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Dissimilatory Fe(III) and Mn(IV) Reduction

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INTRODUCTION

Microbial dissimilatory reduction of Fe(III) and Mn(IV) greatly influences the biogeochemical cycles of carbon and many metals. In fact, the reduction of Fe(III) has been termed the most important chemical change that takes place in the development of anaerobic soils and sediments (261). As discussed in detail below, Fe(III) reduction may have been the first globally significant mechanism for the oxidation of organic matter to carbon dioxide, and Fe(III) and Mn(IV) reduction continues to be an important mechanism for the oxidation of natural or contaminant organic compounds in diverse aquatic environments. Dissimilatory Fe(III) and Mn(IV) reduction have a major influence on the distribution of iron and manganese in aquatic sediments, submerged soils, and groundwater. Furthermore, the extent of Fe(III) and/or Mn(IV) reduction can strongly influence the distribution of toxic trace metals and phosphate, an impor-

tant nutrient in aquatic environments. Microbial Fe(III) reduction also contributes to many other phenomena of environmental and/or practical importance.

Although the ability of some microorganisms to bring about the reduction of Fe(III) and Mn(IV) has been known since the 19th century (2, 7, 129), until recently, even microbiologists considered that much of the Fe(III) and Mn(IV) reduction in natural environments was the result of nonenzymatic processes (99, 112, 341). This hypothesis was due, at least in part, to the fact that there were no microorganisms known which could effectively couple the oxidation of organic matter to the reduction of Fe(III) or Mn(IV). However, organisms which can obtain energy for growth by the complete oxidation of organic compounds to carbon dioxide with Fe(III) or Mn(IV) as the sole electron acceptor have now been described, and it is becoming increasingly apparent that the metabolism of these organisms is responsible for most of the Fe(III) and Mn(IV) reduction taking place in sedimentary environments.

TYPES OF DISSIMILATORY Fe(III) AND Mn(IV) REDUCERS

Dissimilatory Fe(III) or Mn(IV) reduction can be defined as the use of Fe(III) or Mn(IV) as an external electron acceptor in metabolism. Fe(III) is reduced to Fe(II). Mn(II) is generally regarded as the end product of Mn(IV) reduction, but in some instances Mn(III) may be an intermediate (88, 121). (As discussed below, both oxidized and reduced iron and manganese can exist in a variety of dissolved forms and particulate minerals. The forms of the iron and manganese products and reactants can greatly influence the energy yield of the reaction [198]. To avoid confusion, the various redox states of iron and manganese will be referred to only in the appropriate valence state, with no specification of the forms of the iron or manganese involved.)

Dissimilatory Fe(III) and Mn(IV) reduction differs from the various forms of assimilatory Fe(III) or Mn(IV) reduction, in which Fe(III) or Mn(IV) is reduced as part of the process of iron or manganese assimilation into enzymes, cofactors, magnetosomes, etc. A distinction between assimilatory and dissimilatory Fe(III) and Mn(IV) reduction is that, under normal physiological conditions, significant quantities of Fe(II) and Mn(II) are only expected to accumulate outside the cell during dissimilatory Fe(III) and Mn(IV) reduction.

A wide variety of fungi and bacteria reduce Fe(III) or Mn(IV) under various conditions. Detailed lists of these organisms may be found in previous reviews (89, 100, 112, 156, 184, 251). These organisms have typically been identified as dissimilatory Fe(III)- or Mn(IV)-reducing microorganisms according to the accumulation of Fe(II) or Mn(II) in cultures growing under anaerobic conditions in organically complex media. Alternatively, resting cell suspensions of aerobically grown cells have been tested for their abilities to reduce Fe(III) or Mn(IV) under anaerobic conditions. In most instances, the physiological function of the Fe(III) or Mn(IV) reduction, if any, has not been characterized. The metabolism of the relatively few Fe(III)- or Mn(IV)-reducing microorganisms that have been studied in more detail are discussed below.

Fermentative Fe(III) and Mn(IV) Reducers

The first organisms shown to use Fe(III) or Mn(IV) as an electron acceptor while growing under anaerobic conditions were microorganisms that have a primarily fermentative metabolism. In early studies, Runov (274) noted Fe(III) reduction in pure cultures of *Escherichia coli*, *Clostridium pasteurianum*, *Lactobacillus lactis*, and other bacteria. Starkey and Halverson (289) found that Fe(III) was reduced in anaerobic cultures of *Clostridium sporogenes* or *E. coli* growing in a glucose or peptone medium, and Roberts (272) found that *Bacillus polymyxa* reduced Fe(III) while fermenting glucose. Subsequent studies found that a wide variety of fermentative microorganisms reduced Fe(III) or Mn(IV) during anaerobic growth (45, 46, 128, 158, 159, 226, 245, 246), and organisms capable of reducing Fe(III) and Mn(IV) under aerobic culture conditions have also been described (76, 82, 86, 316).

Fe(III) and Mn(IV) reduction is only a minor pathway for electron flow in the microorganisms that are known to reduce Fe(III) or Mn(IV) while metabolizing fermentable sugars or amino acids. In the first quantitative study of this metabolism, Roberts (272) found that *B. polymyxa* reduced 41 mol of Fe(III) per 100 mol of glucose metabolized in a defined glucose-asparagine medium. The complete oxidation of a mole of glucose to carbon dioxide with Fe(III) serving as the electron acceptor is as follows:

$$C_6H_{12}O_6 + 24Fe(III) + 12H_2O \longrightarrow 6HCO_3^- + 24Fe(II) + 30H^+$$

This reaction results in the reduction of 24 mol of Fe(III). Thus, it is apparent that Fe(III) reduction was not an important electron acceptor for glucose metabolism in *B. polymyxa*. Most of the electron equivalents initially present in glucose were recovered as ethanol, 2,3-butylene glycol, lactic acid, formic acid, and hydrogen (272).

Fermentative *Vibrio* spp. transferred only 0.13 or 0.03% of the reducing equivalents available in glucose or malate to Fe(III) (159). Malate fermentation resulted primarily in the accumulation of organic acids and ethanol. With malate as the substrate, there was a decrease in ethanol formation in the presence of Fe(III) and a 28% increase in the molar growth yield. However, it was concluded that the small amount of Fe(III) reduced could not account for the increased growth (159).

Fe(III) added to the medium stimulated the anaerobic growth of *Fusarium oxysporum* on glucose, and added Mn(IV) stimulated glucose metabolism under anaerobic conditions (125). However, it was not determined whether Fe(III) or Mn(IV) reduction was linked to electron transport and directly yielded energy to support growth.

It has been calculated (184, 198) from the data of others (223, 226, 316) that other fermentative Fe(III)- and Mn(IV)-reducing microorganisms transfer less than 5% of the reducing equivalents from their substrates metabolized to Fe(III) or Mn(IV). None of these organisms have been found to gain energy to support growth from the reduction of Fe(III) or Mn(IV). Fe(III) and Mn(IV) appear to serve as minor electron sinks in a primarily fermentative metabolism.

It was previously argued that there should be Fe(III)-reducing microorganisms which, by themselves, could completely oxidize fermentable compounds to carbon dioxide with Fe(III) as the sole electron acceptor, because such organisms could potentially obtain more energy per mole of glucose metabolized than organisms which fermented only glucose (184). However, numerous attempts to isolate or even enrich for organisms which could effectively couple the oxidation of fermentable substrates to the reduction of Fe(III) have been unsuccessful (186). This lack of success could be attributed to a lack of proper culturing techniques.

However, studies of glucose metabolism in various sediments in which Fe(III) reduction was the terminal electron-accepting process found that trace quantities of [U-^{14}C]glucose were invariably fermented to ^{14}C-fatty acids (199). There was no evidence for complete direct oxidation of glucose to carbon dioxide. Thus, if there are Fe(III)-reducing microorganisms which, by themselves, can completely oxidize glucose with Fe(III) as the electron acceptor, their metabolism does not appear to be an important factor in Fe(III)-reducing sediments.

The initial metabolism of glucose (and possibly other fermentable sugars and amino acids) by fermentative microorganisms in Fe(III)-reducing environments is similar to metabolism in methanogenic and sulfidogenic sedimentary environments (190, 332). McInerny and Beaty (215) have suggested that glucose is first fermented in methanogenic and sulfidogenic environments because the important thermodynamic consideration in the competition for organic substrates is not the amount of energy available per mole of substrate metabolized but, rather, the amount of energy released per mole of electrons transferred. According to this hypothesis, sulfate reducers and methanogens that could completely metabolize fermentable sugars or amino acids to methane and/or carbon dioxide would not be competitive with fermentative microorganisms because, per electron transferred, fermentation has a greater potential energy yield than the conversion of these compounds to methane and/or carbon dioxide (215). Thermodynamic calculations suggest that, per electron transferred, glucose fermentation is also more energetically favorable than complete oxidation of glucose with the reduction of Fe(III) (199). Accordingly, organisms attempting to completely oxidize fermentable compounds with Fe(III) as the electron acceptor may be unable to grow as fast or may have some other competitive disadvantage in comparison with fermentative microorganisms (199). Thermodynamic calculations further suggest that the metabolic strategy of using Fe(III) as a minor electron sink can provide a slightly greater energy yield than fermentation alone. This may explain the wide diversity of fermentative microorganisms that can divert a small proportion of their electron flow to Fe(III) reduction. Whether similar arguments can be applied to Mn(IV) reduction does not appear to have been investigated.

Sulfur-Oxidizing Fe(III) and Mn(IV) Reducers

With elemental sulfur (S^0) as the electron donor, *Thiobacillus thiooxidans*, *Thiobacillus ferrooxidans*, and *Sulfolobus acidocaldarius* reduced Fe(III) to Fe(II) while growing under aerobic conditions (*T. thiooxidans* and *S. acidocaldarius*) or after aerobically grown cells were placed under anaerobic conditions (*T. ferrooxidans*) (44). The reaction was presumed to be as follows:

$$S^0 + 6Fe(III) + 4H_2O \longrightarrow HSO_4^- + 6Fe(II) + 7H^+$$

HQNO (2-*n*-heptyl-4-hydroxyquinoline *N*-oxide) inhibited Fe(III) reduction with sulfur in *T. ferrooxidans*, and this fact has been provided as evidence that *T. ferrooxidans* should be able to conserve energy from this reaction (68). However, neither *T. thiooxidans* (169) nor *T. ferrooxidans* (277, 303) was able to obtain energy to support growth from sulfur oxidation coupled to Fe(III) reduction. A hydrogen sulfide-ferric ion oxidoreductase involved in sulfur oxidation has been purified (300, 301). *T. thiooxidans* (325) and *T. ferrooxidans* (302) also oxidized sulfur with the reduction of Mn(IV). However, in both instances it was concluded that the Mn(IV) was nonenzymatically reduced by Fe(II) (302, 325), sulfite (302), or other soluble components (325).

Hydrogen-Oxidizing Fe(III) and Mn(IV) Reducers

There is abundant evidence that there are microorganisms living in sediments which can couple the oxidation of hydrogen to the reduction of Fe(III) or Mn(IV). Addition of hydrogen to sediments containing microbially reducible Fe(III) oxides stimulates Fe(III) reduction (194, 275). Furthermore, the addition of Fe(III) or Mn(IV) to methanogenic or sulfidogenic sediments results in a drop in the steady-state hydrogen concentration to levels much lower than those in the unamended sediments (189, 195). This can be attributed to the high affinity of Fe(III) and Mn(IV) reducers for hydrogen.

A *Pseudomonas* sp. (17) and *Shewanella* (formerly *Alteromonas*) *putrefaciens* (201) oxidized H_2 with Fe(III) as the sole electron acceptor. In both organisms, growth coincided with Fe(III) reduction, and the stoichiometry of hydrogen consumption and Fe(II) accumulation was consistent with the following reaction:

$$H_2 + 2Fe(III) \longrightarrow 2H^+ + 2Fe(II)$$

The *Pseudomonas* sp. did not oxidize H_2 with Mn(IV) as a potential electron acceptor, but *S. putrefaciens* did. Although the stoichiometries of H_2 consumption and Mn(IV) reduction were not determined, it is likely that H_2 is oxidized according to this reaction:

$$H_2 + Mn(IV) \longrightarrow 2H^+ + Mn(II)$$

Neither of the H_2-oxidizing, Fe(III)-reducing microorganisms was capable of autotrophic growth. The *Pseudomonas* sp. required 0.025% yeast extract for growth, and, as previously determined (229), *S. putrefaciens* required the addition of 0.02 g each of L-arginine hydrochloride, L-glutamine, and L-serine per liter. The *Pseudomonas* sp. was capable of growth on a wide variety of carbohydrates, organic acids, and alcohols under aerobic conditions, but the ability of the organism to metabolize these compounds with Fe(III) as the electron acceptor was not reported. *S. putrefaciens* can also metabolize a variety of organic compounds with O_2 as the electron acceptor (282) but is very restricted in the organic compounds which can be oxidized with Fe(III) as the sole electron acceptor (see below).

A gram-negative, coccobacillus which might have the ability to couple the oxidation of H_2 to the reduction of Fe(III) was also reported (158). However, growth and Fe(III) reduction were weak. After 28 days of incubation, less than 40 μmol of Fe(II) per liter had been produced. In contrast, *S. putrefaciens* typically produces 1,000-fold-more Fe(II) over a period of 24 h while growing on H_2 (201). No evidence was provided for H_2 consumption by the coccobacillus. In fact, there was no difference in the amount of Fe(II) produced (7 μmol/liter) in the presence or absence of H_2 in an acetate-Fe(III) medium. Although there was slightly more Fe(II) produced (38 μmol/liter) in H_2-yeast extract-Fe(III) medium than in N_2-yeast extract-Fe(III) medium (27 μmol/liter), there was no evidence presented that this difference was statistically significant. Furthermore, the addition of Fe(III) did not consistently stimulate growth, and there was no evidence that when the addition of Fe(III) stimulated ATP production, the increased ATP production could be attributed to Fe(III) reduction.

Organic-Acid-Oxidizing Fe(III) and Mn(IV) Reducers

The first Fe(III)- and Mn(IV)-reducing microorganisms found to effectively couple the oxidation of organic compounds to the reduction of Fe(III) or Mn(IV) were organic-acid oxidizers. Early evidence that Fe(III)-reducing microorganisms had the potential to completely oxidize organic compounds to carbon dioxide was the finding that, in anaerobic rice paddy soils containing Fe(III) oxides, the disappearance of added acetate and the production of $^{14}CO_2$ from [2-^{14}C]acetate were concurrent with the accumulation of Fe(II) (164). Addition of estuarine water to an anaerobic mineral medium that contained acetate as the sole potential electron donor and MnO_2 as a potential electron acceptor resulted in the accumulation of dissolved manganese over time (337). Enrichment cultures that reduced Mn(IV) in the presence of lactate, succinate, or acetate were recovered from coastal marine sediments (51). However, the medium also contained substrate level of yeast extract and peptone as well as 10% H_2 in the headspace. The metabolisms of these potential substrates were not investigated, so the actual electron donors for Mn(IV) reduction were unknown. Enrichment cultures that reduced Fe(III) in the presence of acetate or succinate were recovered from estuarine sediments, but in most instances, the cultures contained yeast extract, and the electron donors for Fe(III) reduction were not determined (320). Enrichment cultures capable of metabolizing acetate, propionate, and butyrate were established with fresh- and brackish-water sediments from the Potomac River, and the addition of these compounds stimulated Fe(III) reduction in the sediments (194). Furthermore, the acetate and butyrate produced from glucose fermentation in a glucose-Fe(III) enrichment culture were metabolized with the reduction of Fe(III) (194).

The first microorganism in pure culture shown to completely oxidize organic compounds to carbon dioxide with Fe(III) or Mn(IV) as the electron acceptor was the isolate GS-15 (198, 205). This microorganism was isolated from an acetate-Fe(III) oxide enrichment that had been initiated with freshwater sediments of the Potomac River. GS-15 is a gram-negative rod that is 2 to 4 by 0.5 μm. Motility and spores have not been observed. The physiological characteristics of this organism are not consistent with those of any previously described genus (198).

Under strict anaerobic conditions, GS-15 oxidized acetate to carbon dioxide with the concurrent reduction of Fe(III) to Fe(II) according to the following equation:

$$\text{acetate}^- + 8\text{Fe(III)} + 4\text{H}_2\text{O} \longrightarrow 2\text{HCO}_3^- + 8\text{Fe(II)} + 9\text{H}^+$$

This metabolism provided energy to support cell growth. Temperature and pH optima for Fe(III) reduction as well as a requirement for direct contact between GS-15 and the Fe(III) oxide were consistent with a direct enzymatic reduction of Fe(III) rather than an indirect, nonenzymatic process (198). Enzymatic Fe(III) reduction was subsequently confirmed by demonstration of electron transport to a membrane-bound, Fe(III) reductase (see below).

GS-15 is a strict anaerobe, does not grow fermentatively, and cannot use fumarate, sulfate, elemental sulfur, or trimethylamine-*N*-oxide as electron acceptors. However, it can obtain energy for growth by coupling the oxidation of acetate to the reduction of Mn(IV) or to the reduction of nitrate to ammonia (198). Recent studies have demonstrated that the oxidized form of uranium, U(VI), can also serve as an electron acceptor to support anaerobic growth of GS-15 (200). In addition to acetate, GS-15 can oxidize propionate, butyrate, valerate, pyruvate, ethanol, and several aromatic compounds (see below). A wide variety of other organic compounds and hydrogen or elemental sulfur cannot serve as electron donors for Fe(III) reduction.

Another acetate-oxidizing, Fe(III)-reducing microorganism was isolated from the deep subsurface sediments of an Atlantic Coastal Plain aquifer (188). This organism also does not appear to fall into any previously described genus. In contrast to GS-15, the subsurface isolate can grow aerobically as well as anaerobically and is motile.

The only other well-documented pure culture of an Fe(III)- or Mn(IV)-reducing microorganism that is known to obtain energy for growth from the oxidation of organic compounds is a pure culture of *S. putrefaciens*. *S. putrefaciens* can oxidize formate to carbon dioxide with Fe(III) or Mn(IV) as the electron acceptor (201) as follows:

$$\text{formate}^- + 2\text{Fe(III)} + \text{H}_2\text{O} \longrightarrow \text{HCO}_3^- + 2\text{Fe(II)} + 2\text{H}^+$$

$$\text{formate}^- + \text{Mn(IV)} + \text{H}_2\text{O} \longrightarrow \text{HCO}_3^- + \text{Mn(II)} + 2\text{H}^+$$

Lactate and pyruvate are incompletely oxidized to acetate and carbon dioxide with either Fe(III) or Mn(IV) (201) as follows:

$$\text{lactate}^- + 4\text{Fe(III)} + 2\text{H}_2\text{O} \longrightarrow \text{acetate}^- + \text{HCO}_3^- + 4\text{Fe(II)} + 5\text{H}^+$$

$$\text{lactate}^- + 2\text{Mn(IV)} + 2\text{H}_2\text{O} \longrightarrow \text{acetate}^- + \text{HCO}_3^- + 2\text{Mn(II)} + 5\text{H}^+$$

$$\text{pyruvate}^- + 2\text{Fe(III)} + 2\text{H}_2\text{O} \longrightarrow \text{acetate}^- + \text{HCO}_3^- + 2\text{Fe(II)} + 3\text{H}^+$$

$$\text{pyruvate}^- + \text{Mn(IV)} + 2\text{H}_2\text{O} \longrightarrow \text{acetate}^- + \text{HCO}_3^- + \text{Mn(II)} + 3\text{H}^+$$

Although early studies indicated that succinate could serve as substrate for growth with Mn(IV) reduction (229), subsequent studies have indicated that this is incorrect (201). There was slow reduction of Fe(III) and accumulation of small amounts of acetate when *S. putrefaciens* was inoculated into an anaerobic Fe(III)-containing medium with amino acid mixtures as potential electron donors (201). However, in natural environments it is unlikely that this amino acid metabolism would be competitive with the rapid metabolism of amino acids by fermentative microorganisms. Fumarate may also serve as an electron donor to support Fe(III) reduction in resting cell suspensions (234), but data to support this were not shown, and it is unknown whether *S. putrefaciens* can use fumarate as an electron donor to support growth. In contrast to GS-15, *S. putrefaciens* is a facultative anaerobe and can also reduce a variety of other electron acceptors including nitrate, nitrite, tetrathionite, glycine, fumarate, trimethylamine-*N*-oxide, thiosulfate, sulfite, chromate, and U(VI) (200, 229, 234, 282).

A gram-negative marine organism, strain SSW_{22}, had been reported to reduce Mn(IV) with acetate, succinate, and

glucose (89), but details on this metabolism have not been published.

Although few organisms capable of obtaining energy for growth from the oxidation of organic acids with the reduction of Fe(III) or Mn(IV) have been isolated, they are likely to be widespread. Acetate-oxidizing, Fe(III)-reducing enrichment cultures have readily been established with a variety of fresh- and brackish-water sediments (194). Organisms capable of oxidizing acetate with the reduction of Fe(III) have been recovered from as deep as 200 m in the Atlantic Coastal Plain (188) as well as from a variety of soils (35).

Aromatic-Compound-Oxidizing Fe(III) Reducers

Aromatic compounds are important constituents of naturally occurring organic matter in sedimentary environments and are common anthropogenic contaminants as well. GS-15 obtains energy to support growth from the oxidation of a variety of aromatic compounds (187, 191). These include common contaminants such as toluene, phenol, and *p*-cresol as well as benzoate, benzylalcohol, benzaldehyde, *p*-hydroxybenzoate, *p*-hydroxybenzylalcohol, and *p*-hydroxybenzaldehyde. Toluene metabolism is of particular interest because GS-15 in pure culture was the first example of an organism which could anaerobically oxidize an aromatic hydrocarbon. Aromatic hydrocarbons are a prevalent groundwater contaminant, and there is abundant evidence that the metabolism of organisms like GS-15 is an important mechanism for the removal of aromatic-compound contaminants from polluted groundwaters (see below).

Studies on the stoichiometry of substrate metabolism and Fe(III) reduction (187, 191) have suggested that GS-15 can completely oxidize all of its aromatic substrates to carbon dioxide with Fe(III) as the sole electron acceptor, since the following reactions have been documented:

$$\text{benzoate}^- + 30\text{Fe(III)} + 19\text{H}_2\text{O} \longrightarrow 7\text{HCO}_3^- + 30\text{Fe(II)} + 36\text{H}^+$$

$$\text{toluene} + 36\text{Fe(III)} + 21\text{H}_2\text{O} \longrightarrow 7\text{HCO}_3^- + 36\text{Fe(II)} + 43\text{H}^+$$

$$\text{phenol} + 28\text{Fe(III)} + 17\text{H}_2\text{O} \longrightarrow 6\text{HCO}_3^- + 28\text{Fe(II)} + 34\text{H}^+$$

$$p\text{-cresol} + 34\text{Fe(III)} + 20\text{H}_2\text{O} \longrightarrow 7\text{HCO}_3^- + 34\text{Fe(II)} + 41\text{H}^+$$

p-Hydroxybenzoate has been detected as a transitory extracellular intermediate during phenol and *p*-cresol metabolism, but no extracellular intermediates have been detected during the metabolism of other aromatic substrates (187, 191). It has been proposed that the first steps of toluene metabolism are the oxidation of the methyl group to yield benzoate. *p*-Cresol metabolism is also thought to start with oxidation of the methyl group to yield *p*-hydroxybenzoate. Phenol is initially carboxylated to yield *p*-hydroxybenzoate.

Purified enrichment cultures metabolized other important natural or contaminant aromatics such as syringic acid, ferulic acid, nicotinic acid, *o*-phthalic acid, *m*-cresol, tyrosine, phenylacetate, and a variety of mono- and dihydroxybenzoates (183, 202). In each instance in which detailed studies of the stoichiometry of substrate loss and Fe(II) accumulation were conducted, the results were consistent with complete oxidation of the aromatic compounds to carbon dioxide with Fe(III) serving as the sole electron acceptor:

$$\text{syringic acid} + 36\text{Fe(III)} + 22\text{H}_2\text{O} \longrightarrow 9\text{HCO}_3^- + 36\text{Fe(II)} + 45\text{H}^+$$

$$\text{ferulic acid} + 42\text{Fe(III)} + 26\text{H}_2\text{O} \longrightarrow 10\text{HCO}_3^- + 42\text{Fe(II)} + 52\text{H}^+$$

$$\text{nicotinic acid} + 22\text{Fe(III)} + 16\text{H}_2\text{O} \longrightarrow 6\text{HCO}_3^- + 22\text{Fe(II)} + \text{NH}_4^+ + 27\text{H}^+$$

$$m\text{-hydroxybenzoate}^- + 28\text{Fe(III)} + 18\text{H}_2\text{O} \longrightarrow 7\text{HCO}_3^- + 28\text{Fe(II)} + 35\text{H}^+$$

$$\text{2,5-dihydroxybenzoate}^- + 26\text{Fe(III)} + 17\text{H}_2\text{O} \longrightarrow 7\text{HCO}_3^- + 26\text{Fe(II)} + 32\text{H}^+$$

$$m\text{-cresol} + 34\text{Fe(III)} + 20\text{H}_2\text{O} \longrightarrow 7\text{HCO}_3^- + 34\text{Fe(II)} + 41\text{H}^+$$

$$o\text{-phthalic acid} + 30\text{Fe(III)} + 20\text{H}_2\text{O} \longrightarrow 8\text{HCO}_3^- + 30\text{Fe(II)} + 38\text{H}^+$$

Under sterile conditions, none of these aromatics reduced Fe(III) oxide at the circumneutral pH (6.7) of the culture medium. The organisms responsible for oxidizing the aromatics were not isolated. The aromatics could be metabolized by a single Fe(III)-reducing microorganism in a manner similar to the metabolism of other aromatic compounds by GS-15. Alternatively, in some instances, a consortium in which Fe(III) reducers carry out the terminal oxidation of the organics may be involved.

Geochemical evidence has suggested that even compounds as recalcitrant as benzene and xylenes may be degraded in Fe(III)-reducing sediments (16, 187). However, attempts to establish enrichment cultures with either of these compounds as the sole electron donor and Fe(III) as the sole electron acceptor were unsuccessful (183).

PATHWAYS FOR MICROBIAL OXIDATION OF SEDIMENTARY ORGANIC MATTER COUPLED TO Fe(III) AND Mn(IV) REDUCTION

As late as 1984, a study on the role of Fe(III) reduction in lake sediments suggested that "the presence of Fe(III) might permit a slight diversion of metabolism to more energetically favorable end products" but that "the scale of iron reduction both in the field and the laboratory was very small compared with the reducing potential of the available substrates" (159). This conclusion was reasonably based on the available evidence that the Fe(III)-reducing microorganisms that were available in culture could only weakly couple the oxidation of hydrogen or the incomplete oxidation of fermentable compounds to the reduction of Fe(III). Fe(III) was not considered to be able to serve as an electron acceptor for the anaerobic oxidation of organic matter in the same manner that nitrate or sulfate can (46). Furthermore, the conclusion that Fe(III) reduction was a minor process for organic-

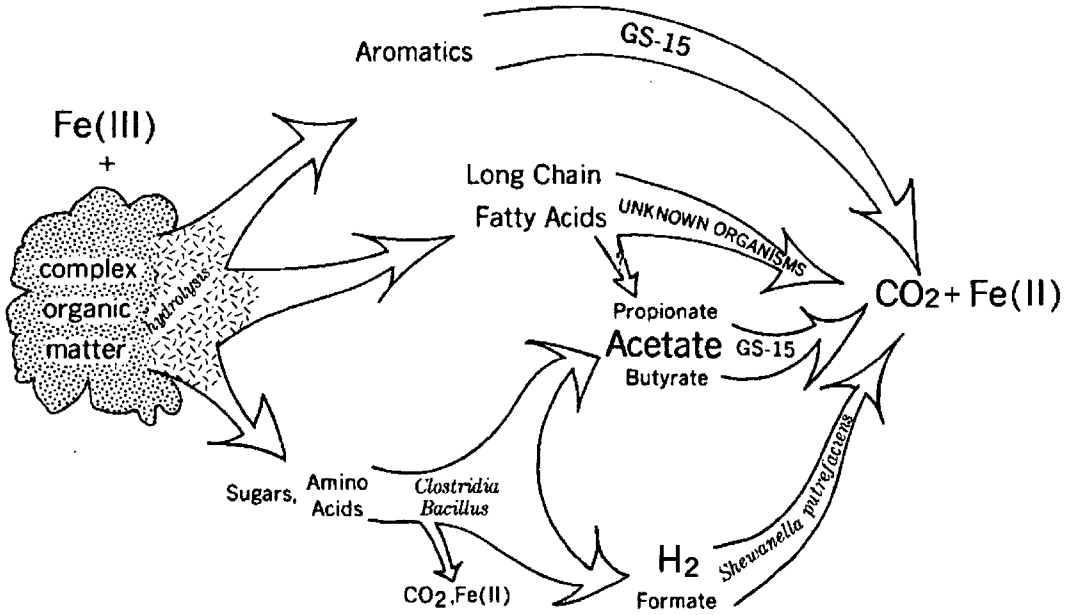

FIG. 1. Model for the oxidation of complex organic matter with Fe(III) serving as the sole electron acceptor, with examples of the organisms that may catalyze the various reactions. A similar model may also apply in sediments in which Mn(IV) reduction is the terminal electron-accepting process.

matter oxidation was supported by studies with lake sediments that indicated that added Fe(III) permitted only minor changes in the fermentative metabolism in sediments to slightly favor the formation of more oxidized fermentation products (159). Fermentation products did not appear to be oxidized with the reduction of Fe(III).

Thus, the prevalent model for metabolism of organic matter by Fe(III)- or Mn(IV)-reducing microorganisms could be summarized as follows (slightly modified from equations in reference 89):

$$\text{fermentable substrate} \longrightarrow ne^- + nH^+$$

$$+ \text{ fermentation products}$$

$$e^- + Fe(OH)_3 + 3H^+ \longrightarrow Fe^{2+} + 3H_2O$$

or

$$2e^- + MnO_2 + 2H^+ \longrightarrow 2Mn^{2+} + 2H_2O$$

In this model, there is relatively little Fe(III) or Mn(IV) reduction, and the metabolism of Fe(III)- and Mn(IV)-reducing microorganisms results in the accumulation of fermentation products.

However, the isolation of microorganisms which can oxidize hydrogen, fatty acids, and aromatic compounds with Fe(III) as the sole electron acceptor has led to the development of a new model which suggests how Fe(III)- and Mn(IV)-reducing microorganisms can completely oxidize complex organic matter to carbon dioxide with Fe(III) or Mn(IV) as the sole electron acceptor. In this model (Fig. 1), the complex organic matrix in the sediments is hydrolyzed to smaller components such as sugars, amino acids, fatty acids, and aromatic compounds through the activity of hydrolytic enzymes that may be produced by a variety of microorganisms. The sugars and amino acids are first metabolized by fermentative microorganisms. These fermentative microorganisms may reduce a small amount of Fe(III) during their metabolism. However, the primary products of the first stage of the metabolism of sugars and amino acids are fermentation acids and possibly hydrogen.

Acetate, which is expected to be the most important fermentation acid (199), can then be oxidized by Fe(III)- or Mn(IV)-reducing microorganisms that have a metabolism similar to that of GS-15. Similar organisms may completely oxidize some of the other minor fermentation acids, like propionate and butyrate. Lactate and pyruvate are not expected to be important fermentation products in Fe(III)-reducing sediments (199) but when present may be incompletely oxidized to acetate by organisms with a metabolism similar to that of *S. putrefaciens*. This ability of *S. putrefaciens* to only incompletely oxidize minor fermentation acids indicates that organisms with this type of metabolism must play a minor role in the oxidation of multicarbon organic compounds in the sediments. The primary role of organisms with a metabolism like *S. putrefaciens* is to oxidize the formate and hydrogen produced by fermentation.

Many aromatic compounds may be directly oxidized to carbon dioxide with the reduction of Fe(III) or Mn(IV) by microorganisms with a metabolism similar to that of GS-15. However, some aromatic compounds, such as the aromatic amino acids, phenylalanine, tryptophan, and tyrosine, which are also readily fermented (21), may first be partially metabolized by fermentative microorganisms.

Long-chain fatty acids (e.g., even-numbered C_{12} to C_{18}) can also be oxidized under Fe(III)-reducing conditions: enrichment cultures with a long-chain fatty acid as the sole electron donor and Fe(III) oxide as the sole electron acceptor were readily established with freshwater sediments (192). Whether short-chain fatty acids are extracellular intermediates of long-chain-fatty-acid oxidation has yet to be determined.

This model is consistent with all of the available geochemical and microbiological information on Fe(III)-reducing environments and microorganisms. Biogeochemical studies have emphasized that there is not a high accumulation of fermentation products in Fe(III)-reducing environments (58, 189, 195). The proposed cooperative activity of fermentative microorganisms and Fe(III) reducers is in agreement with the metabolic capabilities and limitations of known Fe(III)- and Mn(IV)-reducing microorganisms, with the two-stage metabolism of glucose in glucose-Fe(III) oxide enrichment cultures (194), and with the metabolism of [U-^{14}C]glucose in various Fe(III)-reducing sediments (199).

However, it must be emphasized that there are few representatives of the microorganisms which, the model suggests, catalyze most of the Fe(III) and Mn(IV) reduction in sedimentary environments. As detailed above, GS-15 is the only organism capable of completely oxidizing fermentation acids or aromatics to carbon dioxide that has been studied in detail, and only one other organism is known to have a similar metabolism. Furthermore, only two hydrogen-oxidizing, Fe(III)-reducing microorganisms are known. It is not known whether any of the organisms that are available in pure culture are the numerically dominant ones in natural environments.

RELATIVE POTENTIAL FOR ENZYMATIC AND NONENZYMATIC REDUCTION OF Fe(III) AND Mn(IV)

In addition to the model proposed above, in which the oxidation of organic matter coupled to the reduction of Fe(III) or Mn(IV) is the direct result of the enzymatic activity of specialized microorganisms, several models in which Fe(III) and Mn(IV) in sedimentary environments are reduced nonenzymatically have also been proposed. The most commonly cited mechanisms for nonenzymatic Fe(III) and Mn(IV) reduction in dark, nonsulfidogenic environments are referred to here as the redox model and the direct-reduction model. In the redox model, the decomposition of organic matter and removal of dissolved oxygen and nitrate by microorganisms lowers the redox potential. According to equilibrium thermodynamics, as the redox potential decreases, the Fe(II)-Fe(III) and Mn(II)-Mn(IV) equilibria are increasingly shifted in favor of Fe(II) and Mn(II) (132, 133, 261, 298, 341). Thus, in the redox model, as microbial metabolism lowers the redox potential, Fe(III) and Mn(IV) are nonenzymatically converted to Fe(II) and Mn(II) in order to maintain equilibrium. In the direct-reduction model, many of the organic compounds in sedimentary environments are considered to react directly with Fe(III) or Mn(IV) to reduce it.

Distinguishing between these nonenzymatic mechanisms and enzymatic Fe(III) reduction in aquatic sediments is difficult. Fe(III) reduction is invariably inhibited when microbial metabolism in aquatic sediments is inhibited (158, 164, 189, 286). However, this result does not necessarily exclude the redox or direct-reduction mechanism. The redox model relies on microbial metabolism to generate the low redox potential for Fe(III) and Mn(IV) reduction. Many of the organic compounds that in the direct-reduction model are most likely to reduce Fe(III) and Mn(IV) are produced or released during microbial decomposition of organic matter (112, 184, 209). The more-definitive approach has been to directly examine the assumptions of the redox and direct-reduction models under defined conditions in the laboratory.

Redox Model

It is ironic that one of the earliest studies on microbial Fe(III) reduction, the much-cited study by Starkey and Halvorson (289), concluded that Fe(III) reduction was nonenzymatic. Halvorson and Starkey (126) had previously developed theoretical equilibrium relationships for the concentrations of dissolved Fe(III), Fe(II), H^+, and O_2 which indicated that as the concentration of dissolved oxygen decreased, the ratio of dissolved Fe(II) to Fe(III) should increase. Their calculations also predicted that low pH would favor Fe(III) reduction. They assumed that the conversion between Fe(III) and Fe(II) was a freely reversible reaction and that Fe(III) would be nonenzymatically reduced once microbial metabolism had established the appropriate conditions by lowering the dissolved oxygen and/or pH. However, no evidence was provided to support this conclusion.

Furthermore, none of the many other studies that have subsequently treated Fe(III) reduction as part of a reversible redox reaction that can be modeled as a function of redox potential and pH (132, 133, 261, 298, 341) have demonstrated that Fe(III) can be nonenzymatically reduced by mere changes in dissolved oxygen or pH. In contrast, there is much evidence that, under the conditions of temperature and pressure that are typical of most sedimentary environments, decreases in dissolved oxygen or pH are insufficient conditions to bring about measurable Fe(III) reduction. For example, many of the techniques for distinguishing between Fe(III) and Fe(II) in sediments rely on the fact that Fe(III) is not reduced to Fe(II) under the highly acidic conditions of the assays (194, 196, 258). Storage of readily reducible, synthetic, poorly crystalline Fe(III) oxides or sterilized sediment containing poorly crystalline Fe(III) oxides under anaerobic conditions does not result in detectable Fe(III) reduction, even after long periods (46, 90, 188, 189, 192, 245). This is true even when electron donors are present in the medium to further lower the redox potential. Although facultative organisms can metabolize any traces of O_2 in anaerobically prepared media, even down to very low concentrations, there is no Fe(III) reduction during growth of many of these microorganisms (46, 272, 318). For example, when a strain of *E. coli* was grown in an anaerobic glucose-Fe(III) oxide medium, there was no detectable Fe(III) reduction (202), even though the metabolism of *E. coli* in the anaerobic medium could be expected to lower the redox potential to −600 mV (146).

The many studies by Ottow and co-workers consistently indicated that Fe(III) in microbial cultures was only the result of direct enzymatic action (223, 224, 226, 241, 242, 244–246, 248). For example, Munch and Ottow (225, 226) found that *B. polymyxa* and *Clostridium butyricum* could reduce small amounts of Fe_2O_3 (hematite) during fermentation of glucose. However, when the Fe_2O_3 was enclosed in dialysis tubing, which prevented direct contact between Fe(III) and the fermentative Fe(III) reducers, Fe(III) was not reduced even though the accumulation of fermentation acids and the lowering of the measured redox potential were as great as in the Fe(III)-reducing cultures in which contact between microorganisms and hematite was permitted. Similar results have been obtained with *S. putrefaciens* and goethite (11) and with *C. pasteurianum* and GS-15 with highly reactive, poorly crystalline Fe(III) oxides (202).

Thus, although modeling Fe(III) reduction as a readily reversible redox reaction is a convenient mechanism for conceptualizing iron geochemistry, the model does not ac-

curately represent reality and has led to much confusion. In nonsulfidic environments, the measured redox potential is typically a response to the Fe(III)-Fe(II) couple (84, 298, 306, 327). Some process, presumably microbial Fe(III) reduction, must generate Fe(II) before a low redox potential will be measured. Therefore, the concept of a low redox potential bringing about Fe(III) reduction is probably the reverse of the actual order of events in sedimentary environments (202).

Reduction by Organic Compounds

The available evidence also indicates that direct reduction of Fe(III) by organic compounds in sediments must be a trivial process in comparison with enzymatic reduction of Fe(III) by microorganisms. As discussed above, in the presence of the appropriate Fe(III)-reducing microorganisms, a wide variety of organic compounds can be oxidized to carbon dioxide with Fe(III) as the electron acceptor. However, at the circumneutral pHs (6 to 8) that are typical of most anaerobic sediments and submerged soils (261), very few organic compounds nonenzymatically reduce Fe(III). Many of the more-reactive microbial metabolites such as formate, citrate, pyruvate, oxalate, and malate, which are frequently cited as being able to nonenzymatically reduce Fe(III) oxides at low pH (89, 112, 149, 156), do not nonenzymatically reduce Fe(III) at circumneutral pH (198, 201, 202). Although aromatic compounds are frequently discussed as potential nonenzymatic reductants of Fe(III), there are, in fact, very few aromatic compounds which can reduce Fe(III) at circumneutral pH (174, 202).

Furthermore, for those organic compounds which can nonenzymatically reduce Fe(III), the nonenzymatic reaction oxidizes much less of the organic compound and reduces much less Fe(III) than is observed in the presence of the appropriate Fe(III)-reducing microorganisms. For example, although compounds, such as cysteine, which contain a sulfhydryl group can reduce Fe(III) at circumneutral pH (69, 219), only one Fe(III) is reduced per cysteine (69):

$$2\text{cysteine} + 2\text{Fe(III)} \longrightarrow \text{cystine} + 2\text{Fe(II)} + 2\ \text{H}^+$$

In contrast, in an Fe(III)-reducing enrichment culture adapted to grow on cysteine as the sole electron donor, the stoichiometry of Fe(III) reduction was consistent with complete oxidation of cysteine to carbon dioxide with Fe(III) serving as the electron acceptor (202):

$$\text{cysteine} + 10\text{Fe(III)} + 7\text{H}_2\text{O} \longrightarrow 3\text{HCO}_3^- + 9\text{Fe(II)} + 1\text{FeS} + \text{NH}_4^+ + 14\text{H}^+$$

Thus, the microbial oxidation of cysteine coupled to Fe(III) reduction reduces 30-fold-more Fe(III) than the nonenzymatic reaction.

In a similar manner, for the few aromatic compounds that can nonenzymatically reduce Fe(III), this is only a two-electron transfer that does not result in any carbon dioxide production (174, 202). In contrast, as shown above, microbial oxidation of aromatic compounds coupled to Fe(III) reduction can result in reduction of ca. 10 to 20 times as much Fe(III) and can completely oxidize the aromatic compound to carbon dioxide. These considerations indicate that microbial oxidation of organic matter coupled to Fe(III) reduction is likely to be a much more important mechanism for Fe(III) reduction than nonenzymatic reduction of Fe(III) by organics.

This conclusion is supported by studies in sediments. ^{14}C-labeled glucose, acetate, and benzoate (typical representatives of sugars, organic acids, and aromatic compounds, respectively) are readily oxidized to $^{14}CO_2$ in Fe(III)-reducing sediments, but these compounds are not oxidized to carbon dioxide if microbial activity is inhibited (202). Pasteurization of marine sediments inhibited the release of Fe(II) into the interstitial water even though the pasteurization process increased the concentration of dissolved organic carbon more than 10-fold (54). The finding that heat sterilization (46, 54, 158, 189, 286) and a variety of metabolic poisons (158, 164) inhibit Fe(III) reduction in sediments and soils and the fact that the temperature optimum for Fe(III) reduction is consistent with that of an enzymatically catalyzed process (158) all further suggest that there are few organic compounds deposited in sediments that can abiologically reduce Fe(III).

Mn(IV) is more reactive with organic compounds than is Fe(III). A number of organic compounds such as oxalate, pyruvate, syringic acid, 3,4-dihydroxybenzoate, 2,5-dihydroxybenzoate, and resorcinol that do not react with Fe(III) at circumneutral pH reduced Mn(IV) at pH 7.2 (295). Reducing sugars such as glucose and xylose may also nonenzymatically reduce Mn(IV) oxides under some conditions (319). However, the vast majority of organic compounds that would commonly be found in sedimentary environments do not appear to abiologically react with Mn(IV).

Furthermore, as with Fe(III), even with organic compounds that do react with Mn(IV), the rate and extent of abiological Mn(IV) reduction is much less than that possible through microbial metabolism. For example, although glucose, pyruvate, and formate may nonenzymatically react with Mn(IV), these compounds are oxidized much faster in the presence of the appropriate Mn(IV)-reducing microorganisms than under sterile conditions (90, 201). Pyruvate is only incompletely oxidized under sterile conditions (294) as follows:

$$\text{pyruvate}^- + \text{MnO}_2 \longrightarrow \text{acetate}^- + \text{MnCO}_3$$

Acetate, however, which does not react abiologically with Mn(IV), can be completely oxidized to carbon dioxide with the reduction of 4 mol more of Mn(IV) in the presence of an Mn(IV) reducer such as GS-15 (see above). As discussed above for Fe(III), the relative disparity between the nonenzymatic and enzymatic reactions is expected to be even greater for aromatic compounds. Thus, even for the few organic compounds that can nonenzymatically react with Mn(IV), microbial metabolism is probably responsible for the majority of the organic-matter oxidation coupled to Mn(IV) reduction in sediments.

Reduction by Reduced Sulfur Compounds

In some marine sediments, abiological reduction of Mn(IV) and possibly Fe(III) by sulfide has the potential to be an important mechanism for Fe(III) and Mn(IV) reduction. Laboratory studies have indicated that sulfide abiologically reduces Mn(IV) and forms elemental sulfur (52) as follows:

$$3\text{H}^+ + \text{MnO}_2 + \text{HS}^- \longrightarrow \text{Mn}^{2+} + \text{S}^\circ + 2\text{H}_2\text{O}$$

Furthermore, S° or various sulfide forms may be completely oxidized to sulfate in marine sediments by as-yet-unidentified microorganisms using Mn(IV) as the electron acceptor (6, 168). At circumneutral pH, sulfide can reduce Fe(III) (28, 115, 268):

$$2FeOOH + H_2S + 4H^+ \longrightarrow 2Fe(II) + S^\circ + 4H_2O$$

There is no oxidation of reduced sulfur forms by Fe(III) to generate sulfate (6, 168, 195).

Circumstantial evidence for Fe(III) and Mn(IV) reduction by sulfide in marine sediments is the accumulation of S° and pyrite (FeS_2) in marine sediments in the zones in which dissolved Mn(II) and Fe(II) accumulate in the interstitial water (287). However, direct oxidation of organic matter coupled to Fe(III) and Mn(IV) reduction was also considered an important processes within these zones (see below). MnO_2 added to marine sediments oxidized $^{35}S^{2-}$ (168), but here did not appear to be any oxidation of $^{35}S^{2-}$ either by added poorly crystalline Fe(III) oxide (168) or by sediment Fe(III) oxides (286).

Laboratory experiments and diagenetic modeling have indicated that microbial oxidation of organic matter coupled to Fe(III) reduction has the potential to be the predominant pathway for Fe(III) reduction even in anaerobic sediments that contain high concentrations of sulfate. In Fe(III)- and sulfate-reducing enrichment cultures growing on acetate-succinate-yeast extract, the rate of Fe(III) reduction was the same when sulfate reduction was inhibited as it was in controls in which sulfate was actively reduced (320). Fe(III) reduction continued when the enrichment culture was placed in sulfate-free medium. Inhibition of sulfate reduction in a variety of marine sediments has been found to have no effect on the rates of Fe(III) reduction (54, 286). Studies of the profiles of Fe(III), Mn(IV), sulfides, and organic matter in marine-shelf sediments off China indicated that Fe(III) and Mn(IV) reduction did not involve sulfides (20). Data from a variety of marine sediments in which there are extensive zones of dissolved Fe(II) accumulation but no sulfate depletion (5, 63, 67, 77, 109, 331) further demonstrate that sulfate reduction need not be an intermediary between organic-matter oxidation and Fe(III) reduction in marine sediments.

Mn(IV) oxides are more reactive with reduced sulfur compounds than Fe(III) oxides are, but the relative importance of Mn(IV) reduction by sulfur compounds versus organic-matter oxidation coupled to Mn(IV) reduction has not been quantified (6). Although there should be good potential for sulfide reduction of Mn(IV) in near-shore sediments where Mn(IV) oxides and anaerobic sediments may frequently be mixed together (6), in many marine sediments the zones of intense sulfide generation are physically well separated from the most-active zone of Mn(IV) reduction (109, 269).

Thus, although there is the potential for sulfide reduction of Fe(III) and Mn(IV), there is also evidence that microbiological Fe(III) and Mn(IV) reduction is an important process in marine sediments. The relative contribution of abiological and biological Fe(III) and Mn(IV) reduction needs to be examined in each marine environment under investigation, as this might vary greatly with environmental conditions.

Mn(IV) Reduction by Fe(II), Nitrite, or Hydrogen Peroxide

A potentially significant mechanism for nonenzymatic reduction of Mn(IV) in sedimentary environments is the reduction of Mn(IV) by Fe(II) (79, 171, 197, 230, 266, 311):

$$2Mn(IV) + 2Fe(II) \longrightarrow 2Mn(II) + 2Fe(III)$$

This reaction takes place rapidly at pH 7 with the Mn(IV) oxides typically found in aquatic sediments (197). Thus, in sediments it would be difficult to distinguish between direct enzymatic reduction of Mn(IV) and enzymatic reduction of Fe(III) followed by nonenzymatic reduction of Mn(IV) by Fe(II).

It has been suggested that Fe(III) reducers preferentially reduce Mn(IV) (128, 226, 247). If so, then direct enzymatic reduction of Mn(IV) would be expected to be the predominant mechanism for Mn(IV) reduction, since most of the Mn(IV) could be enzymatically reduced prior to production of Fe(II). However, in the cases examined, Fe(III)-reducing microorganisms appeared to reduce Fe(III) in the presence of Mn(IV) (197, 230).

These results raise the question of whether some Mn(IV) reducers have a separate Mn(IV) reductase or whether Mn(IV) reduction is a consequence of Fe(III) reduction. This is not an issue for some organisms which reduce only Mn(IV) but not Fe(III) (318). *S. putrefaciens* MR-1 also appears to have separate Mn(IV) and Fe(III) reductase systems, since mutants which had lost the ability to reduce Fe(III) but could still reduce Mn(IV) were generated (230). Further evidence for distinct mechanisms for Mn(IV) and Fe(III) reduction was the finding that cell suspensions of *S. putrefaciens* MR-1 that had been cultured on nitrate translocated protons with Mn(IV) as the electron acceptor but not with Fe(III) (231).

Circumstantial evidence suggests that Mn(IV) reduction can be distinct from Fe(III) reduction in aquatic sediments. The steady-state concentrations of dissolved H_2 in sediment pore waters are controlled by the predominant terminal electron-accepting process in the sediment (189). Addition of Mn(IV) to sediments in which Fe(III) reduction was the predominant terminal electron-accepting process resulted in a steady-state dissolved-hydrogen concentration that was significantly lower than that observed in Fe(III)-reducing sediments (189). This result suggested that, with the addition of Mn(IV), hydrogen metabolism was linked to Mn(IV) reduction rather than Fe(III) reduction.

Nitrite can reduce Mn(IV) (22) either to Mn(II) or to Mn(III) as follows:

$$NO_2^- + MnO_2 + 2H^+ \longrightarrow Mn^{2+} + NO_3^- + H_2O$$

$$NO_2^- + 2MnO_2 \longrightarrow Mn_2O_3 + NO_3^-$$

Mn(IV) reduction to Mn(III) appeared to predominate when MnO_2-to-nitrate ratios were high. Mn(IV) oxidation of nitrite could explain the fact that nitrite does not generally accumulate in aerobic soils even when the activity of *Nitrobacter* sp. is low (22). Mn(IV) is frequently reduced within the nitrate reduction zone of aquatic sediments (31, 94, 109, 170, 253), and this could potentially be the result of nitrite reduction of Mn(IV).

Nonenzymatic reduction of Mn(IV) by H_2O_2 occurs as follows:

$$H_2O_2 + MnO_2 + 2H^+ \longrightarrow Mn(II) + 2H_2O + O_2$$

This may be the mechanism for Mn(IV) reduction in cultures of H_2O_2-producing *Bacillus* sp. (112). However, since H_2O_2 is produced only in the presence of O_2, this reaction is unlikely to be a mechanism for Mn(IV) reduction in the Mn(IV)-reducing zone of sediments (233).

In summary, there are some potential mechanisms for nonenzymatic reduction of Fe(III) and Mn(IV). However, under the environmental conditions typically found in anaerobic sediments and soils, there is a much greater potential for microbially catalyzed Fe(III) and Mn(IV) reduction.

ENVIRONMENTAL SIGNIFICANCE OF MICROBIAL Fe(III) and Mn(IV) REDUCTION

Fe(III) as the First Organic-Matter Oxidant in the Archaean Biosphere

Fe(III) reduction may have been the first globally important mechanism for microbial oxidation of organic matter to carbon dioxide. Walker (329, 330) emphasizes that under the anaerobic conditions of the first 2 billion years of Earth's history, there was abundant dissolved Fe(II) but little dissolved sulfur, oxygen, or nitrate. As photosynthesis emerged, there was a concurrent oxidation of Fe(II) to Fe(III) as inorganic carbon was reduced to organic carbon (254). Oxygen produced by photosynthetic microorganisms could have oxidized Fe(II) (62), or a form of Fe(II)-based photosynthesis could have directly oxidized Fe(II) as a source of electrons for carbon fixation (130). In either event, there would have been no accumulation of free oxygen, and Fe(II) would have been the net electron donor for photosynthesis. There is abundant evidence that once the coproduced organic matter and Fe(III) oxides sedimented, the organic matter was oxidized to carbon dioxide with Fe(III) serving as the electron acceptor (23, 254, 328, 330) (see discussion of magnetite formation below). Thus, there could have been a nearly closed biogeochemical cycle in which Fe(II) served as the electron donor for primary production and Fe(III) served as the electron acceptor for organic matter oxidation (330). The sedimentation of Fe(III) would have concentrated the oxidant for organic matter in the sediments. Thus, in comparison with the modern environment, in which the atmosphere and surface environments are oxidizing and the sedimentary environments are reduced, the Archaean biosphere may have been "upside down," with a relatively reducing atmosphere and surface and the primary oxidant for organic matter concentrated in the sediments (330).

Most of the oxidation of organic matter coupled to Fe(III) reduction in Precambrian sedimentary environments was probably the result of the activity of dissimilatory Fe(III)-reducing microorganisms which could completely oxidize organic compounds to carbon dioxide with Fe(III) as the sole electron acceptor (185, 205). As discussed above, all of the available evidence suggests that neither the metabolism of fermentative Fe(III)-reducing microorganisms nor abiological mechanisms could have accounted for the oxidation of large amounts of organic matter with Fe(III) as the sole electron acceptor. Thus, the geochemical evidence suggests that respiratory dissimilatory Fe(III) reduction may have evolved prior to other respiratory processes such as sulfate reduction, nitrate reduction, or aerobic respiration (23, 328).

Decomposition of Organic Matter in Modern Sedimentary Environments

Fe(III) reduction continues to be an important process for organic-matter oxidation in many modern sedimentary environments. However, a wide range of electron acceptors for organic-matter oxidation may now be available (Fig. 2). In most stable sedimentary environments, in which there is little sediment mixing and the rates of organic inputs are not excessively high, the different processes for decomposition of organic matter are segregated in space or time or both. This pattern has been observed in a variety of aquatic sediments and waterlogged soils (261, 269) as well as in pristine and contaminated aquifers (15, 16, 56, 58, 187).

In many soils, aquatic sediments, and aquifers, Fe(III) is an abundant, if not the most abundant, potential electron acceptor for organic matter oxidation (41, 269, 306, 323). Although Mn(IV) oxides are typically only 10% as abundant as Fe(III) oxides, a higher proportion of Mn(IV) oxides may be available for microbial reduction. Thus, with the development of anaerobic conditions, Fe(III) and Mn(IV) reduction have the potential to be major processes for organic matter decomposition.

Fe(III) reduction is clearly an important process for organic-matter decomposition in marine and estuarine sediments in the Southern Hemisphere (5, 136). In continental-shelf sediments near the mouth of the Amazon River, there were extensive sediment intervals (1 to 2 m) with high concentrations of dissolved Fe(II) (5). In most cases, there was no evidence for sulfate reduction within these high-Fe(II) zones despite high (20 to 28 mM) sulfate concentrations in the waters overlying the sediments. In some instances, the alkalinity of the pore water increased within the high-Fe(II) zones, which suggested that organic matter was being oxidized to carbon dioxide with Fe(III) as the electron acceptor. The predominance of Fe(III) reduction over sulfate reduction in these sediments was attributed to massive physical reworking of the sediments which periodically reoxidized Fe(II) as well as to concentrations of reactive organic matter slightly lower and concentrations of reactive Fe(III) slightly higher than those found in previously studied temperate sediments.

Fe(III) reduction also dominated sulfate reduction in sediments of four estuaries in northeast Brazil (136). The greater importance of Fe(III) reduction in these sediments in comparison with similar sediments from the northern temperate zone of the United States was attributed to the finding that more of the iron in the soils in the Southern Hemisphere was in the form of poorly crystalline Fe(III) oxides. It has been proposed that, overall, Fe(III) reduction is a more important process than sulfate reduction for organic-matter oxidation in such sediments (136).

Subsequent studies have indicated that Fe(III) [and possibly Mn(IV)] reduction can also be an important process for organic-matter decomposition in temperate continental-shelf sediments (135). At one of the sites studied, denitrification was active in surficial sediments but diminished at depths below 2 cm. Sulfate reduction did not become active until a depth of 6 cm. Rates of glucose turnover remained high within the 2- to 6-cm depth interval, indicating active decomposition within this zone. Fe(II) accumulated in the pore water in the 2- to 6-cm interval. Rates of Fe(III) reduction were not directly measured. However, the results were consistent with Fe(III) reduction serving as the terminal electron-accepting process for organic matter decomposition in the 2- to 6-cm depth interval, which contained an important fraction of the sediments with the highest rates of glucose turnover (135). Studies at other sites also indicated that Fe(III) or Mn(IV) reduction was an important decomposition process. The authors (135) suggest that Fe(III) and Mn(IV) reduction play a significant role in organic-matter decomposition in most continental-shelf areas and emphasize that this is of global significance because continental-shelf zones receive a substantial fraction of the global marine productivity as well as terrestrial inputs from rivers.

A similar intermediate zone, in which dissolved Mn(II) and Fe(II) accumulated below the nitrate reduction zone and above the zone of sulfide accumulation, was noted in coastal sediments of northern Denmark (287). It was suggested that the overall rates of organic-matter decomposition in the intermediate zone must be at least as high as those in the zone of sulfide accumulation, and thus it appeared that

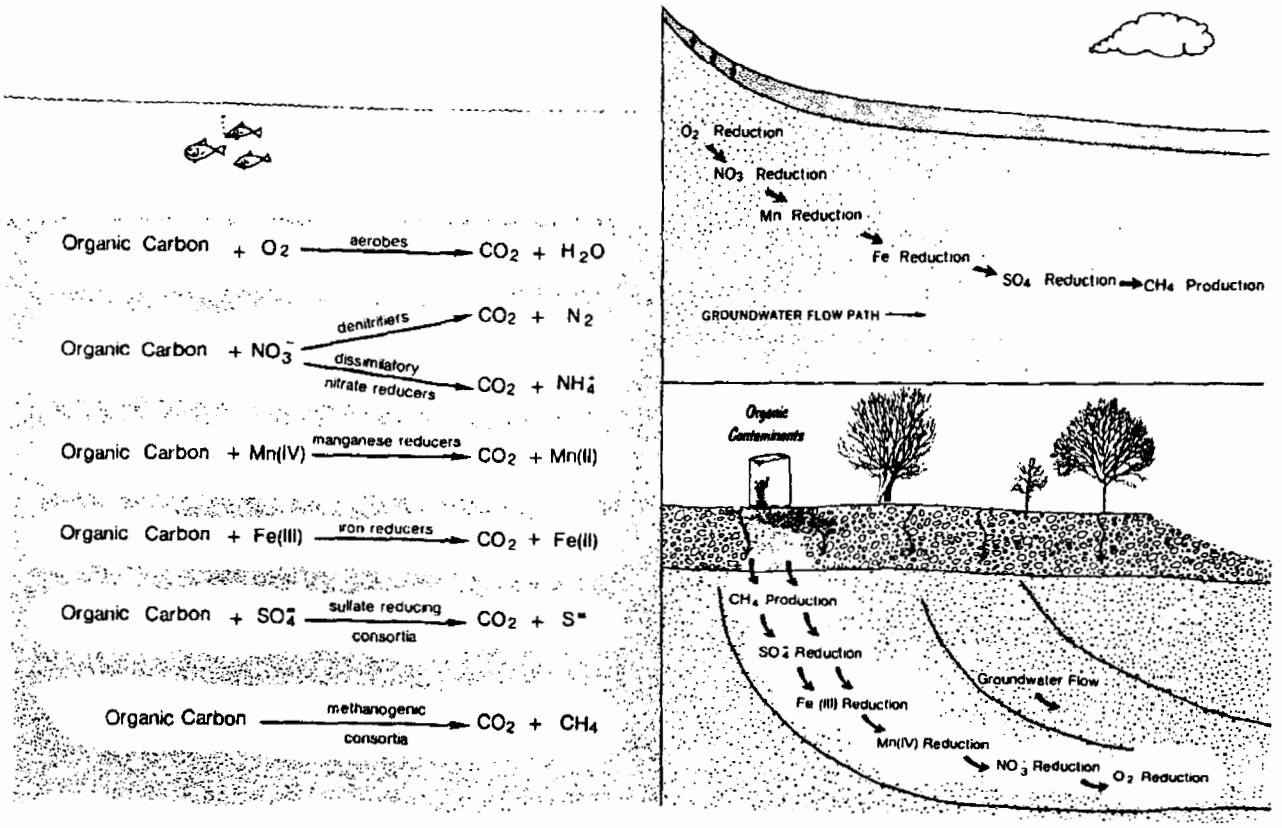

FIG. 2. Typical distribution of terminal electron-accepting process in aquatic sediments (left-hand panel), deep pristine aquifers (upper-right-hand panel), and shallow aquifers contaminated with organic compounds (lower-right-hand panel).

organic matter was being oxidized with the reduction of Mn(IV) and Fe(III) in the intermediate zone.

Although Mn(IV) reduction was considered to account for less than 10% of the oxidation of sedimentary carbon in bioturbated near-shore muds near Long Island, the constant mixing of sediments to resupply Mn(IV) oxides resulted in Mn(IV) reduction accounting for essentially all of the organic-matter oxidation in bioturbated sediments in the Panama Basin (4). Oxidation of organic matter coupled to Mn(IV) reduction in marine sediments formed during the Jurassic is considered to have generated the massive manganese carbonate deposits in the Molango deposit, Mexico (240).

Modeling of iron and manganese geochemistry in Toolik Lake, Alaska, indicated that up to 50% of the anoxic sediment metabolism was the result of Fe(III) or Mn(IV) reduction (72). When the extent of organic-matter decomposition via oxygen respiration, nitrate reduction, sulfate reduction, and methanogenesis in the profundal sediments of a eutrophic lake was estimated by measurements of oxygen loss, nitrate loss, and sulfide and methane accumulation in the hypolimnetic water, only 66% of the carbon dioxide accumulation could be accounted for (154). It was suggested that Fe(III) reduction may have contributed to the remaining 44% of the organic-matter decomposition (154).

Fe(III) reduction is an important process for organic-matter decomposition during the initial development of anaerobic conditions in flooded rice paddy soils (261, 306, 339). Carbon dioxide production and Fe(III) reduction were highly correlated for approximately 2 weeks after flooding (339). Calculations from the paddy soil data of Kamura and co-workers (164) and Saito and Wada (275) indicated that, during the period of active Fe(III) reduction, Fe(III) reduction accounted for 35 to 65% of the organic-matter oxidation to carbon dioxide (184). Studies with a swamp soil indicated that about 50% of the total oxygen consumption in the soil could be attributed to oxidation of dissolved and solid Fe(II) (141). This suggests that Fe(III) reduction was as important as aerobic respiration in the decomposition of organic matter in this environment.

Fe(III) and Mn(IV) reduction were considered to be minor processes for organic-matter decomposition in some lake sediments (156, 158, 159, 167, 326). However, this conclusion was based on measurements of the accumulation of dissolved Fe(II) and Mn(II), which greatly underestimate the extent of Fe(III) and Mn(IV) reduction.

Most of the Fe(II) that is produced as the result of Fe(III) reduction in sediments is not recovered as dissolved Fe(II) (3, 141, 197, 232, 261, 323, 335). For example, during microbial reduction of Fe(III) in freshwater sediments of the Potomac River, the accumulation of dissolved Fe(II) over time represented less than 2% of the total HCl-extractable Fe(II) that accumulated (197). The concentration of sodium acetate-extractable iron in anaerobic, water-saturated soils was 25-fold greater than the concentration of dissolved iron (252).

The Fe(II) that remains in solid forms is not well characterized (153, 323) and may be in the form of various Fe(II) minerals such as vivianite (30, 71, 95, 131, 265), siderite (30,

129, 265, 326), hydroxides (323), mixed Fe(II)-Fe(III) compounds (182, 193, 261, 264, 323), and Fe(II) silicates (30, 122, 261) as well as amorphous Fe(II) adsorbed onto various solid phases, including clays and organic matter (134, 306, 323). Iron sulfides can be important sinks of Fe(II) in marine environments and may also be important in freshwater environments such as swamps, which are unusually rich in organic compounds and have substantial releases of sulfur from organic matter (29). Insoluble Mn(II) in the form of Mn_3O_4, $MnCO_3$, Mn(II) adsorbed onto Mn(IV) or Fe(III) oxides or other minerals, and various forms of MnS may constitute a significant portion of the Mn(II) generated from Mn(IV) reduction (26, 29, 30, 78, 153, 217, 228, 232, 261, 263, 312, 326). Thus, measurements of the accumulation of dissolved Fe(II) and Mn(II) in interstitial waters of sediments or the water overlying sediment can provide a useful indication of Fe(III) and Mn(IV) reduction (109, 122, 144, 156, 158, 159, 167, 173, 220, 276, 290, 315, 326) but cannot be used to calculate the contribution of Fe(III) or Mn(IV) reduction to the overall organic-matter decomposition in the sediments.

Another factor that could lead to the assumption that Fe(III) reduction is a minor process for organic-matter oxidation in organic-compound-rich aquatic sediments is the general finding that the zones of Fe(III) and Mn(IV) reduction are restricted to a narrow band of several centimeters or less in such sediments (5, 91, 127, 173, 193, 287, 291, 315). However, the rates of organic-matter decomposition are also typically highest in surficial sediments. For example, in laboratory incubations of freshwater sediments collected from the Potomac River in autumn, Fe(III) reduction was initially as important a pathway for organic-matter oxidation as methane production in the upper 4 cm, even though Fe(III) reduction was primarily restricted to the 0- to 0.5-cm depth interval (193). Similar results were observed in sediments of an Antarctic lake (91).

The restriction of Fe(III) and Mn(IV) reduction to narrow zones near the sediment-water interface in many aquatic sediments makes determination of the in situ rates of Fe(III) and Mn(IV) reduction difficult. Rates of Fe(III) and Mn(IV) reduction in sediments have typically been estimated from the accumulation of Fe(II) and Mn(II) in anaerobic laboratory incubations of sediments (91, 159, 164, 193, 197, 211, 275, 286). However, such measurements do not account for the fact that the supply of electron acceptors for potentially competing processes such as oxygen and nitrate reduction have been eliminated (193). Furthermore, in many organically rich and bioturbated sediments in which the aerobic sediments are in close contact or are mixing with the Fe(III)- and Mn(IV)-reducing sediments, there may be rapid recycling between reduced and oxidized iron and manganese forms.

A better technique for estimating in situ rates of Fe(III) and Mn(IV) reduction might be to follow the fate of radiolabeled Fe(III) or Mn(IV) added as a tracer to undisturbed sediment cores (193). However, in laboratory measurements of Fe(III) reduction in various soils, microorganisms reduced ca. 70% of the added $Fe^{59}Cl_3$, whereas only 20% of the chemically reducible Fe(III) was microbially reduced (160). These results demonstrate that tracer studies of Fe(III) reduction will need to carefully define the pool of Fe(III) that is available for microbial reduction. Techniques for distinguishing Fe(III) in sediments in this matter have recently been developed (see below).

In environments in which the zones of Fe(III) and Mn(IV) reduction are broader and better defined, diagenetic modeling based on geochemical profiles of solid and dissolved iron and manganese species (3, 29, 50, 139, 163, 271, 304) has the potential to provide accurate estimates of the rates of Fe(III) and Mn(IV) reduction.

Inhibition of Methane Production and Sulfate Reduction

Sulfate reduction and methane production are generally inhibited in sediments in which organic-matter oxidation is being coupled to Fe(III) reduction (109, 261, 269, 339). The available evidence suggests that the inhibition of sulfate reduction and methane production in Fe(III)-reducing sediments can be attributed primarily to Fe(III)-reducing microorganisms maintaining the concentration of electron donors at levels too low for sulfate reducers or methanogens to metabolize them.

When reactive Fe(III) oxides were added to sediments in which sulfate reduction or methane production was the predominant terminal electron-accepting process, then, depending on the sediments and the type of Fe(III) added, methane production or sulfate reduction was inhibited 50 to 100% (6, 91, 168, 194, 195). The added Fe(III) did not inhibit the overall rate of organic-matter decomposition, since the inhibition of electron flow to sulfate reduction or methane production was associated with a corresponding increase in electron flow to Fe(III) reduction (194, 195). The inhibition of sulfate reduction or methane production could be relieved with the addition of hydrogen or acetate. This result demonstrated that the Fe(III) oxides were not directly toxic to sulfate reducers or methanogens. The steady-state concentrations of hydrogen and acetate were significantly lower in Fe(III)-amended sediments than in controls not receiving Fe(III), and if hydrogen or acetate was added, it was rapidly metabolized back down to the low steady-state levels of the Fe(III)-amended sediments (195). In a wide variety of aquatic sediments and groundwater, hydrogen and acetate concentrations were lower in Fe(III)-reducing zones than in sulfate-reducing or methanogenic zones (58, 189).

These results indicate that Fe(III) reducers prevent sulfate reduction and methane production by outcompeting sulfate reducers and methanogens for electron donors. Further evidence for the ability of Fe(III) reducers to metabolize electron donors down to concentrations below those that can be metabolized by sulfate reducers or methanogens was the finding that, with Fe(III) as the electron acceptor, *S. putrefaciens* could metabolize hydrogen down to a minimum threshold 25- and 150-fold lower than the minimum threshold for sulfate reducers and methanogens, respectively (201).

In addition to hydrogen and acetate, other short-chain fatty acids, aromatic compounds, and fermentable substrates may be important substrates for sulfate-reducing microorganisms (257). It would be interesting to determine if Fe(III)-reducing organisms also have a competitive advantage for these substrates when Fe(III) is not limiting.

Sulfate reduction and methane production are also expected to be limited by electron donor availability in the Mn(IV)-reducing zone of sediments. Hydrogen concentrations are even lower in sediments in which Mn(IV) reduction is the terminal electron-accepting process than they are in Fe(III)-reducing sediments (189). Additions of MnO_2 inhibited sulfate reduction in marine sediments, even when reduced sulfur reoxidation was inhibited (6, 168). This suggested that Mn(IV) reduction was directly outcompeting sulfate reduction for organic compounds and hydrogen (6, 168).

Since the inhibition of sulfate reduction and methane production in the presence of Fe(III) or Mn(IV) is merely the

result of competition for electron donors, these processes can proceed concurrently with Fe(III) or Mn(IV) reduction when the ability of Fe(III)- or Mn(IV)-reducing microorganisms to maintain low concentrations of electron donors is limited. Sulfate reduction or methane production that is simultaneous with Fe(III) or Mn(IV) reduction has been observed in a variety of laboratory and field studies (65, 168, 187, 189, 194, 195, 287, 320). A major limitation of Fe(III)-reducing microorganisms is the suitability of the Fe(III) forms available in the sediment. Additions of poorly crystalline Fe(III) oxides to sediments result in a greater inhibition of methane production and sulfate reduction than do additions of more-crystalline, less-reactive Fe(III) oxides (6, 194, [illegible].

Role of Fe(III) Reduction in Controlling Global Methane Fluxes

Shallow freshwater environments such as rice paddies, marshes, and swamps are estimated to account for ca. 40% of the annual flux of methane into the atmosphere (61). An important characteristic of these shallow freshwater environments is that they typically undergo alternating cycles of flooding conditions, when the sediments are exposed to air and are oxidized, and wet conditions, when the sediments are covered with water and anaerobic conditions develop (61). The Fe(III) oxides that are generated during the oxidation period permit Fe(III) reducers to divert electron flow away from methanogenesis during the initial stages of the anaerobic, flooded period. The extent of this diversion depends on such factors as the temperature and the organic-matter and iron content of the soils. Because of increasing interest in the contribution of methane to global warming and thus interest in attempting to model the flux of methane from shallow freshwater environments to the atmosphere, the potentially important controlling effect Fe(III) reduction is likely to have on methane production in these environments needs to be considered.

Oxidation of Organic Contaminants in Groundwater

The contamination of groundwater with organic compounds frequently leads to the development of anaerobic conditions (15, 299). With the onset of anaerobic conditions, Fe(III) is the most abundant potential electron acceptor for organic-matter decomposition in many subsurface environments. The accumulation of high concentrations of dissolved Fe(II) and Mn(II) is a common observation in aquifers contaminated with organic compounds (15, 73, 85, 142, 187, 281). Fe(III)- and Mn(IV)-reducing microorganisms presumably catalyze this oxidation of organic contaminants coupled to Fe(III) or Mn(IV) reduction, since the most common organic contaminants do not nonenzymatically react with Fe(III) or Mn(IV) oxides (see above).

The importance of Fe(III) reduction in metabolizing groundwater contaminants was obvious in a shallow aquifer located in Bemidji, Minn. (16, 187). As the result of a break in an oil pipeline, there was a lens of crude oil on top of the water table. Aromatic hydrocarbons leached out of the oil lens and into the surrounding groundwater, which was anaerobic immediately downgradient from the oil lens. Despite the anaerobic conditions, some process other than adsorption or volatilization selectively removed aromatic hydrocarbons such as benzene, xylenes, and toluene from the groundwater. The accumulation of isotopically light dissolved inorganic carbon in the contaminated groundwater suggested that the mechanism for removal of the aromatics was oxidation of the isotopically light hydrocarbons to carbon dioxide. Associated with the apparent anaerobic oxidation of the aromatic hydrocarbons was an accumulation of dissolved Fe(II) over time and depletion of Fe(III) oxides from the contaminated sediments.

Evidence for a microbial role in oxidizing aromatic compounds with the reduction of Fe(III) was the finding that in the Fe(III)-reducing aquifer sediments the removal of added toluene and the oxidation of [*ring*-^{14}C]benzoate to $^{14}CO_2$ was dependent on active microbial metabolism (187). Furthermore, the pure-culture Fe(III) reducer GS-15, which can oxidize a variety of aromatic compounds including toluene, coupled the oxidation of aromatics to the reduction of Fe(III) oxides in aquifer sediments from the Bemidji site.

The widespread availability of Fe(III) as a potential electron acceptor in subsurface environments (15, 56, 58, 145, 188, 189, 261, 323) suggests that microbial oxidation of contaminants coupled to Fe(III) reduction may be an important natural mechanism for contaminant removal and may be a metabolism that could be stimulated in bioremediation efforts.

Oxidation of Organic Matter and Release of Dissolved Fe(II) and Mn(II) in Pristine Aquifers

The reduction of highly insoluble Fe(III) and Mn(IV) oxides to more-soluble Fe(II) and Mn(II) forms releases dissolved Fe(II) and Mn(II) to the surrounding water. This is one of the most important geochemical events in all anaerobic sedimentary environments (261). It is of particular practical importance in groundwater, where undesirably high concentrations of dissolved Fe(II) and Mn(II) are a major groundwater quality problem worldwide (148, 327). When Fe(II) and Mn(II) in anaerobic groundwaters contact oxygen, they are oxidized to Fe(III) and Mn(IV) oxides, which clog wells and plumbing, discolor clothing and fixtures, and impair taste (9). This problem is generally expensive to remediate (270).

The potential for microbial Mn(IV) reduction resulting in the release of Mn(II) in groundwater was demonstrated in a study in which gravel from an aquifer was incubated in an anaerobic peptone medium (148). Under sterile conditions, there was only a slight release of dissolved manganese, whereas microbial activity resulted in a large release of manganese. Fe(III)- and/or Mn(IV)-reducing microorganisms were also recovered from the loose rock forming aquifers in Germany (119, 120) and from aquifer sediments in France (82).

Fe(III) reducers are also active in the deep subsurface. Geochemical studies have suggested that the oxidation of organic matter coupled to Fe(III) reduction is an important process in many deep aquifer systems (14, 56, 58, 176, 188, 260). For example, in a zone of high levels of dissolved Fe(II) in the Middendorf aquifer in South Carolina, there was a direct correlation between the accumulation of dissolved Fe(II) along the groundwater flow path and the accumulation of dissolved inorganic carbon (58). The dissolved inorganic carbon had the same isotopic composition as the organic matter in the sediments. This suggested that the source of dissolved inorganic carbon was organic-matter oxidation. Further evidence for ongoing organic matter oxidation coupled to Fe(III) reduction within this zone was the finding that [2-^{14}C]acetate injected into the sediments was oxidized to $^{14}CO_2$ by an anaerobic process that was not inhibited with molybdate (58, 59).

In sampling three separate deep core holes in South Carolina that were in or near aquifers with high levels of dissolved Fe(II), acetate-oxidizing Fe(III)-reducing microorganisms were recovered only from the aquifer sediments (as deep as 200 m) in which there was evidence of Fe(III) reduction (188). Not only could the organisms obtain energy for growth by coupling the oxidation of acetate to the reduction of synthetic, poorly crystalline Fe(III) oxide, but they could also use the Fe(III) oxides present in the deep subsurface sediments as electron acceptors. In conjunction with the geochemical (58) and biogeochemical (59) evidence for ongoing organic-matter oxidation coupled to Fe(III) reduction in the zones with high levels of dissolved Fe(II), these results suggest that the ongoing activity of Fe(III)-reducing microorganisms living in deep subsurface sediments is the most likely mechanism for the accumulation of high levels of dissolved Fe(II).

The finding that when the appropriate conditions develop, Fe(III)-reducing bacteria can become active in sediments of considerable age suggests that Fe(III)-reducing microorganisms may be responsible for other instances of late postdepositional Fe(III) reduction (188). For example, the Fe(III)-depleted zones of variegated redbeds and reduction spots may develop when dissolved organic matter percolates into previously deposited Fe(III)-containing sedimentary material (138, 213, 259, 310). A modern analog of such late postdepositional Fe(III) reduction is the microbial reduction of Fe(III) in the sediments of a sand-and-gravel aquifer following the introduction of soluble hydrocarbon components into the groundwater (187).

Release of Trace Metals and Phosphate into Water Supplies

Microbial reduction of Fe(III) and Mn(IV) plays an important role in the flux of phosphate and trace metals into water supplies. Fe(III) oxides tenaciously bind phosphate in oxidized sediment (40, 220). With the development of anaerobic conditions, Fe(II) and phosphate are simultaneously released into the surrounding water (40, 220). This release of phosphate is significant because phosphate is frequently the nutrient limiting the development of nuisance algal blooms in freshwater environments. Silica may be released from sediments in a similar manner (261).

In the presence of the nitrate-reducing microorganism *Pseudomonas fluorescens* or an *Alcaligenes* sp., dissolved ^{55}Fe and ^{32}P were simultaneously released from artificially generated $^{55}Fe(III)$-$^{32}PO_4$ precipitates (147). Although it was assumed that the release of dissolved iron was the result of Fe(III) reduction to Fe(II), it was not determined whether either of these organisms could reduce Fe(III). Furthermore, there was significant release of dissolved ^{55}Fe and ^{32}P under sterile conditions and in the presence of culture filtrates.

Although the concept of Fe(III) reduction resulting in phosphate release in sediments was proposed more than 50 years ago (220) and had been generally accepted (40), it is now being suggested that phosphate release during Fe(III) reduction in sediments is merely coincidental (110). In this alternative hypothesis, sediment microorganisms store large amounts of phosphate under aerobic conditions and then release intracellular phosphate when anaerobic conditions develop. With the recent development of new methods for distinguishing forms of iron in sediments and with enhanced understanding of the mechanisms of Fe(III) reduction in sediments, it might be possible to more accurately assess the role of Fe(III) reduction in phosphate flux.

Fe(III) and Mn(IV) oxides in sediments strongly adsorb a wide variety of toxic trace metals (151, 206, 216, 278, 284, 308). Furthermore, coprecipitation of toxic metals with Fe(III) and, to a lesser extent, Mn(IV) is a common treatment for a variety of wastes (104, 284). There is substantial evidence for release of trace metals into the interstitial water of aquatic sediments (1, 19, 70, 122, 124, 162, 170, 207, 267), anoxic bottom waters (313), or groundwater (42) as the result of Fe(III) and Mn(IV) reduction. This remobilization of heavy metals is a potential hazard to aquatic ecosystems and drinking-water supplies (284).

In laboratory studies, as *Bacillus* strain GJ33 reduced the Mn(IV) in ferromanganese concretions, nickel, cobalt, copper, and Mn(II) were released into solution (90). Chromium release was associated with Fe(III) reduction in laboratory studies on the effect of anaerobic conditions on coal-cleaning wastes (103). Cadmium, nickel, and zinc were released from an artificially generated coprecipitate of trace metals and goethite when a *Clostridium* sp. reduced Fe(III) (104). Laboratory studies using *S. putrefaciens* to specifically reduce Fe(III) oxides in uranium mill tailings demonstrated that microbial Fe(III) reduction can release radium from such wastes (175). These results demonstrate that a variety of metal-containing wastes may not be stable if disposed of in anaerobic environments.

Soil Gleying

As waterlogged soils become anaerobic, Fe(III) is reduced and Fe(II) compounds accumulate, resulting in grey, discolored zones (36, 37, 245, 324). This phenomenon is termed gleying. Fe(III)-reducing fermentative bacteria have been isolated from gleyed soils (46, 245, 246), and artificially gleyed soils have been generated by stimulating microbial Fe(III) reduction (7, 36, 243, 245). It has also been suggested that soil gleying could, at least in part, be the result of dissolved organic compounds abiologically reducing Fe(III) in the soil (37). All of these studies on soil gleying have been conducted under highly artificial conditions. Studies on the processes taking place in naturally gleying soils are warranted.

Generation of Iron and Manganese Minerals

Microbial Fe(III) reduction can result in the generation of several important Fe(II)-containing minerals in sedimentary environments. Most studied has been magnetite, a magnetic mineral with the formula $Fe(II)Fe(III)_2O_4$. Magnetite formation during dissimilatory Fe(III) reduction was first reported for Fe(III)-reducing enrichment cultures from the Potomac River (193). However, magnetite in Fe(III)-reducing cultures may have been observed (but not recognized as magnetite) as early as 1913 (222). In that early study, a synthetic Fe(III) hydroxide was converted to a black solid during the fermentation of peptone by a *Bacillus* sp. Magnetite accumulation was subsequently observed in various pure cultures (185, 205) and consortia (27) of Fe(III)-reducing microorganisms. Under the appropriate conditions, magnetite is an end product of all Fe(III)-reducing microorganisms that have been examined (185).

Magnetite is not an obligatory end product of dissimilatory Fe(III) reduction, and Fe(III)-reducing bacteria do not direct the synthesis of magnetite through enzymatically catalyzed reactions (27, 185). It has been argued that magnetite formation during dissimilatory Fe(III) reduction is an abiological reaction which can be explained on the basis of equilibrium thermodynamics (27). However, magnetite formation during

dissimilatory Fe(III) reduction has routinely been observed under conditions that are inconsistent with the predictions of equilibrium thermodynamics (185). Although magnetite may be formed abiologically by the reaction of Fe(II) with Fe(III) under the appropriate anaerobic conditions (280, 307), actively metabolizing cells were required for magnetite formation under the conditions used to culture Fe(III)-reducing microorganisms (185, 205). The metabolism of Fe(III)-reducing microorganisms may provide localized conditions of high Fe(II) and pH at the cell-Fe(III) oxides interface which serves to stimulate magnetite formation (185).

Magnetite production has been studied most in GS-15. Large amounts of magnetite accumulated during growth of GS-15 in a phosphate-buffered medium with poorly crystalline Fe(III) oxide as the electron acceptor (205). GS-15 could not reduce the Fe(III) in magnetite, which appears to be stable under Fe(III)-reducing conditions (185, 193, 198). The stoichiometry of Fe(II) accumulation and preservation of Fe(III) in magnetite-forming cultures suggested that GS-15 could quantitatively convert poorly crystalline Fe(III) oxide to magnetite (185).

Microbial production of magnetite during dissimilatory Fe(III) reduction is distinct from the assimilatory production of magnetite in magnetotactic bacteria, which form intracellular chains of pure single-domain magnetite (33, 107). Novel magnetotactic bacteria which can form magnetic iron sulfide minerals have also recently been described (97, 210). Intracellular magnetite permits the magnetotactic bacteria to orient themselves in Earth's magnetic field, and magnetotactic bacteria swim along magnetic field lines. The finding that magnetotactic bacteria living in the Northern Hemisphere seek magnetic north and that those in the Southern Hemisphere seek magnetic south has led to the suggestion that the magnetotactic bacteria produce magnetite as a navigational device to help them find their preferred environment (107). Magnetotactic bacteria produce magnetite with well-defined, species-specific morphologies and size (40 to 120 nm) (33, 107, 293). The magnetite is produced within a membrane-bound structure, the magnetosome (118), by a complex process that includes sequential reduction and reoxidation of iron within the cell (108).

In contrast to that produced by the magnetotactic bacteria, all of the magnetite produced by GS-15 is extracellular, with no evidence of cellular material associated with it (205, 288). The magnetite crystals are round or oval and typically vary in diameter from 6 to 50 nm (205, 288). The magnetite has a significant single-domain component (205), but many of the smaller magnetite crystals are superparamagnetic (221).

Magnetite formation in a magnetotactic bacterium is typically limited to the formation of 10 to 20 intracellular crystals. The Fe(III) reduction associated with magnetite formation does not appear to be an important mechanism for energy conservation (see below). In contrast, magnetite is an end product of the energy-generating metabolism of GS-15. As a consequence, on a per-cell basis, the metabolism of GS-15 typically generates 5,000-fold-more magnetite than a magnetotactic microorganism produces (106). Another important distinction is that magnetotactic bacteria are known to produce magnetite only under microaerobic conditions (34) or with N_2O as the electron acceptor (25), whereas dissimilatory Fe(III)-reducers produce magnetite under strict anaerobic conditions (185, 205).

Magnetite formation during dissimilatory Fe(III) reduction may explain a number of important geological phenomena. For example, the extensive deposition of magnetite in banded iron formations probably resulted from the activity of dissimilatory Fe(III)-reducing microorganisms (23, 185, 205, 328). There is a strong correlation between the accumulation of magnetite in banded iron formations and the accumulation of isotopically light carbonates (23, 254). This suggests that magnetite formation was the result of the oxidation of isotopically light organic matter coupled to the reduction of Fe(III) oxides (254). The fine-scale variability of the carbonate isotopes indicates that the oxidation of organic matter was an early diagenetic reaction (23), and thus the oxidation of organic matter coupled to Fe(III) reduction is consistent with the metabolism of organic-matter-oxidizing, Fe(III)-reducing microorganisms (23, 328). Although it has been suggested that magnetotactic bacteria might have been the source of magnetite in the banded iron formations (105), the magnetotactic bacteria do not significantly oxidize organic matter with the reduction of Fe(III). Thus, magnetotactic bacteria do not have the type of metabolism that is required to generate such large quantities of magnetite. In contrast, the production of copious quantities of magnetite under strict anaerobic conditions with the concurrent production of carbon dioxide from organic carbon is consistent with the metabolism of dissimilatory Fe(III)-reducing microorganisms.

Magnetic anomalies around hydrocarbon seeps are useful in locating hydrocarbon deposits, and these magnetic anomalies have been attributed to the accumulation of magnetite (83). Magnetite formation appears to be associated with the biodegradation of hydrocarbon components, with the concurrent reduction of Fe(III) to ultrafine-grained magnetite and larger crystal aggregates (93, 214). The capacity of dissimilatory Fe(III) reducers to metabolize a variety of hydrocarbon components has yet to be investigated. However, the ability of GS-15 to generate copious quantities of ultrafine-grained magnetite while growing on the hydrocarbon toluene (191) lends support to the hypothesis that microbial metabolism is responsible for the accumulation of magnetite near hydrocarbon deposits.

An area of intense debate is the potential for dissimilatory Fe(III) reduction to contribute to the magnetization of freshwater and marine sediments and soils (185, 293). Not only do Fe(III)-reducing microorganisms isolated from aquatic sediments produce magnetite under laboratory conditions, but there is evidence for magnetite accumulation during Fe(III) reduction in both marine and freshwater sediments (166, 203). However, the relative importance of Fe(III) reduction, magnetite-producing magnetotactic bacteria (25, 34, 166, 255, 292), iron sulfide-producing magnetotactic bacteria (97, 210), and soil-derived magnetite (produced abiologically [208], by Fe(III) reducers [185, 204], or by magnetotactic bacteria [98]) has yet to be resolved (185, 204, 293).

Rhodochrosite ($MnCO_3$) and two Fe(II) minerals, siderite ($FeCO_3$) and vivianite [$Fe_3(PO_4) \cdot 8H_2O$], that are commonly found in anaerobic sediments (see above) have also been observed to form in cultures of Fe(III)- or Mn(IV)-reducing microorganisms under appropriate conditions (2, 100, 198, 256). Microbial Fe(III) or Mn(IV) reduction has been proposed as the source of siderite or rhodochrosite in aquatic sediments (92, 285). Whether, as in magnetite formation, the Fe(III)- or Mn(IV)-reducing microorganisms serve any function other than to generate the Fe(II) or Mn(II) necessary for the formation of these minerals does not appear to have been investigated.

Reduction of Structural Fe(III) in Clays

The reduction of Fe(III) in clay minerals is a critical step in the diagenesis of clay minerals in sedimentary environments (74). The reduction of Fe(III) in clays might be directly coupled to the oxidation of organic matter, but the actual reduction mechanisms are unknown (74, 296). Fe(III)-reducing microorganisms can readily reduce the Fe(III) oxide coatings on the exterior of clay particles (193, 195). However, there is only weak evidence that microorganisms may be capable of reducing some of the structural Fe(III) in clay minerals (297, 338).

In one study (297), clays were incubated in nutrient broth (5 g of peptone and 3 g of beef extract per liter of water) under either air or N_2. Fe(III) was reduced in an unsterilized smectite clay or when an organism, designated P-1, was added to smectite, montmorillonite, or nontronite clay. Slightly less, but significant, amounts of Fe(III) were reduced under sterile conditions. In the sterile, aerobic incubations, there was reoxidation of some of the Fe(II) that had initially been produced (297). Furthermore, it was not shown whether P-1 was an Fe(III)-reducing microorganism. The slightly higher level of Fe(II) that was observed in nonsterile aerobic incubations might be explained by microbial metabolism maintaining lower oxygen concentrations and thus providing less opportunity for Fe(II) oxidation than in the controls. Under anaerobic conditions in which Fe(II) oxidation was not a factor, the percentage of Fe(II) generated in nonsterile clay (ca. 12%) was only slightly greater than that in the sterile controls (8%) after 28 days of incubation.

In a second study, Fe(III) was reduced when nontronites were incubated in a soil-water extract amended with sucrose (338). However, no controls were conducted, and thus there was no evidence that the reduction was the result of microbial metabolism. The potential for microbial reduction of Fe(III) in clays warrants further investigation.

Immobilization of Uranium

The reduction of soluble U(VI) to insoluble U(IV) is an important sink for the removal of dissolved uranium from aquatic environments and for the formation of some uranium ores (8, 38, 63, 66, 140, 152, 161, 177, 321, 331). Until recently, U(VI) reduction was considered to be an abiological reaction in which various reduced compounds such as sulfide, hydrogen, or organic matter nonenzymatically reduce U(VI) (140, 152, 177, 212). However, the Fe(III)-reducing microorganisms GS-15 and *S. putrefaciens* can use U(VI) as an electron acceptor via electron transport mechanisms that appear similar to those for Fe(III) reduction (200). This enzymatic reduction of U(VI) reduction was much faster than reduction by potential nonenzymatic mechanisms under defined laboratory conditions and in aquatic sediments (200). On the basis of these findings and previous geochemical observations (38, 63, 66, 138, 161, 331), the reduction of U(VI) by Fe(III)-reducing microorganisms has been proposed to account for the deposition of uranium in such environments as marine sediments, roll-type uranium deposits, and the bleached reduction spots of otherwise red, Fe(III)-rich rocks (200). Immobilization of uranium by microbial U(VI) reduction is a potential method for the bioremediation of uranium-contaminated waters (200).

Factors Controlling the Rate and Extent of Fe(III) and Mn(IV) Reduction

Alternative electron acceptors. Although some microorganisms may reduce Fe(III) or Mn(IV) under aerobic conditions (76, 316, 319), generally there is no significant net oxidation of organic matter coupled to Fe(III) or Mn(IV) reduction in sedimentary environments until anaerobic conditions develop (89, 109, 261, 269, 306, 339). In the case of Fe(III)- and Mn(IV)-reducing microorganisms that are capable of using oxygen as an electron acceptor, it is likely that electron transport is preferentially diverted to oxygen reduction when oxygen is available (12, 75). Oxygen is toxic to some Fe(III) and Mn(IV) reducers (198). Furthermore, the organisms which can effectively couple the oxidation of organic matter to the reduction of Fe(III) or Mn(IV) primarily metabolize fermentation products which are not produced in abundance in aerobic environments.

Nitrate inhibits Fe(III) reduction in sediments (91, 158, 286, 340). It is commonly considered that the inhibition of Fe(III) reduction is the result of preferential nitrate reduction by nitrate- and Fe(III)-reducing organisms (91, 128, 158, 226, 286). However, this cannot account for the complete inhibition of Fe(III) reduction, because nitrate does not inhibit Fe(III) reduction in all Fe(III)-reducing microorganisms (128, 158, 226, 236). Nitrite produced from nitrate reduction may prevent Fe(II) accumulation by oxidizing Fe(II), but this does not appear to be an important reaction in marine sediments (286). Alternatively, it has been demonstrated that the concentration of hydrogen is much lower in sediments in which nitrate is available than in those in which Fe(III) reduction is the predominant terminal electron-accepting process (189). Thus, in analogy to the inhibition of sulfate reduction and methane production in the presence of Fe(III) (see above), Fe(III) reduction may be inhibited in the presence of nitrate because, with nitrate as the terminal electron acceptor, the concentration of electron donors is maintained at too low a level to support Fe(III) reduction. The inhibition of Fe(III) reduction in the presence of Mn(IV) in sediments (109, 261, 269, 306, 339) has been attributed to the rapid Mn(IV) oxidation of Fe(II) (see above) and the lower concentration of electron donors in the presence of Mn(IV) reduction (197).

In contrast to Fe(III) reduction, Mn(IV) reduction can proceed in the presence of nitrate in sediments (31, 94, 109, 170, 253). This is consistent with the finding that both pure (226, 319) and enrichment (51) cultures can simultaneously reduce nitrate and Mn(IV). Furthermore, as discussed above, nitrite produced as an intermediate in nitrate reduction may abiologically reduce Mn(IV) (22). In some sediments there appears to be no Mn(IV) reduction until nitrate is depleted (77, 306).

Forms of Fe(III) and Mn(IV). The rate and extent of Fe(III) and Mn(IV) reduction in sediments may be limited not only by the concentrations of Fe(III) and Mn(IV) in sediments (6, 189, 194, 195, 197) but also by the forms of Fe(III) and Mn(IV) available. Most of the Fe(III) and Mn(IV) in sediments and soils is in the form of insoluble oxides, but organically complexed and colloidal forms may also be abundant in some instances (53, 60, 101, 133, 181, 227, 280, 284). There are a multitude of Fe(III) and Mn(IV) oxides, often in complex mixtures with each other, that range widely in degree of crystallinity, particle size, available surface area, reactivity, and oxidation state (48, 53, 90, 153, 163, 178, 180, 181, 227, 228, 263, 280, 284). These oxides are often considered to exist as coatings on clays and other particles

(49, 55, 151, 284). However, detailed studies using density gradient centrifugation and electron microscopy have suggested that most of the Fe(III) in the clay fraction of soils exists as discrete particles rather than coatings (150).

Numerous studies have demonstrated that the more crystalline the Fe(III) oxide, the slower the rate of Fe(III) reduction and that soluble, chelated Fe(III) forms are reduced the fastest (11, 13, 76, 155, 158, 193, 194, 198, 224, 225, 242, 256, 319, 334). The form of the Mn(IV) oxides is also likely to affect the rate and extent of Mn(IV) reduction, but this does not appear to have been investigated systematically. Some Fe(III)-reducing microorganisms grown on complex medium reduced highly crystalline Fe(III) oxide forms such as hematite (Fe_2O_3) and goethite (α-FeOOH) (11, 13, 128, 155, 226, 245, 246, 256, 320). In contrast, with acetate as the electron acceptor, GS-15 could only actively reduce (within 4 days) natural or synthetic poorly crystalline Fe(III) oxides (198). After 53 days of incubation, akaganeite (β-FeOOH) was slightly reduced and there was no reduction of goethite, hematite, or magnetite. These results are consistent with the finding that, with hematite as the Fe(III) source, active Fe(III)-reducing enrichment cultures from aquatic sediments could be established only for some electron donors (glucose and hydrogen) but not others (acetate and butyrate) (194).

Poorly crystalline Fe(III) oxides appear to be the primary source of Fe(III) in the Fe(III) reduction zone of soils and sediments. Measurements of redox potential and iron solubilities in sediments of groundwaters in which Fe(III) reduction was the predominant terminal electron-accepting process have suggested that either amorphous $Fe(OH)_3$ (43, 264) or an ill-defined Fe(III) oxide with a stability intermediate between those of amorphous $Fe(OH)_3$ and α-FeOOH (14, 322) is the Fe(III) form that is reduced in these environments. Further indirect evidence that poorly crystalline Fe(III) oxides are the form of Fe(III) supporting Fe(III) reduction in the Fe(III)-reducing zone was the finding that poorly crystalline but not highly crystalline Fe(III) oxides permit Fe(III)-reducing microorganisms to outcompete sulfate reducers and methanogens for electron donors in sediments (6, 194, 195).

Using a hydroxylamine extraction procedure that is specific for poorly crystalline Fe(III) oxides, it was found in fresh- and brackish-water aquatic sediments, as well as in aquifer sediments, that there was a strong relationship between the amount of hydroxylamine-extractable Fe(III) oxides and the extent of microbial Fe(III) reduction (196). Oxalate, another commonly used extractant for poorly crystalline Fe(III), extracted approximately fourfold-more Fe(III) from fresh- and brackish-water sediments than hydroxylamine (193, 258). However, only ca. 25% of the oxalate-extractable Fe(III) appeared to be reduced in the sediments. Most of the oxalate-extractable Fe(III) persisted with depth and was not reduced even when electron donors were added in an attempt to stimulate Fe(III) reduction (193). In a similar manner, only ca. 20% of the iron that was extracted from soils with a citrate-dithionite procedure was reduced when the soils were amended with organic matter and artificially flooded (160).

On the basis of thermodynamic analyses that have suggested that mixed Fe(III)-Fe(II) compounds such as $Fe_4(OH)_{10}$ and $Fe_3(OH)_8$ are the predominant solid iron phase in reduced, anaerobic sediments (43, 245, 264, 279) and the finding that oxalate extracts Fe(III) from mixed Fe(III)-Fe(II) forms which Fe(III) reducers cannot reduce (193), it was suggested that the oxalate-extractable Fe(III) that was unavailable for microbial reduction in sediments of the Potomac River was primarily in the form of mixed Fe(III)-Fe(II) compounds (193). However, subsequent studies found that, although oxalate does not extract crystalline Fe(III) oxides such as goethite and hematite when these are the only forms present, oxalate extracts these Fe(III) forms in the presence of catalytic quantities of Fe(II) (305). Oxalate extracts highly crystalline, synthetic Fe(III) oxides from Potomac River sediments which contain Fe(II) (192). These results suggest that the oxalate-extractable Fe(III) that is not microbially reducible is in the form of crystalline Fe(III) oxides. However, Mossbauer analysis of the sediments from various Canadian lakes suggested that the Fe(III) that was resistant to microbial reduction was in the form of poorly crystalline Fe(III) oxide (64). Further studies to determine the factors protecting much of the Fe(III) in sediments from microbial reduction are warranted.

MISCELLANEOUS APPLIED ASPECTS OF THE METABOLISM OF Fe(III)- AND Mn(IV)-REDUCING MICROORGANISMS

Iron and Manganese Mining

Microbial Fe(III) reduction has been proposed as a mechanism for extraction of Fe(III) from ore (11). Fe(III)-reducing organisms could potentially selectively dissolve the Fe(III) in the ore, yielding a soluble iron form, Fe(II), which would require less reduction for conversion to elemental iron than would be required for Fe(III). Microbial reduction of the Mn(IV) in ferromanganese concretions and crusts from the surface of marine sediments as well as in terrestrial manganese oxides results in the release of dissolved manganese, cobalt, copper, and nickel, but existing methods of microbial leaching are too slow to be competitive with chemical processes (87, 273, 314). Microbial reduction of Fe(III) and Mn(IV) in lake ore has also been described (319).

Corrosion of Steel

S. putrefaciens isolated from crude oil stimulated the corrosion of steel (237–239, 334). In the absence of *S. putrefaciens*, an Fe(III) oxide film formed on the surface of the metal and protected it from further corrosion. *S. putrefaciens* reduced the Fe(III) oxide film and thus exposed the bare metal to the environment. This Fe(III) reduction led to anodic depolarization of the steel (239). Corrosion may also have been stimulated by the attachment of the Fe(III)-reducing microorganisms to the steel surface, which may have caused further anodic depolarization and the pitting of the steel surface (237, 334). The close similarity between the corrosion brought about in the presence of *S. putrefaciens* and the corrosion of steel by mixed natural microbial populations suggested that the metabolism of *S. putrefaciens* (and similar organisms) was an important mechanism for the corrosion of oil pipelines (237, 239, 334).

Removal of Iron Impurities from Clays

Fe(III) impurities in kaolins used in the production of ceramics detracts from the whiteness of porcelain (137). A microbial process for removing Fe(III) impurities from low-quality kaolins has been developed (137). A mixed culture containing *Enterobacter* (formerly *Aerobacter*) *aerogenes* and *Leuconostoc mesenteroides* was inoculated into a nutrient medium containing molasses, urea, and kaolin. As the

Fe(III) impurities were reduced, the supernatant was periodically siphoned off to remove the dissolved Fe(II) that had been generated, and fresh medium was added. Depending on the kaolins utilized, the microorganisms removed from 27.5 to 43.9% of the initial Fe(III). The amount of Fe(III) removed was directly proportional to the initial Fe(III) content of the kaolins. This treatment did not cause any unfavorable modifications in the chemical and physical characteristics of the kaolins, and it increased the whiteness of the kaolins upon burning.

Toxicity Assays

The extent of Fe(III) or Mn(IV) reduction in submerged soils in the presence of various compounds has been employed as a sensitive assay for the toxicity of the compounds to the anaerobic microbial community (249, 333, 342). Pesticides or other compounds of interest are added to the soil with or without additional amendments of organic substrates and Fe(III) or Mn(IV). The extent of Fe(III) or Mn(IV) reduction is compared with that of controls without the added test compound. Fe(III) reducers or microbial food chains with Fe(III) reducers in the terminal position may also be involved in pesticide degradation, since the extent of parathion degradation in waterlogged soils was directly related to the capacity of the soils for Fe(III) reduction (335).

Rice Culturing

The Fe(II) that accumulates as the result of Fe(III) reduction in rice paddies is beneficial to rice growth at low levels but toxic at higher levels (261, 262, 323, 339). The addition of nitrate or Mn(IV) to paddy soils inhibited Fe(III) reduction and concomitantly remedied physiological disorders in rice plants (340). The pH increase often associated with Fe(III) reduction may stimulate rice growth in some instances (323). Given the importance of rice to the world's food supply and the important controlling influence that Fe(III) reduction has on the geochemistry of rice paddies (164, 261, 306, 323, 339), further study of the microbial ecology of Fe(III) reduction in rice paddies seems warranted.

ENUMERATION AND CULTURING OF Fe(III) AND Mn(IV) REDUCERS

Despite the environmental significance of Fe(III)- and Mn(IV)-reducing microorganisms, very few organisms capable of obtaining energy for growth from Fe(III) and Mn(IV) reduction are available for study, and there is little information on the distribution or diversity of these organisms. Fe(III)- and Mn(IV)-reducing microorganisms have been enumerated and cultured on a wide variety of solid, semisolid, and liquid media that are similar in composition to those used for other heterotrophic microorganisms but with the modification that some form of Fe(III) or Mn(IV) was included in the medium. Naturally occurring Fe(III) or Mn(IV) oxides have been employed, as have hematite, goethite, lepidocrocite, synthetic poorly crystalline Fe(III) oxides, $FePO_4$, $FeCl_3$, and Fe(III) citrate (17, 45, 91, 116, 155, 172, 198, 236, 246, 256, 282, 285, 318). For agar media, the Fe(III) or Mn(IV) may be added as an overlay (89, 230). In agar media, Fe(III) or Mn(IV) reduction can be detected as zones of clearing or discoloration in the Fe(III) or Mn(IV) suspension (89, 116, 230, 272, 318). Alternatively, the production of Fe(II) can be monitored by such techniques as placing strips of filter paper soaked with 2,2'-dipyridyl on the colonies (46) or spraying ferrozine on the agar surface (81). In the case of Mn(IV), Berbelin blue-I (*N,N'*-dimethylamino-*p,p'*-triphenylmethane-*o''*-sulfonic acid), which reacts with Mn(IV) but not Mn(II), may be added to plates after growth to enhance visualization of the zones of Mn(IV) reduction (172).

In liquid culture, the production of Fe(II) by Fe(III) reduction can be monitored with reagents such as ferrozine (193, 197), 2,2'-dipyridyl (46, 241), 1,10-phenanthroline (10, 236), or 2,4,6-tripyridyl-1,3,5-trazine (158), which react specifically with Fe(II). For quantitative measurements of the amount of Fe(II) formed, it is necessary to extract insoluble Fe(II) from the samples with HCl or an anaerobic solution of ammonium oxalate (46, 193, 198, 320), because most of the Fe(II) that is produced is found in solid forms under many culture conditions (46, 197, 320). Loss of Fe(III) can also be monitored by one of several techniques (196, 198). Reduction of Mn(IV) is typically monitored as the production of dissolved manganese, since Mn(II) is much more soluble than Mn(IV). However, as with Fe(II), much of the Mn(II) produced from Mn(IV) reduction is typically not dissolved. Copper or magnesium sulfate solutions have been used to desorb Mn(II) from solids (47, 51, 172, 197, 229). However, copper sulfate does not extract some important Mn(II) forms such as $MnCO_3$ (197). The Mn(II) in $MnCO_3$ is solubilized in 0.5 N HCl, which in short-term extractions was found to dissolve $MnCO_3$ but not MnO_2 (198).

In freshwater liquid medium with poorly crystalline Fe(III) oxide as the electron acceptor, Fe(III) reduction can also be monitored by observing the conversion of the reddish brown, nonmagnetic Fe(III) oxide to the intensely black and highly magnetic mineral magnetite (193, 205). This technique may be inappropriate for monitoring Fe(III) reduction in mixed cultures in media which contain significant quantities of sulfate, because sulfate reduction could lead to the formation of black iron sulfides, some of which are magnetic. In liquid medium with Fe(III)-citrate as the electron acceptor, the medium changes from orange-brown to green as Fe(III) is reduced (198, 282). At the completion of Fe(III) reduction, the medium typically clears because the Fe(II) forms vivianite [$Fe_3(PO_4)_2 \cdot 8H_2O$], a white precipitate (201). In medium containing a bicarbonate-CO_2 buffer, Mn(IV) reaction can also be detected visually because the brown MnO_2 is converted to a white precipitate of MnCO (2, 198).

For Fe(III)- or Mn(IV)-reducing microorganisms that obtain their energy for growth from Fe(III) or Mn(IV) reduction, isolation of microorganisms on solid medium is hampered by the difficulty in providing enough Fe(III) or Mn(IV) to support colony development. For example, *S. putrefaciens* MR-1 formed clearing zones but no visual colonies in an MnO_2-containing medium (229). Alternatively, Fe(III)- and Mn(IV)-reducing microorganisms which can use nitrate as an electron acceptor can be isolated as colonies with nitrate as the electron acceptor (198). With a soluble form of Fe(III) such as Fe(III) citrate, enough Fe(III) to support the growth of visible colonies can be incorporated into solid medium (192). However, this technique may not be useful for the direct isolation of Fe(III)-reducing microorganisms from sediments because of the widespread occurrence of citrate-degrading microorganisms which consume the citrate with the precipitation of the Fe(III).

Given the anaerobic nature of most Fe(III)- and Mn(IV)-reducing environments and the finding that some Fe(III)- and Mn(IV)-reducing microorganisms are inhibited by O_2 (198), anaerobic techniques are preferred for isolation or enumer-

ation. Fe(II) or sulfide is a suitable reducing agent (191, 205). Although isolations have been conducted in anaerobic chambers (229), the H_2 atmosphere typically in these systems may favor the growth of H_2-oxidizing Fe(III)- and Mn(IV)-reducing microorganisms. Fe(III)- and Mn(IV)-reducing microorganisms have also been cultured by using bench-top anaerobic techniques (18, 143, 218) which do not require H_2 in the gas phase (17, 158, 198).

ELECTRON TRANSPORT TO Fe(III) and Mn(IV)

An understanding of the mechanisms of electron transport to Fe(III) and Mn(IV) is important for understanding the factors controlling the rate and extent of Fe(III) and Mn(IV) reduction in various environments. Furthermore, the suggestion that microbial Fe(III) reduction may have been the first globally significant mechanism for oxidation of organic matter to carbon dioxide (see above) indicates that some of the first microbial electron transport chains to evolve may have served to couple organic-matter oxidation to Fe(III) reduction.

Electron Transport to Fe(III)

Electron transport from organic compounds to Fe(III) has been investigated in a wide variety of microorganisms. However, with few exceptions, these studies have been conducted with microorganisms that were grown under aerobic conditions. Membrane-bound Fe(III) reductases are likely to be important mechanisms for non-siderophore-mediated assimilatory iron uptake in many organisms (96). Thus, in studies on aerobically grown cells, there is the possibility that the Fe(III) reduction that was investigated had an assimilatory rather than dissimilatory function.

One of the earliest studies giving evidence consistent with enzymatic Fe(III) reduction was conducted by Bromfield (45). Fe(III) reduction in cell suspensions of aerobically grown *Bacillus circulans* was not the result of the release of extracellular compounds. Substrates that resulted in rapid reduction of methylene blue also resulted in the most rapid Fe(III) reduction. Inhibitors of methylene blue reduction inhibited Fe(III) reduction. Similar results were observed with *Bacillus megaterium* and *E. aerogenes*. These organisms could not reduce Fe(III) oxide unless an Fe(III) chelate was present. Therefore, it must be questioned whether the electron transport to Fe(III) that was observed in these organisms is a useful model for dissimilatory Fe(III)-reducing microorganisms living in natural environments, where insoluble Fe(III) oxides are the predominant Fe(III) form.

Membrane-bound Fe(III) reductases which could reduce Fe(III) citrate were discovered in *Spirillum itersonii* (75) and *Staphylococcus aureus* (179). A link between organic-matter oxidation and Fe(III) reduction was established, since functional dehydrogenases were required for Fe(III) reduction. Fe(III) reduction was not inhibited by HQNO, which indicated that Fe(III) reduction occurred at a site(s) on the respiratory chain prior to cytochrome *c*. Oxygen (*S. itersonii*) or nitrate (*S. aureus*) preferentially oxidized components of the electron transport chain and prevented Fe(III) reduction. However, Fe(III) was reduced in the presence of these alternative electron acceptors if their reduction was prevented with inhibitors. The Fe(III) reductase activities in these organisms were studied in the context of iron assimilation, and these organisms have not been reported to obtain energy for growth from dissimilatory Fe(III) reduction.

Several of the fermentative organisms that use Fe(III) as a minor electron sink were considered to contain Fe(III)-reducing enzymes because Fe(III) reduction could not be accounted for by the generation of a low pH or the release of extracellular products (76, 159, 225, 226, 320). In addition to apparent enzymatic reduction of hematite by a *Clostridium* sp., the low-pH (3.1) spent culture fluid of this organism also solubilized iron (102). It was not reported whether the dissolved iron was Fe(II) or just Fe(III) that had been dissolved at the low pH. In a study with a glucose-fermenting *Vibrio* sp., extracellular components released into the medium reduced Fe(III) at a rate that was ca. one-third of the rate in the presence of the organism (158). Although a variety of inhibitors of electron transport inhibited Fe(III) reduction in the *Vibrio* sp., this organism did not conserve energy through Fe(III) reduction (158).

An indirect indication of electron transport to Fe(III) in *Aquaspirillum magnetotacticum*, *Bacillus subtilis*, and *E. coli* was the finding that when Fe(III) was added to anaerobic cell suspensions, protons were translocated (283). Azide inhibited proton translocation, suggesting that a terminal oxidase was involved in Fe(III) reduction.

However, it is unlikely that electron transport is coupled to energy-yielding, dissimilatory Fe(III) reduction in any of these organisms. *A. magnetotacticum* was subsequently found to be incapable of growth under anaerobic conditions with Fe(III) as the electron acceptor (123). Furthermore, the Fe(III) reductase activity in *A. magnetotacticum* is not associated with cell respiration (24, 250). *E. coli* is also unable to obtain energy for growth from Fe(III) reduction, and respiratory chains are not involved in Fe(III) reduction in this organism (336). Although *B. subtilis* does contain soluble Fe(III) reductases that are probably involved in the release of Fe(III) from siderophores (111), *B. subtilis* does not produce extracellular Fe(II) under anaerobic conditions (192).

Ferricyanide can accept electrons from electron transport chains in membrane vessicles of *B. subtilis* (32) and *E. coli* (39). It is possible that the Fe(III)-quinate that was used in the proton translocation studies can interact with electron transport components in a similar manner. Although this type of Fe(III) reductase activity is probably not related to dissimilatory Fe(III) reduction, it could have accounted for the observed proton translocation.

Electron transport has also been investigated in *S. putrefaciens* 200 (previously referred to as *Pseudomonas* sp. strain 200, *Pseudomonas ferrireductans*, and *Alteromonas putrefaciens* sp. strain 200). Aerobically grown cells of *S. putrefaciens* reduced Fe(III) when placed under anaerobic conditions (236). *S. putrefaciens* did not release soluble components capable of reducing Fe(III), and direct contact between the organism and insoluble Fe(III) oxides was required for Fe(III) reduction (11). Circumstantial evidence for the involvement of cytochromes in electron transport to Fe(III) was the finding that when *S. putrefaciens* was grown aerobically in media that stimulated the production of *c*- and *b*-type cytochromes, Fe(III) was reduced at a higher rate than in cells that had a lower concentration of cytochromes (235). The diminished production of *b*- *c*-, and *d*-type cytochromes in a mutant of *S. putrefaciens* that had diminished Fe(III) reductase activity was also proposed as evidence for a role of cytochromes in electron transport to Fe(III) (81).

HQNO and carbon monoxide inhibited Fe(III) reduction in anaerobic suspensions of aerobically grown *S. putrefaciens* (235, 236). Also working with aerobically grown cells (dissolved oxygen concentration of ≥80% saturation with air), Arnold and co-workers (10) found that dicyclohexylcar-

odiimide and dicumarol also inhibited Fe(III) reduction but that quinacrine, cyanide, or azide had little affect. Inhibition of cytochrome oxidase with cyanide permitted *S. putrefaciens* to reduce Fe(III) in the presence of oxygen (12). When *S. putrefaciens* was grown at lower oxygen concentrations (<2% saturation) Fe(III) reduction was not greatly inhibited by HQNO, carbon monoxide, dicyclohexylcarodiimide, or dicumarol (10). However, quinacrine was more inhibitory to Fe(III) reduction in the cells that had been grown at the low oxygen concentration. These results were interpreted as indicating that the Fe(III) reductase activity that was expressed during growth at high oxygen concentrations was linked to electron transport and that the Fe(III) reductase activity that predominated at the low oxygen concentration served only as a sink for cellular reducing power and was not involved in oxidative phosphorylation via proton translocation (10).

Subsequent studies have indicated that the Fe(III) reductase that is produced constitutively in aerobically grown *S. putrefaciens* is also not involved in energy transduction. When aerobically grown cells of *S. putrefaciens* were put under anaerobic conditions in the presence of Fe(III), 95 to 98% died within 2 days, and there was no significant subsequent growth (11). Studies with the MR-1 strain of *S. putrefaciens* indicated that aerobically grown cells did not synthesize electron transport chains capable of proton translocation with Fe(III) as the electron acceptor (231). Thus, although the Fe(III) reductase activities in aerobically grown cells of *S. putrefaciens* are typically characterized as dissimilatory (10, 12), there is no evidence that the Fe(III) reductases and associated electron transport chains that are produced in aerobically grown cells are involved in energy transduction.

However, anaerobically grown cells of two strains (MR-1 and ATCC 8071) of *S. putrefaciens* must produce Fe(III) reductases that are involved in energy transduction, since these organisms grew under anaerobic conditions with Fe(III) as the sole electron acceptor (201, 229). Strain MR-1 translocated protons when Fe(III) was provided to suspensions of cells that had been grown anaerobically with fumarate as the electron acceptor (231). Although this is consistent with coupling of electron transport to Fe(III) reduction, it is not "unequivocal evidence for energy generation linked to Fe(III) respiration" as has been claimed (231) because, as discussed above, cell suspensions of microorganisms in which Fe(III) reduction is not involved in energy transduction have also been observed to translocate protons when provided with Fe(III).

The studies cited above demonstrate that even organisms such as *S. putrefaciens*, which have the potential to obtain energy to support growth from Fe(III) reduction, may produce Fe(III) reductases that are not involved in energy transduction. Therefore, it seems essential that studies on mechanisms for electron transport to Fe(III) be carried out on cells that have been grown with Fe(III) as the electron acceptor. Only such cells are certain to produce the Fe(III) reductase and associated electron transport chain that is involved with energy transduction during growth on Fe(III).

Only one preliminary study on GS-15 has investigated electron transport to Fe(III) in anaerobically grown cells that had obtained their energy for growth from Fe(III) reduction (117). GS-15 was assumed to conserve energy for growth via electron transport to Fe(III) because it grew with acetate, a nonfermentable substrate, as the sole electron donor and Fe(III) as the sole electron acceptor (198). Direct contact between Fe(III) oxide and GS-15 were required for Fe(III) reduction (198). The Fe(III) reductase activity in GS-15 was localized in the membrane fraction (117). In cell suspensions, Fe(III) reduction was irreversibly inhibited by oxygen. HQNO, azide, or cyanide had no effect at the concentrations examined. Rotenone inhibited Fe(III) reduction ca. 30%. GS-15 contained a *b*-type cytochrome. The addition of Fe(III) to cells in which the cytochrome had been reduced oxidized the cytochrome. However, added Fe(III) did not oxidize the cytochrome if the Fe(III) reductase activity was first inhibited by brief exposure to air. These results suggest that GS-15 obtains energy for growth via electron transport involving a *b*-type cytochrome and a membrane-bound Fe(III) reductase (117).

Nitrate Reductase as an Fe(III) Reductase?

There is little information on the properties of Fe(III) reductases in dissimilatory Fe(III)-reducing microorganisms. It is frequently suggested that Fe(III) may be reduced by the nitrate reductase of some organisms (128, 223, 226, 241, 242, 246, 248). However, there is no direct evidence to support this suggestion, and in many instances it is clear that the Fe(III)-reducing microorganisms contain an Fe(III) reductase that is distinct from the nitrate reductase. The primary evidence given for involvement of nitrate reductase in Fe(III) reduction has been the inhibition of Fe(III) reduction in the presence of added nitrate (223, 226, 241, 242, 246, 248). However, this inhibition can also be attributed to preferential electron flow to nitrate reductase rather than Fe(III) reductase (179) or to chemical oxidation of Fe(II) by nitrite produced during nitrate reduction (179, 236). The other claim for involvement of nitrate reductase was that in a medium without nitrate, mutants that could no longer reduce nitrate produced less Fe(II) than did their wild-type counterparts (244). However, examination of the data indicates that this was not true in all instances. Furthermore, in the instances in which mutants that did not reduce nitrate did produce less Fe(II), it is not clear that the slightly smaller amounts of Fe(II) produced were significantly lower than the amounts of Fe(II) produced by the wild type. Even if there had been an instance in which the loss of the ability to reduce nitrate had also resulted in the loss of the ability to reduce Fe(III), this would not be good evidence for involvement of nitrate reductase in Fe(III) reduction unless it could be demonstrated that the mutation had directly affected nitrate reductase rather than other electron transport components.

There is direct evidence that Fe(III) reductase is distinct from nitrate reductase in many Fe(III)-reducing organisms. This is clear for species of *Bacillus*, *Clostridium*, *Bacteriodes*, *Desulfotomaculum*, which can reduce Fe(III) but not nitrate (76, 157, 226, 242, 244). Furthermore, many organisms which have a nitrate reductase do not reduce Fe(III) (157, 192).

In *S. aureus*, azide, a competitive inhibitor of nitrate reductase, did not inhibit Fe(III) reductase activity (179). Furthermore, Fe(III) was reduced in the absence of hemin in membrane fractions from an *S. aureus* mutant which required hemin for nitrate reduction. Cyanide or azide, inhibitors of nitrate reductase, did not significantly inhibit Fe(III) reductase activity in *B. circulans* (45), and cyanide did not inhibit Fe(III) reductase activity in *E. coli* (336). However, as discussed above, the Fe(III) reductase activities under study in these organisms were probably not involved in energy transduction.

Nitrate reductase in GS-15 does not function as an Fe(III) reductase (117). Cell suspensions of GS-15 grown with

Fe(III) as the sole electron acceptor actively reduced Fe(III) but had no nitrate reductase activity. Cells grown with nitrate as the sole electron acceptor had nitrate reductase activity but only half the Fe(III) reductase activity found in Fe(III)-grown cells. Azide and cyanide inhibited electron transport to nitrate but had no effect on electron transport to Fe(III) in either Fe(III)- or nitrate-grown cells. These results indicated that the nitrate reductase and Fe(III) reductase are two separate enzymes.

Electron transport to nitrate and Fe(III) also appear to utilize different electron transport components in *S. putrefaciens*. *S. putrefaciens* cells grown with nitrate as the electron acceptor did not translocate protons when Fe(III) was provided as an electron acceptor, whereas fumarate-grown cells did (231). Cyanide and azide, typically inhibitors of nitrate reductase, did not inhibit Fe(III) reduction in anaerobic suspensions of aerobically grown *S. putrefaciens* (231).

Electron Transport to Mn(IV)

Electron transport of Mn(IV) has been intensively investigated in the marine microorganism *Bacillus* strain 29 (112–114, 316, 317), and these studies have recently been reviewed in detail elsewhere (112). The electron transport system contains a dicumarol-sensitive quinone; an Mn(II)-inducible, Atebrine-sensitive flavoprotein; and a cyanide- and azide-sensitive MnO_2 reductase (112). *Bacillus* strain 29 may also reduce MnO_2 through the production of H_2O_2, which can nonenzymatically reduce MnO_2 (112). *Bacillus* strain GJ33 and several gram-negative marine bacteria also reduce Mn(IV) through an unspecified electron transport pathway (88). Another *Bacillus* sp. reduces Mn(IV) through an electron transport chain that involves both *b*- and *c*-type cytochromes (80). The physiological role of Mn(IV) reduction in these *Bacillus* spp. has not been identified. However, as discussed above, the available evidence suggests that Mn(IV) reduction is a minor side reaction in the metabolism of these organisms and does not provide energy to support growth. Therefore, the relevance of the electron transport to Mn(IV) in these organisms to the bulk of dissimilatory Mn(IV) reduction in sedimentary environments is uncertain.

Enzymatic Mn(IV) reduction was studied in cell extracts of *Acinetobacter calcoaceticus* (165). Details on the role of Mn(IV) reduction in the metabolism of this organism were not provided. The cells that were studied were grown on molasses without Mn(IV). It was not specified whether the cells were grown aerobically or anaerobically. With glucose as a potential electron donor, cell extracts reduced Mn(IV) faster than heat-killed controls. The rate of Mn(IV) reduction was proportional to the amount of cell protein provided, and the temperature and pH optima were consistent with an enzymatic reaction. *A. calcoaceticus* could also reduce nitrate to ammonia. Addition of nitrate inhibited Mn(IV) reduction at an Mn(IV)/nitrate ratio (mole/mole) of 5:1, but Mn(IV) reduction resumed as the Mn(IV) concentration was increased. Mn(IV) had no effect on nitrate reduction. Cells grown on defined medium without molybdenum had lower nitrate and Mn(IV) reductase activities than cells grown with molybdenum, and additions of molybdenum to extracts of cells grown without molybdenum stimulated both nitrate and Mn(IV) reduction. These results were considered to demonstrate that in *A. calcoaceticus*, Mn(IV) is reduced by the assimilatory nitrate reductase. However, as discussed above for Fe(III) reduction, preferential electron flow to nitrate reductase and away from a distinct Mn(IV) reductase could also explain such results.

There has been little work on electron transport to Mn(IV) in organisms which can obtain energy for growth from Mn(IV) reduction. Growth of GS-15 on acetate, a nonfermentable substrate, coincide with Mn(IV) reduction (198). This indicated that electron transport to Mn(IV) yielded energy to support growth. Growth of *S. putrefaciens* coincided with Mn(IV) reduction, and a variety of electron transport inhibitors partially inhibited Mn(IV) reduction in anaerobic cell suspensions (229). Azide (1 mM) only partially (36%) inhibited Mn(IV) reduction in strain MR-1 (229), whereas a higher concentration (10 mM) completely inhibited Mn(IV) reduction in enrichment cultures of Mn(IV)-reducing microorganisms (51).

Proton translocation was observed in anaerobic cell suspensions of *S. putrefaciens* MR-1 with lactate as the electron donor and Mn(IV) as the electron acceptor (231). This proton translocation was noted in cells grown anaerobically with either nitrate or fumarate as the electron acceptor. Proton translocation was not observed in Mn(IV)-grown cells, but this was attributed to damage of the cells during chemical removal of the MnO_2 electron acceptor from the cultures. The protonophore carbonyl cyanide *m*-chlorophenylhydrazone and the electron transport inhibitors HQNO and antimycin A eliminated proton pulses. Although these results were considered to provide direct biochemical proof that MR-1 can obtain energy from Mn(IV) reduction (231), as discussed above for previous studies on electron transport to Fe(III), acidification of the medium in response to an added electron acceptor does not necessarily demonstrate that the organism obtains energy to support growth from the reduction of that electron acceptor.

Basic questions about dissimilatory Mn(IV) reduction such as whether Mn(IV) is most commonly directly reduced to Mn(II) or whether Mn(III) (120) is a typical intermediate are yet to be answered (88). As with dissimilatory Fe(III) reduction, no enzymes directly responsible for Mn(IV) reduction have been isolated and characterized (89).

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GS-15 oxidizes acetate via the citric acid cycle (57). GS-15 cultured either with nitrate or Fe(III) as the electron acceptor contained catabolic levels of the citric acid cycle enzymes. In contrast, enzyme activities representative of the carbon monoxide dehydrogenase pathway were absent. This is only the third example of a strict anaerobic microorganism which uses the citric acid cycle in preference to the carbon monoxide dehydrogenase pathway (57). The presence of the citric acid cycle in GS-15 is consistent with the fact that the standard redox potentials for the Fe(III)-Fe(II), Mn(IV)-Mn(II), and NO_3^--NH_4^+ couples are probably too high to permit the carbon monoxide dehydrogenase pathway to function (309). GS-15 activates acetate to acetyl coenzyme A for the citric acid cycle by the action of acetate kinase and phosphotransacetylase (57).

No other studies on catabolic pathways in Fe(III) reducers appear to have been conducted. Of interest are the mechanisms for the oxidation of aromatic compounds, especially the aromatic hydrocarbon toluene, in GS-15. Studies on the source of electrons for Fe(III) reduction in the fermentative Fe(III)-reducing microorganisms would elucidate whether Fe(III) reduction in these organisms results in an energetic advantage over fermentative microorganisms which do not reduce Fe(III).

CONCLUSIONS

The environmental significance of organic-matter oxidation coupled to Fe(III) and Mn(IV) reduction is being increasingly documented. It is also clear that in typical sedimentary environments, Fe(III) reduction [and probably Mn(IV) reduction] is primarily the result of the enzymatic activity of specialized Fe(III)- and Mn(IV)-reducing microorganisms. Although Fe(III)- and Mn(IV)-reducing microorganisms have been studied for more than 60 years, organisms with the type of respiratory metabolism likely to be responsible for most of the Fe(III) and Mn(IV) reduction in sedimentary environments have only recently been isolated. Only a few isolates are available from what is likely to be a diverse assemblage of organisms which can effectively couple the oxidation of organic compounds to the reduction of Fe(III) or Mn(IV). Little is known about the physiology of Fe(III) and Mn(IV) reduction or the enzymes involved in this novel form of metabolism.

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