volume of liquid and concentrate it into a very pure, compact solid.

D. desulfuricans was chosen for detailed studies of microbial removal of U(VI) from contaminated environments because of the ease in mass culturing this organism and because its U(VI)-reducing capacity is extremely stable. For example, freeze-dried cells kept under air at room temperature lose none of their potential for U(VI) reduction even after six months of storage (81). *D. desulfuricans* effectively reduces U(VI) both at very high (24 mM) and at relatively low (< 50 nM) concentrations (81). Of the wide variety of potentially inhibiting anions and cations evaluated, only exceptionally high concentrations (> 20 μM) of copper inhibited U(VI) reduction. *D. desulfuricans* readily removed soluble U(VI) from several mine drainage waters and contaminated ground waters from a Department of Energy site.

In addition to treating uranium-contaminated waters, microbial U(VI) reduction can be used as part of a technique to concentrate uranium from contaminated soils (D. R. Lovley, E. J. P. Phillips & E. R. Landa, in preparation). In this process, uranium is leached from the soils with a bicarbonate solution and then microbial U(VI) reduction precipitates the uranium from the extract.

Microbial U(VI) reduction has several advantages over other previously proposed treatment techniques (81). These include: the ability to precipitate uranium from U(VI)-carbonate complexes; the recovery of uranium in a highly concentrated and pure form; high uranium removal per amount of biomass; the potential to simultaneously treat organic contaminants and uranium by using the organic as an electron donor for U(VI) reduction; and the potential for in situ remediation of both ground and surface waters.

Se(VI), Se(IV), AND Se(0) REDUCTION

Although selenium is classified a metalloid rather than a true metal, it is included here because of the obvious parallels between dissimilatory selenium reduction and dissimilatory metal reduction and because of its environmental significance. The discovery that toxic concentrations of selenium were adversely affecting bird populations at the Kesterson National Wildlife Refuge in California led to a surge in research on microbial metabolism of selenium. The predominant redox states of selenium in natural environments are Se(VI) (selenate, SeO_4^{-2}), Se(IV) (selenite, SeO_3^{-2}), Se(0) (elemental selenium), and Se(−II) (selenide) (29). The first three of these can potentially serve as electron acceptors for microbial metabolism.

Se(VI)-, Se(IV)-, and Se(0)-Reducing Microorganisms

A high percentage of the heterotrophic microorganisms that can be isolated from soil can reduce selenate to elemental selenium (13). *Clostridium* (56), *Citrobacter, Flavobacterium,* and *Pseudomonas* species (21) as well as an

unidentified gram-negative rod (89) all reduce selenate. However, the mechanisms for selenate reduction in these organisms were not studied in detail and no evidence of selenate-dependent growth was provided.

Several organisms that can conserve energy to support growth via selenate reduction have been described. Strain SeS was purified from intertidal sediments of San Francisco Bay (105). SeS obtains energy to support growth by the reaction:

$$4\ CH_3COO^- + 3\ SeO_4^{-2} \rightarrow 3\ Se^0 + 8\ CO_2 + 4\ H_2O + 4\ H^+.$$

Pseudomonas sp. AX, which was isolated from biological reactors used for selenium removal (88) has a different mode of selenate reduction, reducing selenate to selenite:

$$CH_3COO^- + H^+ + 4\ SeO_4^{-2} \rightarrow 2\ CO_2 + 4\ SeO_3^{-2} + 2\ H_2O.$$

When *Pseudomonas* sp. AX is cocultured with a selenite-reducing microorganism, selenate is rapidly reduced to elemental selenium (88). Another selenate-reducing microorganism, designated SeS-3, grows in defined medium with lactate as the electron donor and selenate as the electron acceptor (104, 122). Selenate is reduced to selenite and elemental selenium.

The ability of microorganisms to reduce selenite to elemental selenium has been known since the turn of the century (67 and references therein) and a wide range of microorganisms are known to catalyze this reaction (29, 104). This metabolism has never been found to be associated with energy conservation and may be a detoxification mechanism (29, 104). Cell suspensions of *T. ferrooxidans* reduce red elemental selenium to selenide (2).

Environmental Significance and Ecology of Selenium Reduction

Selenate and selenite reduction to insoluble elemental selenium has been documented in a variety of soils and aquatic sediments, and the evidence suggests that this reduction is enzymatically catalyzed (30, 89, 105, 123). Because of the low concentrations of selenate and selenite in the environment, they are not important electron acceptors for organic matter oxidation (105). However, microbial selenate and selenite reduction play a central role in affecting the fate of selenium in aquatic environments and may be the principle mechanism for the removal of selenate and selenite from agricultural wastewater evaporation ponds and high-selenium ground waters (104, 106, 107).

Various lines of evidence indicate that sulfate-reducing microorganisms are not responsible for selenate reduction in sediments. Selenate and selenite are

reduced at shallower depths than the zone of sulfate reduction (105, 106). At the depths where selenate is reduced, the sulfate concentrations are at levels that inhibit selenate reduction by sulfate reducers (143). Furthermore, molybdate, a specific inhibitor of sulfate reduction, did not inhibit selenate reduction (105).

Nitrate and Mn(IV) are preferred electron acceptors for selenate reducers (105, 122). Selenate reduction might be catalyzed by enzyme(s) that are also involved in dissimilatory nitrate reduction (104). The distribution of the maximum potential for denitrification and selenate reduction potentials in sediments are similar (106, 123), and the inhibition of selenate reduction in some sediments by tungstate but not molybdate might indicate the involvement of a molybdenum-containing nitrate reductase (105, 123). However, nitrate does not always inhibit selenate reduction at the low concentrations found in pore waters (106).

The immobilization of selenium that results when selenate is reduced to elemental selenium may be exploited to enhance removal of selenate from contaminated waters (89). For example, at Kesterson, flooding of previously exposed pond sediments with selenium-free water immobilized 66–110% of the dissolved selenium that had been present in the upper 1.22 m of soil (69). Immobilization is thought to result from the development of anaerobic conditions, which should stimulate selenate reduction. However, in situ stimulation of selenate reduction may not be a suitable long-term solution to the selenium-contamination problem as elemental selenium may enter the food chain through the bottom-feeding organisms (87).

Ex situ treatment processes for contaminated waters are also possible. Selenate is microbially reduced to insoluble elemental selenium when uranium-mine discharge water is passed through a soil column (56). Several investigators have suggested using combined algal-bacterial systems to remove selenium from contaminated waters (35, 106). Nitrate, which inhibits selenate reduction, is removed via the growth of algae. The water thus treated is then fed into an anaerobic digestor where a portion of the algae are oxidized and thereby become electron donors for removal of any remaining nitrate (through denitrification), and then selenate is reduced and precipitated as elemental selenium.

Cr(VI) REDUCTION

Chromium contamination of the environment is extensive (12). The reduction of highly toxic and mobile Cr(VI) to the less toxic, less mobile Cr(III) is likely to be a useful process for the remediation of contaminated waters and soils (108). This problem has stimulated interest in microorganisms that can use Cr(VI) as an electron acceptor.

Cr(VI)-Reducing Microorganisms

Early investigations demonstrated that facultative anaerobes such as *Pseudomonas dechromaticans* (117), *Pseudomonas chromatophila* (66), and *Aeromonas dechromatica* (64) remove Cr(VI) from solution by the formation of a Cr(III) precipitate, presumably $Cr(OH)_3$. Subsequent studies have demonstrated that the capacity for Cr(VI) reduction is widespread and found in such organisms as *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Achromobacter eurydice, Micrococcus roseus,* and *Escherichia coli* (43), as well as *Pseudomonas ambigua* (48), *Pseudomonas fluorescens* (17), *Enterobacter cloacae* (137), *Streptomyces* spp. (26), *Pseudomonas putida* (52), *D. desulfuricans,* and *D. vulgaris* (D. R. Lovley & E. J. P. Phillips, in preparation).

Many of these organisms reduce Cr(VI) better under aerobic conditions than under anaerobic conditions and the physiological role of this aerobic Cr(VI) reduction has not been well defined. Cr(VI) reduction is not a Cr(VI)-resistance mechanism (17), and Cr(VI) reduction in *P. putida* and possibly other Cr(VI) reducers is a side activity for enzymes that have other, as yet unidentified, natural substrates (23, 52).

Although some microorganisms reduce Cr(VI) during anaerobic growth in media in which Cr(VI) is provided as the sole electron acceptor, in no instance has Cr(VI) reduction definitely been shown to yield energy to support anaerobic growth. For example, *Pseudomonas chromatophila* purportedly uses Cr(VI) as an electron acceptor to support growth under anaerobic conditions with a variety of electron acceptors, including the nonfermentable substrate, acetate (66). However, in the only data shown, most of the Cr(VI) reduction took place after growth stopped. No studies demonstrating that anaerobic growth depended upon the presence of Cr(VI) were presented.

Pseudomonas fluorescens LB300, which reduces Cr(VI) while growing aerobically in a glucose medium, also grows in an anaerobic chamber containing oxygen-free N_2 on agar plates containing acetate as a potential electron donor and Cr(VI) as the potential electron acceptor (17). However, no Cr(VI) reduction occurs in anaerobic liquid cultures. Neither acetate oxidation nor Cr(VI) reduction under anaerobic conditions was documented. Further studies on whether electron transport to Cr(VI) can yield energy to support growth of this organism seem warranted given the difficulties in maintaining oxygen-free conditions in anaerobic chambers under a N_2 atmosphere.

Enterobacter cloacae strain HO1 reduces Cr(VI) while growing anaerobically in a medium that contains acetate and Casamino acids as potential electron donors (137). O_2 rapidly inhibits Cr(VI) reduction, but reduction

resumes when O_2 is removed (62). Amino acid mixtures are the best electron donors for Cr(VI) reduction (102). The fate of the electron donors (i.e. whether they are oxidized to carbon dioxide or whether they are fermented) has not been reported. *E. cloacae* can grow anaerobically in the absence of added Cr(VI) and no evidence for Cr(VI)-dependent growth has been presented (137). In fact, in one study, the number of viable cells decreased in the initial stages after Cr(VI) was added, and ~50% of the added Cr(VI) was reduced before viable cell numbers increased over what was present prior to Cr(VI) addition (100).

Enzymatic Mechanisms for Cr(VI) Reduction

Cr(VI) reduction is an enzymatically catalyzed reaction in the Cr(VI) reducers that have been studied in detail. For example, spent medium from cultures of *P. fluorescens* LB300 does not reduce Cr(VI), and washed cell suspensions reduce Cr(VI) only if glucose or another suitable electron donor is provided (17). Cyanide (10^{-2} M) or azide (10^{-3} M) inhibit Cr(VI) reduction in crude cell extracts, and the Cr(VI)-reducing capacity is lost when the membrane fraction is removed.

Evidence for an enzymatic role in Cr(VI) reduction in *E. cloacae* is that: Cr(VI) is reduced faster with higher cell densities; no Cr(VI) reduction occurs in cell-free controls; and inhibition of growth with antibiotics inhibits Cr(VI) reduction (137). Cell-free filtrates of cultures do not reduce Cr(VI) (138). Several metabolic poisons as well as molybdate, vanadate, and tellurate inhibit Cr(VI) reduction. Cr(VI) also inhibits Cr(VI) reduction—the rate of reduction declines as the concentration rises above 1 mM (100). Temperature and pH optima for Cr(VI) reduction are characteristic of an enzymatically catalyzed reaction (63).

The Cr(VI) reductase activity in *E. cloacae* is located in the membrane fraction (138). When membrane vesicles are reduced with NADH and then exposed to Cr(VI), the *c*- and *b*-type cytochromes are oxidized (139). Further analyses have suggested that of the identifiable cytochromes in the membrane vesicles (c_{548}, c_{549}, c_{550}, b_{555}, b_{556}, and b_{558}), c_{548} might be specifically involved in Cr(VI) reduction, serving as a branch point between Cr(VI) and O_2 reduction.

In contrast to *P. fluorescens* and *E. cloacae*, the Cr(VI)-reducing activity in *P. ambigua* and *P. putida* is in the soluble fraction of the cell (48, 52). A 65-kDa protein has been purified from *P. ambigua* that can reduce Cr(VI) with NADH or NADPH serving as the electron donor (134). The enzyme initially reduces Cr(VI) to Cr(V), which is subsequently reduced to Cr(III). NADH also reduces Cr(VI) to Cr(V) in the absence of the enzyme, but at slower rates than with the enzyme.

Washed cell suspensions of *D. desulfuricans* and *D. vulgaris* rapidly reduce

Cr(VI) to Cr(III) under anaerobic conditions with H_2 as the electron donor (D. R. Lovley & E. J. P. Phillips, in preparation). H_2-dependent, Cr(VI) reductase activity in the soluble, cell-free fraction of *D. vulgaris* is lost when the soluble fraction is passed over a cation-exchange column that removes cytochrome c_3, a periplasmic protein. The capacity for Cr(VI) reduction is restored when cytochrome c_3 is added back. In the presence of H_2 and an excess of hydrogenase, cytochrome c_3 reduces Cr(VI) at a rate 50-fold faster than the maximum rate for Cr(VI) reduction by the Cr(VI) reductase purified from *P. ambigua*.

Environmental Significance of Cr(VI) Reduction

Most of the earth's chromium is tied up as insoluble Cr(III) (12). In unpolluted fresh and sea waters the concentrations of chromium are generally less than 50 nmol/liter (113). Therefore, microbial Cr(VI) reduction is of primary concern in contaminated environments or for the treatment of Cr(VI)-containing wastes. Cr(VI) is readily reduced to Cr(III) in anaerobic aquatic sediments, but nonenzymatic processes such as Cr(VI) reduction by Fe(II) are likely to be at least as important as enzymatic Cr(VI) reduction (91).

The most extensive studies on the potential use of Cr(VI)-reducing microorganisms for the removal of Cr(VI) from waste streams have been conducted with *E. cloacae* strain HO1. Early studies with this organism revealed that during Cr(VI) reduction the yellow medium turns white and turbid, presumably as the result of the formation of insoluble Cr(III) hydroxide (137). However, the Cr(III) does not readily precipitate and cannot be effectively removed with centrifugation (61). Cr(III) is removed more effectively if *E. cloacae* is placed on one side of a semipermeable membrane and the Cr(VI)-containing medium is on the other (61). Cr(VI) diffuses into culture and *E. cloacae* reduces the Cr(VI) to Cr(III), which is retained on the culture side, especially when an anion-exchange membrane that does not permit passage of Cr(III) is used (60). Despite successful removal of Cr(VI) from culture medium with *E. cloacae*, removal from industrial effluents has been problematic as heavy metals and sulfate in the effluents can inhibit Cr(VI) reduction (45, 101). Whether microbial Cr(VI) reduction would have advantages over simple chemical Cr(VI) reduction in the removal of Cr(VI) does not appear to have been evaluated.

Hg(II) REDUCTION

Many aerobic and facultative microorganisms reduce soluble ionic Hg(II) to volatile Hg(0) as a detoxification mechanism (20, 114, 132). The Hg(II) is reduced during aerobic growth and Hg(II) reduction is not linked to energy-conserving electron transport. In contrast to most types of dissimilatory metal

reduction, about which very little is known, many of the mechanisms for Hg(II) reduction have been beautifully elucidated for both gram-negative and gram-positive bacteria. This includes understanding of the mechanisms for Hg(II) transport into the cell and the intracellular reduction of Hg(II), as well as mapping of the organization of the mercury-resistance (*mer*) operons and determination of the structure of the Hg(II) reductase. These topics have been reviewed frequently (for recent updates see 119, 121, 131 and references therein) and thus, because of space limitations, are not discussed further here. As recently suggested, the respiratory metal-reducing microorganism, *G. metallireducens,* may be able to reduce Hg(II) through its dissimilatory metal-reducing pathway (76).

Environmental Significance and Ecology of Microbial Hg(II) Reduction

Microbial reduction of Hg(II) may play an important role in the fate of mercury in both aquatic and terrestrial environments (11, 38). For example, microbial Hg(II) reduction in ocean waters has been proposed to be a significant source of atmospheric mercury (57). However, the study of Hg(II) reduction in the environment is complicated by the fact that the mercury cycle is much more complex than the geochemical cycles of other redox sensitive metals such as iron (51). In addition to microbial Hg(II) reduction, microbially catalyzed methylation and demethylation reactions as well as a myriad of abiotic reactions also influence mercury cycling (11, 38). Furthermore, although some of the Hg(II) reduction in natural waters clearly depends upon the presence of living microorganisms, in some instances Hg(II) reduction is primarily a nonbiological process (6, 8, 11, 112).

Mercury contamination of the environment generally enhances the capacity for microbial Hg(II) reduction. In uncontaminated waters the rate of biological Hg(II) reduction typically lags for 6–24 h until the microbial population can adapt (6, 11). This lag is in contrast to the immediate microbial Hg(II) reduction in water samples from contaminated sites or pristine waters that have been artificially preexposed to Hg(II). Mercury-contaminated environments have increased abundance of Hg(II)-resistant bacteria and/or *mer* genes as well as increased rates of Hg(II) reduction (4–6, 8–11, 103, 115). Although many of the Hg(II)-reducing microorganisms in mercury-contaminated environments do not have genes that hybridize to *mer* gene probes under highly stringent hybridization conditions (7, 8, 103, 115), current evidence indicates that these organisms also volatilize Hg(II) with a NADPH-dependent Hg(II) reductase (7).

One proposed bioremediation strategy for removing mercury from contaminated environments would be to stimulate *mer*-mediated reduction of Hg(II)

(11, 38). This could be accomplished by increasing the density of working *mer* operons, either by adding Hg(II) reducers, stimulating the growth of indigenous populations, amplifying the number of *mer* operons in indigenous microorganisms, or increasing the percentage of indigenous *mer* operons that are expressed (11, 38). However, these approaches assume that Hg(II) reduction is enzyme limited. A greater limitation in the use of microbial Hg(II) reduction to remove Hg(II) from some contaminated environments is that not all of the Hg(II) may be available for microbial reduction. For example, typically less than half and sometimes less than 10% of the Hg(II) in contaminated waters is volatilized by microbial reduction (11, 112). Much of the mercury may be bound in nonreducible forms to suspended particulate matter in the water (11). Mercury in aquatic sediments may also be unavailable for microbial reduction because sterile sediment added to water inhibits Hg(II) reduction (11) and sediment-water incubation systems volatilize less mercury than systems with water alone (112). Further evidence that mercury was in nonreducible forms in aquatic sediments was the finding that the abundance of *mer* genes in contaminated bottom sediments was two orders of magnitude lower than in the water column even though the sediment had very high mercury concentrations (11).

Preliminary experiments demonstrated that microbial Hg(II) reduction has the potential to volatilize Hg(0) from Hg(II)-containing mine- and industrial-waste waters (133) as well as residential sewage (44). However, I have been unable to find documentation of any instance in which microbial Hg(II) reduction is currently being applied for the removal of mercury from contaminated waters or waste streams.

Tc(VII) REDUCTION

Technetium is a long-lived (half-life, 2.15×10^5 years) radioactive contaminant in the environment that is a by-product of fission reactions occurring in atomic explosions and in nuclear power stations (135). Under aerobic conditions, technetium is primarily in the form of pertechnetate [Tc(VII); TcO_4^-), which is highly soluble (135). The reduced form of technetium, Tc(IV) is highly insoluble (135). Several studies have demonstrated that the development of reducing conditions in soils greatly decreases the solubility and mobility of technetium, presumably as the result of Tc(VII) reduction to Tc(IV) (120 and references therein).

One of the few studies that have directly examined the potential for microorganisms to reduce Tc(VII) found that a mixed culture of anaerobic bacteria produced more insoluble and/or adsorbed technetium from pertechnetate than mixed or pure cultures of aerobic bacteria (46). Forms of technetium

other than pertechnetate were also associated with dissolved organics. However, if pertechnetate was added to bacteria-free filtrates of the mixed anaerobic cultures, no organic-associated technetium was formed. These results suggested that Tc(VII) might have served as an electron acceptor in anaerobic respiration.

Tc(VII) was reduced during the stationary growth phase of *Moraxella* and *Planococcus* spp. as oxygen was depleted from the medium (109). Studies with heat-killed cells and incubations with live cells at different temperatures suggested that Tc(VII) reduction resulted from an enzymatically catalyzed reaction.

Sulfate-reducing bacteria may also be involved in Tc(VII) reduction. The sulfate-reducing microorganisms, *Desulfovibrio gigas* and *D. vulgaris*, were even more effective than a mixed anaerobic microbial culture in converting Tc(VII) to insoluble and/or adsorbed forms (46). Molybdate, a specific inhibitor of sulfate reducers, inhibited Tc(VII) reduction in a mixed culture of anaerobic marine microorganisms (109). Both studies suggested that the ability of sulfate-reducers to remove technetium from solution was due, at least in part, to the formation of insoluble technetium sulfides and/or the release of other reducing agents. However, the only evidence for nonenzymatic reduction of Tc(VII) was the association of some technetium with dissolved organics when pertechnetate was added to bacterial-free filtrates of the sulfate-reducing cultures (46). Given the recent finding that sulfate reducers can enzymatically reduce other metals [see sections on Fe(III), U(VI), and Cr(VI) reduction, above], sulfate reducers might also be able to enzymatically reduce Tc(VII).

V(V) REDUCTION

Although microbial V(V) reduction has not been studied intensively, this metabolic capability may be widespread. Almost all of the heterotrophic bacteria and fungi isolated from a silty clay loam soil could reduce V(V) (13). However, the mechanisms for V(V) reduction were not determined.

Two V(V)-reducing microorganisms have subsequently been studied in greater detail (142). *Pseudomonas vanadium reductans* was isolated from the effluent of a metallurgical factory, and *Pseudomonas isachenkovii* was isolated from sea water. Evidence for V(V) reduction is primarily qualitative. During the anaerobic growth of these microorganisms, the V(V)-containing medium changes color from the pale yellow characteristic of dissolved V(V) to blue, which is characteristic of V(IV). With further incubation the medium turns black and opaque, presumably from the accumulation of colloidal V(III) particles. All of the V(V) is reduced within 10 days of incubation; there is

no loss of V(V) in sterile controls. Several sugars, amino acids, lactate, glycerol, H_2, and CO can serve as electron donors for V(V) reduction.

Given the similarities between the geochemistry of uranium and vanadium (50), microbial vanadium reduction could be responsible for the commonly observed (47, 50) precipitation of vanadium in anaerobic environments, as seen with uranium reduction (see above), and this metabolism could potentially be used to remove vanadium from ore-processing waste streams.

Mo(VI) REDUCTION

Pseudomonas guillermondii and a *Micrococcus* sp. reduce Mo(VI) to blue reduced molybdenum [presumably Mo(V)] during growth on aerobic heterotrophic medium or in cell suspensions (13). The mechanisms for Mo(VI) reduction were not determined.

At high temperature (60°C), low pH (< 2), and with S^0 as the potential electron donor, *Sulfolobus brierleyi* and *S. acidocaldarius* reduce Mo(VI) to Mo(V) under aerobic or anaerobic conditions (18). Mo(VI) is not reduced when the organisms are grown on yeast extract instead of S^0.

Cell suspensions of the mesophile *T. ferrooxidans* also reduce Mo(VI) with S^0 (129). The rate of Mo(VI) reduction is proportional to the amount of cell protein added and the initial S^0 and Mo(VI) concentrations. The hydrogen sulfide:ferric ion oxidoreductase [see section on Fe(III) reduction] purified from *T. ferrooxidans* catalyzes S^0 oxidation to sulfite coupled to Mo(VI) reduction (129). However, Fe(III) is considered to be the physiological electron acceptor for this enzyme because the rate of Mo(VI) reduction is 20-fold lower than the rate of Fe(III) reduction.

Apparently no ecological investigations have examined the role of microbial Mo(VI) reduction in the environment. The reduction of Mo(VI) under acidic conditions could potentially influence molybdenum cycling during ore leaching. Microbial Mo(VI) reduction at circumneutral pH might be involved in the concentration of insoluble molybdenum in anaerobic marine sediments (15) and the reduction spots found in rocks (47).

Cu(II) REDUCTION

Washed cell suspensions of *T. ferrooxidans* reduce Cu(II) to Cu(I) in the presence of S^0 as a potential electron donor (128). Cu(II) is reduced under both aerobic and anaerobic conditions. However, only net reduction occurs under aerobic conditions when azide or cyanide are added to prevent the iron oxidase from oxidizing Cu(I). The hydrogen sulfide:ferric ion oxidoreductase that reduces Fe(III) and Mo(VI) (see above) also reduces Cu(II) (128). Cu(II) reduction by *T. ferrooxidans* may play a role in copper leaching (124).

Au(III), Au(I), AND Ag(I) REDUCTION

Bacillus subtilis, *Aspergillus niger*, *Cholorella vulgaris*, and *Spirulina platentis* reduce Au(III) to Au(0) (16, 34, 41). Within 5 min of exposure of isolated walls of *B. subtilis* to Au(III) chloride, granules of elemental gold form within the walls (16). Au(III) and Au(I) adsorb onto cells of *C. vulgaris* with subsequent reduction to Au(0) (41). Reduction of Au(III) to Au(I) is much faster than the reduction of Au(I) to A(0), which takes place over several days. Gold crystals form on the cell surfaces of *C. vulgaris* and within the cells (49). In some instances the gold crystals are larger than the organisms themselves (34). The mechanisms for Au(III) reduction in these organisms have not been elucidated.

In a similar manner to Au(III) and Au(I), Ag(I) adsorbs onto the cell walls of bacteria and fungi with the subsequent reduction of Ag(I) to colloidal Ag(0) (92, 93). Reduction of Ag(I) to Ag(0) through the production of unknown reducing compounds may account for silver resistance in some bacteria (14).

Dissimilatory Fe(III)-reducing microorganisms can probably also reduce Au(III) and Ag(I). Addition of Au(III) or Ag(I) resulted in the oxidation of the *c*-type cytochrome(s) in *G. metallireducens*, suggesting that the *c*-type cytochromes or a terminal reductase can transfer electrons to Au(III) (76). Washed cell suspensions of *D. vulgaris* rapidly reduce Au(III) to Au(0), and the c_3 cytochrome from *D. vulgaris* reduces Au(III) with the accumulation of Au(0) (D. R. Lovley, P. K. Widman & J. C. Woodward, submitted).

Microbial Au(III) reduction may lead to the formation of gold deposits (140) and could potentially be used for the removal of gold from waters and waste streams (34, 41, 90).

CONCLUSIONS

The intrinsic activity of dissimilatory metal-reducing microorganisms that use Fe(III), Mn(IV), U(VI), or Se(VI) as terminal electron acceptors can greatly influence the fate of these metals in aquatic sediments and groundwater. Fe(III) [and possibly Mn(IV)] reduction can also be an important mechanism for the oxidation of naturally occurring organic matter and organic contaminants in these environments. Dissimilatory reduction of U(VI), Se(VI), Cr(VI), Hg(II), Tc(VII), V(V), Au(III), and Ag(I) is a potential mechanism for removing these metals from contaminated environments or waste streams. Although microorganisms are available in pure culture that can serve as models for the reduction of each of these metals, there is no substantive information about which microorganisms are important in catalyzing metal reduction in natural environments. Furthermore, with the exception of Hg(II) reduction, very little is known about the physiology and biochemistry of dissimilatory metal reduction.

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