
Influence of dissimilatory metal reduction on fate of organic and metal contaminants in the subsurface

Derek R. Lovley · Robert T. Anderson

Abstract Dissimilatory Fe(III)-reducing microorganisms have the ability to destroy organic contaminants under anaerobic conditions by oxidizing them to carbon dioxide. Some Fe(III)-reducing microorganisms can also reductively dechlorinate chlorinated contaminants. Fe(III)-reducing microorganisms can reduce a variety of contaminant metals and convert them from soluble forms to forms that are likely to be immobilized in the subsurface. Studies in petroleum-contaminated aquifers have demonstrated that Fe(III)-reducing microorganisms can be effective agents in removing aromatic hydrocarbons from groundwater under anaerobic conditions. Laboratory studies have demonstrated the potential for Fe(III)-reducing microorganisms to remove uranium from contaminated groundwaters. The activity of Fe(III)-reducing microorganisms can be stimulated in several ways to enhance organic contaminant oxidation and metal reduction. Molecular analyses in both field and laboratory studies have demonstrated that microorganisms of the genus *Geobacter* become dominant members of the microbial community when Fe(III)-reducing conditions develop as the result of organic contamination, or when Fe(III) reduction is artificially stimulated. These results suggest that further understanding of the ecophysiology of *Geobacter* species would aid in better prediction of the natural attenuation of organic contaminants under anaerobic conditions and in the design of strategies for the bioremediation of subsurface metal contamination.

Résumé Des micro-organismes simulant la réduction du fer ont la capacité de détruire des polluants organi-

ques dans des conditions anérobies en les oxydant en dioxyde de carbone. Certains micro-organismes réducteurs de fer peuvent aussi dé-chlorer par réduction des polluants chlorés. Des micro-organismes réducteurs de fer peuvent réduire tout un ensemble de métaux polluants et les faire passer de formes solubles à des formes qui sont susceptibles d'être immobilisées dans le milieu souterrain. Des études d'aquifères pollués par du pétrole ont montré que des micro-organismes réducteurs de fer peuvent être des agents efficaces pour éliminer les hydrocarbures aromatiques des eaux souterraines dans des conditions anérobies. Des études en laboratoire ont montré que des micro-organismes réducteurs de fer avaient la capacité d'éliminer l'uranium d'eaux souterraines polluées. L'activité de micro-organismes réducteurs de fer peut être stimulée de différentes manières pour augmenter l'oxydation de polluants organiques et la réduction de métaux. Des analyses moléculaires concernant des études de terrain et de laboratoire ont montré que des micro-organismes du genre *Geobacter* deviennent les membres dominants de la communauté microbienne quand les conditions de réduction en Fe(III) sont réalisées à la suite d'une pollution organique, ou lorsque la réduction en Fe(III) est stimulée artificiellement. Ces résultats laissent penser que des connaissances supplémentaires sur l'écophysologie des espèces *Geobacter* devraient aider à une meilleure prédiction de la diminution naturelle des teneurs en polluants organiques dans des conditions anérobies, ainsi qu'à la définition de stratégies de dépollution biologique de pollutions souterraines par les métaux.

Resumen Algunos microorganismos Fe(III)-reductores son capaces de destruir selectivamente determinados contaminantes orgánicos en condiciones anaerobias, oxidándolos a dióxido de carbono. Otros de estos microorganismos Fe(III)-reductores pueden reducir, bien compuestos clorados, bien una gran variedad de metales, que dejan de ser solubles y se inmovilizan en el subsuelo. Estudios realizados en acuíferos contaminados por petróleo muestran que los microorganismos Fe(III)-reductores pueden ser unos agentes muy eficientes para eliminar los hidrocarburos aromáticos de las aguas subterráneas en condiciones anaerobias, mientras que estudios de laboratorio

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muestran el potencial de estos microorganismos para eliminar uranio. La actividad de los microorganismos Fe(III)-reductores se puede estimular para conseguir una mayor eficiencia en la oxidación de contaminantes orgánicos y en la reducción de metales. Diversos análisis moleculares en estudios de campo y de laboratorio muestran que los microorganismos del género *Geobacter* se convierten en los miembros dominantes de la comunidad microbiana cuando se desarrollan condiciones Fe(III)-reductoras, bien como resultado de la contaminación orgánica, bien por estimulación artificial. En consecuencia, se hace necesario un mayor entendimiento de la ecofisiología de los microorganismos del género *Geobacter* para mejorar las predicciones sobre atenuación natural de los contaminantes orgánicos bajo condiciones anaerobias y para el diseño de estrategias de biorremediación del subsuelo en los casos de contaminación por metales.

Key words microbial processes · contamination · metals · hydrocarbons · bioremediation

Introduction

Of the various forms of microbial respiration that have an important impact on the geochemistry of subsurface environments, dissimilatory Fe(III) reduction is one of the most recently discovered. During dissimilatory Fe(III) reduction, microorganisms transfer electrons to Fe(III), reducing it to Fe(II) for purposes other than assimilation of iron into cellular components. Fe(III) reduction is not linked to energy conservation in all microorganisms that are capable of dissimilatory Fe(III) reduction (Lovley 1987, 1991). However, the term “dissimilatory Fe(III) reduction” is often used in a more narrow sense to refer to the process in which microorganisms conserve energy to support their growth from the oxidation of organic compounds and/or H₂ coupled to the reduction of Fe(III). This more restrictive definition is used here.

Dissimilatory Fe(III) reduction significantly influences the fate of both organic and inorganic compounds in both pristine and contaminated subsurface environments. For example, in deep pristine aquifers Fe(III) reduction can be an important process for oxidation of organic matter, increasing the concentrations of dissolved inorganic carbon and dissolved Fe(II) while at the same time preventing the accumulation of sulfide (Lovley et al. 1990; Chapelle and Lovley 1992; Lovley and Chapelle 1995). These and other roles of dissimilatory Fe(III) reduction in pristine aquifers have recently been reviewed (Lovley and Chapelle 1995; Lovley 1997a) and are not discussed further here. In polluted aquifers, dissimilatory Fe(III) reduction can remove organic contaminants by serving as an electron acceptor for contaminant oxidation. Fe(III)-reducing microorganisms can also immobilize a variety of contaminant metals and metalloids by

reducing them to less soluble forms. Significant advances have recently been made in understanding the microbiology and geochemistry of contaminant removal from groundwater by dissimilatory Fe(III)-reducing microorganisms. This review focuses on the role of Fe(III) reducers in natural and engineered remediation of organic and metal contamination in the subsurface.

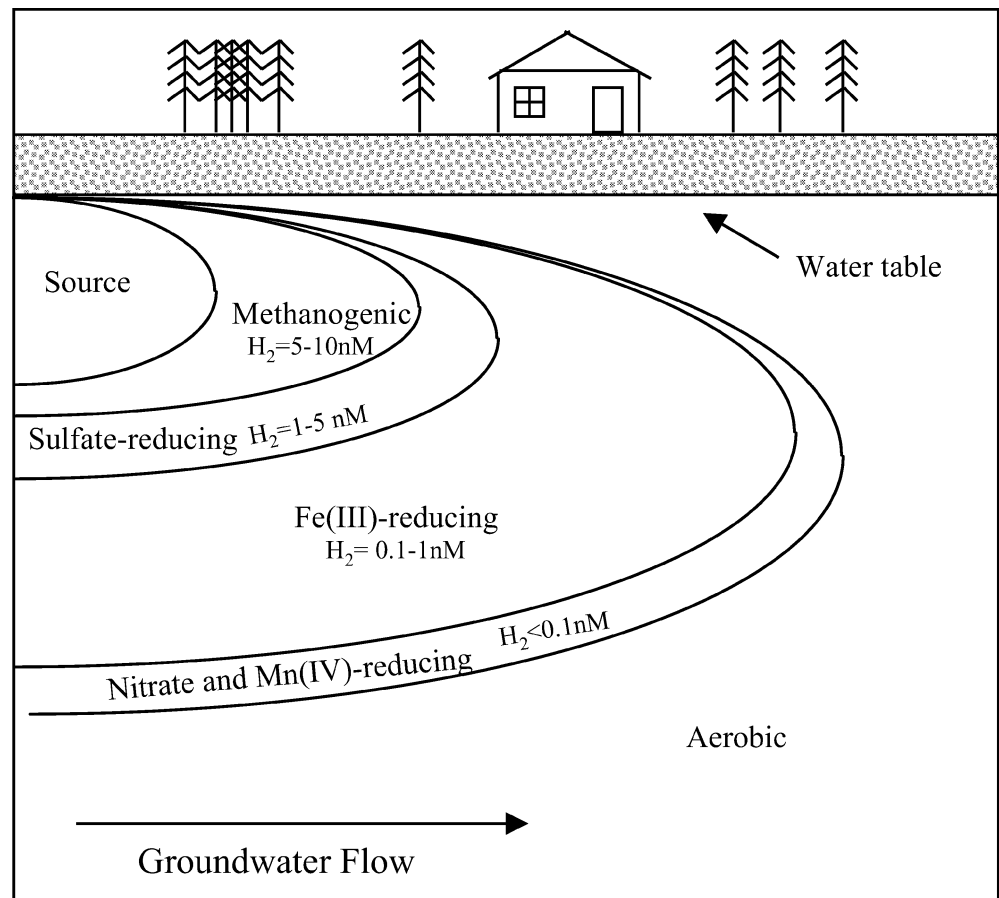
Oxidation of Organic Contaminants Coupled to Fe(III) Reduction

Microbial Fe(III) reduction is an important process only under anaerobic conditions. Thus, Fe(III) reduction generally does not have an important influence on the water quality of shallow pristine aquifers, which are typically aerobic. However, when shallow aquifers are contaminated with soluble organic compounds, such as those in landfill leachates or leaking underground storage tanks, then anaerobic conditions may develop. With the development of anaerobic conditions, Fe(III) is generally the most abundant potential electron acceptor for organic-matter oxidation (Lovley 1991, 1997b). Thus, aquifers contaminated with organic compounds typically contain extensive zones in which Fe(III) reduction is the predominant terminal electron-accepting process (TEAP) (Christensen et al. 1994; Anderson and Lovley 1997). In general, the zone in which Fe(III) reduction predominates is found downgradient of zones of methane production and sulfate reduction, and upgradient of zones of nitrate and Mn(IV) reduction, as shown in *Figure 1*. The reason for this distribution is that methanogenesis and sulfate reduction can only become important TEAPs when microbial Fe(III) reduction becomes limited by the availability of Fe(III), whereas typically no net Fe(III) reduction occurs in the presence of Mn(IV) and nitrate (Lovley and Chapelle 1995).

Geochemical Evidence for Fe(III) Reduction in Contaminated Aquifers

The potential importance of dissimilatory Fe(III) reduction in contaminant degradation in polluted aquifers is visually apparent in many instances. Prior to contamination, most shallow aquifers are aerobic and the sandy aquifer material is often tan or reddish, due to Fe(III) oxide coatings on the sand grains. However, the aquifer material in the heavily contaminated portions of many aquifers no longer has an Fe(III) oxide hue and is typically gray, because the Fe(III) oxides have been reduced to Fe(II). Unlike Fe(III), Fe(II) is relatively soluble and some of the Fe(II) is removed as groundwater flows through the zone of Fe(III) reduction. The remainder of the Fe(II) forms minerals such as vivianite, siderite, or mixed Fe(III)-Fe(II) oxides, which are generally white, gray, or greenish in color (Lovley 1991; Baedeker et al. 1992).

Figure 1 Generalized distribution of terminal electron-accepting processes within aquifers contaminated with petroleum or landfill leachate (adapted from Lovley 1991) and steady-state concentrations of dissolved hydrogen typically associated with these processes. (Lovley and Chapelle 1995)



More direct evidence for Fe(III) reduction in contaminated aquifers is the depletion of Fe(III) from the sediments, the accumulation of dissolved Fe(II) in the groundwater, and the formation of Fe(II) minerals in the aquifer sediments. For example, initial evidence for the potential role of microbial Fe(III) reduction in the degradation of aromatic hydrocarbons in a petroleum-contaminated aquifer in Bemidji, Minnesota, USA, was the progressive accumulation of dissolved Fe(II) in the groundwater for several years following the initial contamination of the site (Lovley et al. 1989). The sediments within the contaminated zone were depleted of Fe(III) in comparison with nearby uncontaminated background sediments (Lovley et al. 1989). Subsequently, more detailed studies revealed a gradation of Fe(III) oxide depletion at this site, with lowest Fe(III) oxide concentrations in sediments closest to the source of contamination and progressively higher Fe(III) concentrations as distance from the source of contamination increases (Anderson and Lovley 1999; Tuccillo et al. 1999). These relationships are shown in *Figure 2*.

As previously reviewed (Christensen et al. 1994; Lovley 1997b), elevated concentrations of dissolved Fe(II) and/or depletion of Fe(III) have been observed in other aquifers contaminated with petroleum or other organic pollutants. Hydrocarbon plumes emanat-

ing from leaking underground storage tanks can be associated with high groundwater Fe(II) concentrations (Borden et al. 1995). Likewise, substantial Fe(II) concentrations have been detected in contaminant plumes downgradient from sanitary landfills (Baedecker and Back 1979; Lyngkilde and Christensen 1992b; Bjerg et al. 1995) and near sewage effluent infiltration beds (Lee and Bennett 1998). However, high concentrations of dissolved Fe(II) in the groundwater within a given portion of an aquifer do not necessarily mean that Fe(III) reduction is ongoing at that particular site. The Fe(II) may result from dissolution of Fe(II) previously deposited at the site during an earlier active period of Fe(III) reduction, and/or dissolved Fe(II) may be coming from a more reduced site upgradient.

Direct indications of whether Fe(III) reduction is currently taking place within a given site can be obtained with groundwater measurements and/or analysis of the sediments. Measurements of dissolved H_2 concentrations in groundwater can accurately determine whether Fe(III) reduction is the predominant TEAP at a given site within contaminated aquifers (Lovley et al. 1994a), because a specific range of H_2 concentrations is associated with Fe(III) reduction as the TEAP (*Figure 1*) (Lovley and Goodwin 1988; Lovley et al. 1994a). H_2 concentrations outside of this

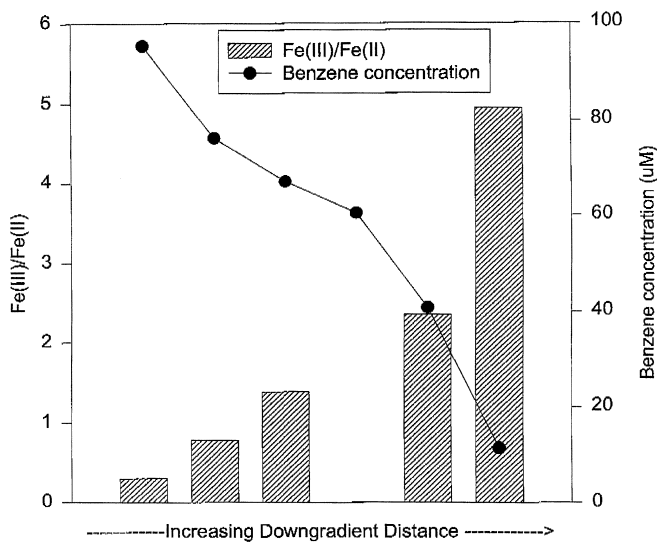
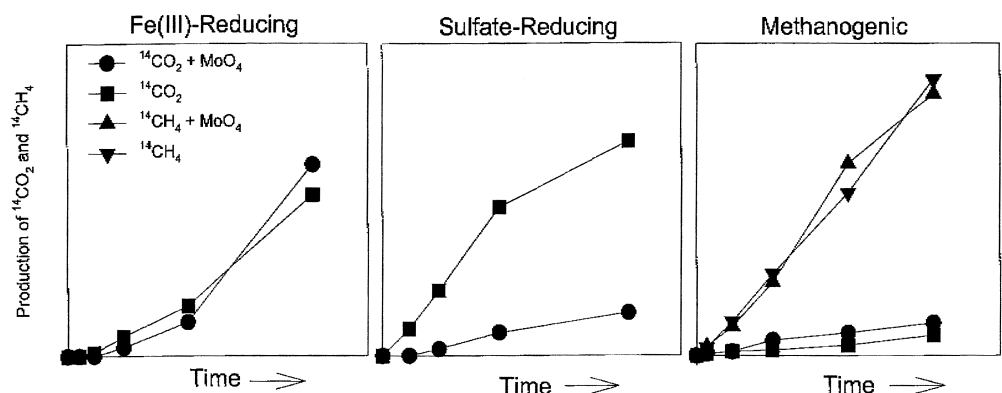


Figure 2 Groundwater benzene concentrations and Fe(III) in sediment collected downgradient from a crude oil spill near Bemidji, Minnesota, USA. Dissolved benzene was determined with a purge-and-trap gas chromatographic procedure (Anderson et al. 1999). Fe(III) and total iron soluble in 0.5 N HCl were determined with ferrozine. (Anderson et al. 1998)

range suggest that processes other than Fe(III) reduction are the predominant TEAP.

If it is possible to sample the sediments, then the potential for ongoing Fe(III) reduction in the sediments may be observed as an accumulation of Fe(II) over time in anaerobic incubations of the sediments (Lovley et al. 1989, 1994a, 1994b; Albrechtsen et al. 1995; Ludvigsen et al. 1998). This technique is often limited by the fact that high initial levels of Fe(II) and slow rates of Fe(III) reduction often require long incubation periods in order to document a detectable increase in Fe(II). Monitoring the fate of [2- 14 C]-acetate added to aquifer sediments often affords a quicker indication of whether Fe(III) reduction is the TEAP because the patterns of $^{14}\text{CO}_2$ and $^{14}\text{CH}_4$ production are different with different anaerobic TEAPs, as shown in Figure 3.

Figure 3 Examples of mineralization of [2- 14 C]-acetate under typical anaerobic conditions in sediments from a petroleum-contaminated aquifer. Production of $^{14}\text{CH}_4$ and $^{14}\text{CO}_2$, in presence or absence of 1 mM molybdate, was monitored with a gas chromatograph coupled to a gas proportional counter. (Anderson and Lovley 1999)



As previously discussed in detail (Lovley 1997b), in methanogenic systems [2- 14 C]-acetate is metabolized to a combination of $^{14}\text{CO}_2$ and $^{14}\text{CH}_4$, whereas only $^{14}\text{CO}_2$ is produced if Fe(III) reduction or sulfate reduction is the TEAP. Sediments in which sulfate reduction and Fe(III) reduction are the TEAP can be distinguished by the fact that molybdate, a specific inhibitor of sulfate reduction, inhibits oxidation of [2- 14 C]-acetate to $^{14}\text{CO}_2$ by sulfate reducers, but not Fe(III) reducers (Figure 3). Although [2- 14 C]-acetate is also oxidized to $^{14}\text{CO}_2$ when nitrate reduction or Mn(IV) reduction is the TEAP, these processes can be ruled out if the sediments are nitrate depleted and Fe(II) is present (Lovley 1997b).

Potential for Contaminant Oxidation Coupled to Fe(III) Reduction

Most of the research on the oxidation of organic contaminants coupled to Fe(III) reduction has focused on the degradation of aromatic hydrocarbons. *Geobacter metallireducens* was the first microorganism in pure culture found to be able to oxidize an aromatic hydrocarbon in the absence of oxygen (Lovley et al. 1989; Lovley and Lonergan 1990). *G. metallireducens* can oxidize toluene to carbon dioxide with Fe(III) serving as the sole electron acceptor. *G. metallireducens* also oxidizes several other monoaromatic compounds to carbon dioxide with Fe(III) as the electron acceptor, including phenol and *p*-cresol, which may be important contaminants in some subsurface environments. *G. metallireducens* conserves energy to support growth from the oxidation of aromatic compounds coupled to Fe(III) reduction. This finding suggests that Fe(III)-reducing microorganisms should be able to respond to contamination from monoaromatic compounds with an increase in numbers of aromatic degraders fueled by energy derived from the degradation of the contaminant.

Benzene is the aromatic hydrocarbon contaminant of greatest concern in most polluted aquifers. Studies with aquifer sediments from the Fe(III) reduction zone of a petroleum-contaminated aquifer (Lovley et

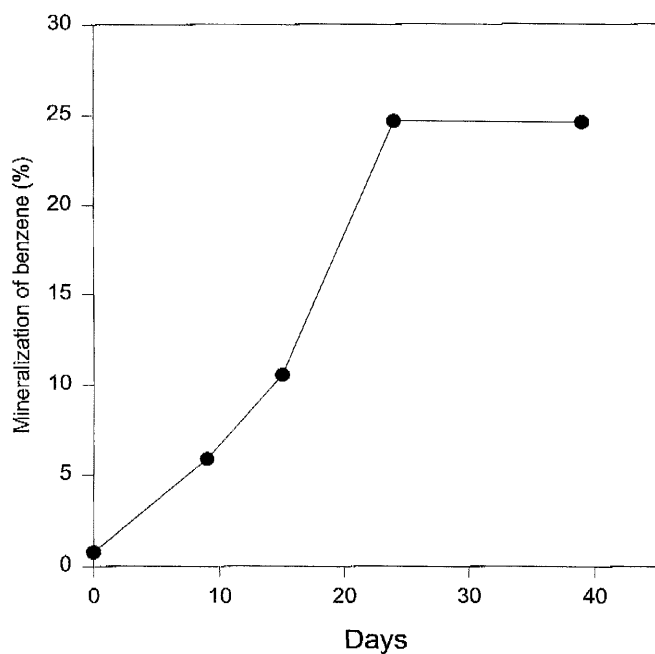


Figure 4 Anaerobic oxidation of [^{14}C]-benzene to $^{14}\text{CO}_2$ in an enrichment culture established from petroleum-contaminated aquifer sediments under Fe(III)-reducing conditions. Production of $^{14}\text{CO}_2$ was monitored as described by Anderson and Lovley (1999)

al. 1994b) demonstrate that benzene could be oxidized to carbon dioxide with Fe(III) serving as the sole electron acceptor. No pure culture of Fe(III)-reducing microorganism capable of growing with benzene as the sole electron donor has as yet been described. However, a highly purified enrichment that could oxidize benzene to carbon dioxide with Fe(III) serving as the electron acceptor was established with sediments from the Fe(III) reduction zone of a petroleum-contaminated aquifer (Rooney-Varga et al. 1999). This enrichment oxidized [^{14}C]-benzene to $^{14}\text{CO}_2$ in a medium in which Fe(III) was provided as the sole electron acceptor, as shown in Figure 4. Analysis of 16S rDNA sequences in the enrichment demonstrated that a *Geobacter* species was the predominant organism (Rooney-Varga et al. 1999).

A variety of other monoaromatic compounds are oxidized under Fe(III)-reducing conditions (Lonergan and Lovley 1991). As discussed in detail below, the rapid oxidation of naphthalene in sediments from the Fe(III) reduction zone of a petroleum-contaminated aquifer suggests Fe(III) reducers are also capable of naphthalene degradation. However, an actual stoichiometry of naphthalene degradation coupled to Fe(III) reduction has not been established in aquifer sediments.

Evidence for Oxidation of Aromatic Hydrocarbons with Reduction of Fe(III) in Situ

The fact that Fe(III)-reducing microorganisms have the potential to oxidize groundwater contaminants under laboratory conditions does not necessarily mean that these compounds are degraded in situ. As discussed in detail below, compounds that are known to be degraded with the reduction of Fe(III) may persist in some aquifers. However, there are data for significant in situ degradation of some contaminants within the Fe(III) reduction zone of some polluted aquifers. For example, groundwater concentrations of aromatic hydrocarbons decreased substantially across the Fe(III) reduction zone downgradient from two sanitary landfills (Lyngkilde and Christensen 1992a; Ruge et al. 1995). Similarly, large decreases in aromatic hydrocarbon concentrations have been observed downgradient from petroleum spill sites in areas where Fe(III) reduction is the dominant microbial process (Cozzarelli et al. 1990; Baedecker et al. 1993; Borden et al. 1995). These geochemical studies suggest that contaminant-degradation processes coupled to microbial Fe(III) reduction can contribute substantially to the overall removal of contaminants from the subsurface.

When sediments from the Fe(III) reduction zone of the Bemidji site were incubated under anaerobic conditions and amended with [^{14}C]-toluene, the microorganisms immediately began oxidizing the toluene to $^{14}\text{CO}_2$ (Anderson and Lovley 1999). The fact that the microbial community did not need an adaptation period prior to metabolizing the added toluene suggests that they were already expressing the enzymes required for toluene oxidation, indicating that they were probably degrading toluene in situ. A similar oxidation of [^{14}C]-toluene has been observed in Fe(III)-reducing sediments from other petroleum-contaminated aquifers (Lovley et al. 1994b; Anderson and Lovley 1999).

Although toluene can be anaerobically oxidized within the Fe(III) reduction zone of most, if not all, contaminated aquifers, current evidence indicates that the potential for anaerobic oxidation of benzene coupled to Fe(III) reduction is found only in some aquifers. Initial studies on benzene degradation in a petroleum-contaminated aquifer in Hanahan, South Carolina, USA, indicate that benzene persisted under anaerobic conditions, including sediments in which Fe(III) reduction was the TEAP (Lovley et al. 1994b, 1996b; Weiner and Lovley 1998a). However, if the Fe(III) chelator nitrilotriacetic acid (NTA) was added to the sediments, then benzene was degraded after a lag period of about 100 days (Lovley et al. 1994b). Stoichiometric studies demonstrate that the benzene was being oxidized to carbon dioxide with Fe(III) serving as the sole electron acceptor. The addition of other Fe(III) chelators or humic acids also stimulated benzene degradation in these sediments, in some instances with a shorter lag period than was observed when NTA was added (Lovley et al. 1996b). The need

for an adaption period prior to benzene degradation and the fact that benzene persisted in the absence of added chelators suggest that the Fe(III)-reducing microbes were not significantly degrading benzene under in situ conditions. As described below, the addition of the Fe(III) chelators or humics greatly altered the availability of Fe(III) which probably accounts for the stimulation of benzene degradation with these amendments.

In a more detailed evaluation of the in situ potential for benzene degradation in four petroleum-contaminated aquifers, evidence was found for in situ degradation of benzene in one of the aquifers (Anderson et al. 1998; Anderson and Lovley 1999). In these studies, aquifer sediments were incubated under conditions designed to represent those found in situ as closely as possible. No significant oxidation occurred of the [^{14}C]-benzene added to sediments from most of the aquifers evaluated. However, in sediments from one portion of the Fe(III) reduction zone in the Bemidji aquifer, [^{14}C]-benzene was oxidized to $^{14}\text{CO}_2$ without a lag. The lack of a requirement for an adaption period in these incubations suggests that the microorganisms were already degrading benzene in situ. Benzene degradation was fastest in the Fe(III)-reducing sediments most distal from the source of the contamination, which were also the sediments with the most Fe(III) and the anaerobic zone in which there was the most rapid decrease in groundwater concentrations of benzene (Figure 2). These findings provide further evidence that the benzene degradation observed in the laboratory incubations is indicative of the potential for in situ degradation.

The possibility of degradation of polycyclic aromatic hydrocarbons (PAHs) under Fe(III)-reducing conditions has not been investigated in as much detail as the degradation of monoaromatic hydrocarbons. One reason for this lack of attention is that, until recently, PAHs were generally regarded as resistant to degradation under strict anaerobic conditions. However, the finding that PAHs could be degraded under nitrate- and sulfate-reducing conditions in soils and sediments (Mihelcic and Luthy 1988; Coates et al. 1996a, 1997) led to investigations of whether PAHs might also be degraded under Fe(III)-reducing conditions in petroleum-contaminated aquifers (Anderson and Lovley 1999). These studies focused on the most water-soluble PAH, naphthalene, because this is the PAH likely to be the most mobile in groundwater.

Investigations of naphthalene degradation in sediments from the Fe(III) reduction zone of three petroleum-contaminated aquifers found that [^{14}C]-naphthalene was readily oxidized to $^{14}\text{CO}_2$ in sediments from the Bemidji aquifer, but not in sediments from aquifers in Hanahan, South Carolina, or Columbus, Mississippi, USA (Anderson and Lovley 1999). In a profile across the Fe(III)-reducing zone of the Bemidji aquifer, rates of naphthalene oxidation were fastest in the sediments with the highest ratio of

Fe(III) to total iron, which was also the zone of the most rapid degradation of benzene. However, naphthalene was also oxidized to carbon dioxide in sediments from other sites within the Fe(III) reduction zone that exhibited little benzene oxidation. The fact that naphthalene was oxidized without a lag period suggests that the microorganisms were adapted for naphthalene degradation in situ. However, geochemical data on naphthalene distributions in the aquifer were not available to provide supporting evidence for in situ degradation. The oxidation of naphthalene in the sediments in which Fe(III) reduction was the TEAP contrasts with a lack of anaerobic naphthalene oxidation in sediments from the methanogenic zone of the aquifer and in sediments from a nearby, uncontaminated portion of the aquifer.

There was no detectable oxidation of [^{14}C]-phenanthrene in any of the sediments evaluated, including those from the Bemidji aquifer, which did oxidize naphthalene (Anderson and Lovley 1999). The low solubility of phenanthrene might result in little transport of phenanthrene to the Fe(III) reduction zone, and this lack of exposure to phenanthrene might account for the lack of potential for phenanthrene degradation in the Fe(III) reduction zone (Anderson and Lovley 1999).

Microorganisms Involved in Aromatic Hydrocarbon Oxidation Coupled to Fe(III) Reduction

Understanding the factors that control the rate and extent of contaminant degradation coupled to Fe(III) reduction in polluted aquifers requires information on the microorganisms responsible for this process and their metabolic capabilities. The development of anaerobic conditions following the contamination of aquifers is accompanied by a general increase in the number of culturable, Fe(III)-reducing microorganisms (Anderson et al. 1998; Bekins et al. 1999). However, only a few pure cultures of Fe(III)-reducing microorganisms have been recovered from contaminated aquifers. Several of these are *Geobacter* species (Coates et al. 1996b; Synoeyenbos-West et al. in press).

A novel Fe(III)-reducing microorganism, *Geothrix fermentans*, was also recovered from sediments within the Fe(III)-reducing zone of the Hanahan aquifer discussed above (Coates et al. 1999). Similar strains were isolated from the petroleum-contaminated aquifer in Bemidji. This organism, which was enriched and isolated on media with acetate as the electron donor and Fe(III) as the electron acceptor, forms a deeply branching, novel line of descent in the *Bacteria* together with its closest relatives, *Holophaga foetida* and *Acidobacterium capsulatum* (Ludwig et al. 1997). Like the *Geobacter* species, *Geothrix fermentans* is a gram-negative strict anaerobe that grows with various simple organic acids as electron donors. It can also grow via fermentation of citrate, fumarate, and succinate in the absence of Fe(III). However, none of the

Geothrix fermentans strains evaluated to date has the ability to oxidize aromatic compounds with Fe(III).

A culturing study of the Fe(III)-reducing populations in the petroleum-contaminated aquifer in Bemidji indicates that significant differences may exist in the distribution of *Geobacter* and *Geothrix* species in polluted aquifers (Anderson et al. 1998). Acetate-oxidizing Fe(III)-reducing microorganisms were enumerated in a fresh-water medium. Acetate was chosen as the electron donor because all Fe(III) reducers that are known to oxidize aromatic compounds can also use acetate, and because growth is faster on acetate than on aromatic compounds. Most-probable-number (MPN) estimates indicate that there were similar numbers of acetate-oxidizing Fe(III) reducers in sediments from the uncontaminated portion of the aquifer and in sediments from the Fe(III) reduction zone in which benzene degradation was most active. However, when the 16S rDNA sequences in the highest positive MPN dilutions were analyzed, only a sequence closely related to *Geothrix fermentans* was recovered in the highest dilutions from the uncontaminated site, whereas sediments from the Fe(III) reduction zone contained a sequence characteristic of *Geobacter* species as well as the sequence closely related to *Geothrix fermentans*. These results suggest that *Geothrix* species were numerous at both the contaminated and uncontaminated site and that there was a specific enrichment of a *Geobacter* species in the Fe(III) reduction zone. This finding is consistent with the known metabolic capabilities of the microorganisms. *Geobacter* species are known to oxidize aromatic compounds with the reduction of Fe(III) and thus would be expected to be enriched within the zone of aromatic hydrocarbon degradation. In contrast, *Geothrix* species are not known to oxidize aromatic compounds, and thus their growth is not likely to be stimulated in the presence of aromatic contaminants. The reasons for the apparent predominance of culturable *Geothrix* over *Geobacter* species in the uncontaminated sediments are unknown.

A potential problem exists in the interpretation of results from such culturing studies. Studies that rely on culturing to describe the composition of microbial communities may yield a biased view because the media employed to recover the microorganisms may be inappropriate for the growth of microorganisms that are important in the environment (Amann et al. 1995). However, studies conducted with molecular techniques that avoid such culture biases also indicate that *Geobacter* species were enriched in the sediments in which benzene degradation was the fastest (Anderson et al. 1998; Rooney-Varga et al. 1999). When 16S rDNA sequences were amplified from sediment DNA with PCR primers designed to amplify a portion of the 16S rDNA of most *Bacteria*, sequences closely related to known *Geobacter* species were among the most abundant sequences recovered (Rooney-Varga et al. 1999). The number of *Geobacter* sequences recov-

ered from the zone of active benzene degradation was several orders of magnitude higher than in a nearby site in which Fe(III) reduction was the TEAP but benzene was not rapidly degraded (Anderson et al. 1998). Analysis of microbial lipids in the sediments also suggests that the benzene-degrading sediments had more *Geobacter* species. As noted above, an Fe(III)-reducing enrichment culture capable of anaerobically oxidizing benzene to carbon dioxide could be established with sediments from the zone of benzene degradation, and the dominant organism in the enrichment was a *Geobacter* species (Rooney-Varga et al. 1999). Attempts to establish benzene-degrading enrichments with sediments from zones in which benzene was not oxidized were unsuccessful.

These studies demonstrate that organisms closely related to known *Geobacter* species were associated with anaerobic benzene degradation in the Bemidji aquifer. This finding is consistent with the fact that *Geobacter* spp. are the only organisms available in culture that are known to oxidize aromatic compounds with the reduction of Fe(III) (Lovley et al. 1989, 1997; Lovley and Lonergan 1990; Coates et al. 1996b). The significant increase in a specific tight phylogenetic cluster of *Geobacter* spp. in the zone of benzene degradation and the finding that a *Geobacter* spp. was the dominant organism recovered in the benzene-oxidizing, Fe(III)-reducing enrichment culture from these sediments suggest that *Geobacter* species might be the organisms responsible for benzene degradation in these sediments.

Factors Controlling the Rate of Aromatic Hydrocarbon Oxidation with Fe(III) Reduction

Several factors influence the rate and extent of organic-contaminant degradation in the Fe(III)-reducing zone of polluted aquifers. The quantity and form of Fe(III) in the sediments are major determinants. Most of the Fe(III) in the subsurface can be expected to be in the form of insoluble Fe(III) oxides (Lovley 1991). Of the various types of Fe(III) oxides, poorly crystalline Fe(III) oxides are the most readily available for microbial reduction (Lovley and Phillips 1986; Lovley and Woodward 1996; Lovley et al. 1998). Crystalline Fe(III) oxides are only poorly reduced by the *Geobacter* species considered to be important in contaminant oxidation (Lovley and Phillips 1988). Therefore, the quantity of organic contaminants that can be oxidized in an aquifer may be limited by the amount of poorly crystalline Fe(III) oxide that is available. Qualitative estimates of the concentration of poorly crystalline Fe(III) oxides that might be reduced by Fe(III)-reducing microorganisms can be obtained with chemical assays (Lovley and Phillips 1987). However, a biological assay in which a suitable electron donor, such as acetate, is added to sediments incubated under anaerobic conditions may provide a more quantitative estimate of the amount of Fe(III) available for microbial reduction.

The effect of a limitation in the availability of Fe(III) on the rate of contaminant degradation is apparent from geochemical data and laboratory studies. In an aquifer contaminated with landfill leachate, a variety of organic contaminants including benzene persisted in the groundwater as it moved through the Fe(III)-depleted methanogenic zone closest to the source of contamination. However, once the groundwater entered the Fe(III)-reducing zone, these contaminants were removed from the groundwater (Lyngkilde and Christensen 1992a), due to the activity of Fe(III)-reducing microorganisms (Nielsen et al. 1995).

Sediments from the methanogenic zone of a petroleum-contaminated aquifer in Ponca City, Oklahoma, USA, converted benzene to a mixture of methane and carbon dioxide (Weiner and Lovley 1998b). When Fe(III) was added to these sediments, the rate of benzene degradation was stimulated (Weiner et al. 1998). Enhanced benzene degradation was associated with a switch in the metabolism of benzene to carbon dioxide as the sole end product. This result is consistent with the Fe(III) additions switching the TEAP from methane production to Fe(III) reduction.

Another limiting factor in the degradation of organic contaminants may be the necessity for Fe(III)-reducing microorganisms to establish direct physical contact with insoluble Fe(III) oxides in order to reduce them. When the Fe(III) chelator nitrilotriacetic acid (NTA) was added to aquifer sediments from the Fe(III) reduction zone of a petroleum-contaminated aquifer, the rate of toluene degradation was greatly accelerated (Lovley et al. 1994b). As mentioned above, after an adaption period, benzene, which otherwise persisted in these sediments, was also oxidized with the reduction of Fe(III). Other Fe(III) chelators had a similar effect (Lovley et al. 1996b).

The stimulation of toluene and benzene degradation with Fe(III) chelators was associated with a stimulation in the rate of Fe(III) reduction. Studies on the mechanism by which the added NTA stimulated aromatic hydrocarbon degradation and Fe(III) reduction demonstrate that NTA solubilized Fe(III) in the sediment (Lovley and Woodward 1996). Thus, in the presence of NTA the microorganisms no longer had to directly contact insoluble Fe(III) oxides in order to reduce Fe(III). Numerous pure culture studies have demonstrated that soluble Fe(III) is reduced faster than insoluble Fe(III), presumably because soluble Fe(III) is more accessible for microbial reduction (Lovley 1991).

In addition to synthetic chelators, the addition of humic substances (humics) also stimulated aromatic hydrocarbon degradation in Fe(III)-reducing sediments (Lovley et al. 1996b). Humics were initially added to the sediments because humics are known to chelate Fe(III), and thus it was expected that humics would stimulate aromatic hydrocarbon degradation in a manner similar to other chelators. However, the

humics stimulated benzene degradation better than any of the synthetic chelators that were evaluated, despite the fact that the humics had a much lower Fe(III) chelation capacity than any of the synthetic chelators. The addition of humics to aquifer sediments also greatly stimulated rates of Fe(III) reduction (Nevin and Lovley in press). Further investigation revealed that Fe(III)-reducing microorganisms could use humics as electron acceptors (Lovley et al. 1996a; Coates et al. 1998; Lovley et al. 1998; Scott et al. 1998). Studies of electron spin resonance indicate that quinone moieties are the primary electron-accepting group in humics for microbial reduction (Scott et al. 1998). Fe(III)-reducing microorganisms can also transfer electrons to a variety of other extracellular quinones (Lovley et al. 1996a, 1998). For example, all Fe(III)-reducing microorganisms that have been evaluated have the ability to reduce anthraquinone-2,6-disulfonate (AQDS) to anthrahydroquinone-2,6-disulfonate. AQDS appears to serve as a good model for humics, because enrichment and isolation of microorganisms from a variety of sedimentary environments with AQDS as the electron acceptor invariably yielded microorganisms that could also reduce humics (Coates et al. 1998).

Once microbially reduced, humics and other extracellular quinones can then transfer electrons to insoluble Fe(III) oxides, which reoxidizes the humics to a form that may again act as an electron acceptor for the Fe(III)-reducing microorganisms. In this manner, when Fe(III) oxides are present, even low concentrations of humics can serve as the electron acceptor for significant amounts of organic-matter oxidation. The reason that organic-matter oxidation and Fe(III) reduction are faster in the presence of humics than in their absence is probably that the electron shuttling by humics alleviates the need for Fe(III)-reducing microorganisms to contact insoluble Fe(III) oxides (Lovley et al. 1996a, 1998; Nevin and Lovley in press). In the presence of humics and Fe(III) oxides, the Fe(III)-reducing microorganisms can transfer their electrons to a soluble electron acceptor, even though insoluble Fe(III) oxides are the ultimate electron acceptor.

Humics may further aid Fe(III) reduction by permitting reduction of Fe(III) forms that Fe(III)-reducing microorganisms do not readily reduce in the absence of humics. For example, reduction of the crystalline Fe(III) oxides goethite and hematite by *G. metallireducens* was accelerated in the presence of humics, as was the reduction of structural Fe(III) in clays (Lovley et al. 1998). Humics may also provide a mechanism to transfer electrons to Fe(III) entrained within pore spaces too small for Fe(III)-reducing microorganisms to enter, as evidenced in studies in which Fe(III) oxides were incorporated into porous beads that had pores too small for *G. metallireducens* to fit through (Nevin and Lovley in press). There was no Fe(III) reduction when *G. metallireducens* was added to media that contained the beads. However,

when the humics analog AQDS, which could enter the pores of the beads, was also added to the cultures, then Fe(III) was readily reduced.

The relative importance of humics and other extracellular quinones in Fe(III) reduction in subsurface environments has yet to be quantified. It was speculated that the presence of electron-shuttling organics and/or chelators in groundwater might explain the more rapid degradation of benzene in the Fe(III) reduction zone in the Bemidji aquifer (Anderson and Lovley 1999). However, electron-shuttling compounds or Fe(III) chelators could not be detected. The finding that the presence of electron-shuttling compounds or soluble chelated Fe(III) stimulated the rate of aromatic hydrocarbon oxidation coupled to Fe(III) reduction in aquifer sediments suggests that the addition of Fe(III) chelators or electron shuttles might be used to enhance in situ degradation of organic contaminants.

A major factor that appears to control the rate of contaminant oxidation coupled to Fe(III) reduction is the composition of the microbial community. As discussed above, the zone of most rapid aromatic hydrocarbon degradation in the Bemidji aquifer was associated with a significant enrichment of *Geobacter* species. Such findings demonstrate that analysis of microbial populations in petroleum-contaminated aquifers may aid in the prediction of which sites are likely to have the potential for anaerobic oxidation of aromatic contaminants coupled to Fe(III) reduction. However, the underlying reasons for enhanced growth and activity of *Geobacter* species at this site have yet to be determined.

Degradation of Chlorinated Organics by Fe(III) Reducers

Some Fe(III)-reducing microorganisms have the ability to use chlorinated organics as terminal electron acceptors. These include a microorganism in the *Geobacteraceae* that could be grown with tetrachlorethylene (PCE) or trichloroethylene (TCE) as the sole electron acceptor (Krumholz et al. 1996). It is not clear how many of the Fe(III)-reducing *Geobacteraceae* have this capacity, because the only other organism in this family that was tested, *Geobacter metallireducens*, did not dechlorinate (Krumholz et al. 1996). *Desulfotobacterium dehalogenans*, which can reductively dechlorinate chlorophenolic compounds (Utkin et al. 1994), also has the ability to reduce Fe(III) (Lovley et al. 1998). Studies to determine whether these organisms preferentially use chlorinated compounds or Fe(III) as an electron acceptor when both are present have not yet been conducted, and the importance of Fe(III) reducers as dechlorinating organisms in contaminated subsurface environments has not been evaluated.

The Fe(II) minerals produced in subsurface environments as the result of the activity of Fe(III) reducers may abiotically donate electrons to react with chlorinated contaminants (Glass 1972; Fredrick-

son and Gorby 1996) or nitroaromatics (Heijman et al. 1993, 1995), but the extent to which this takes place in contaminated aquifers is not known.

Fe(III)-reducing microorganisms have been implicated in the anaerobic oxidation of dichlorethene (DCE) and vinyl chloride, two intermediates often observed in contaminated aquifers in which PCE and TCE are being reductively dechlorinated. Anaerobic oxidation of vinyl chloride to carbon dioxide was stimulated with the addition of Fe(III), humics, or the humics analog AQDS to aquifer sediments (Bradley and Chapelle 1996; Bradley et al. 1998a). Anaerobic oxidation of DCE was stimulated with the addition of Mn(IV) or humics, but not Fe(III) (Bradley and Chapelle 1996; Bradley et al. 1998a, 1998b). These results suggest that Fe(III)-reducing microorganisms using either Fe(III), Mn(IV), or humics as the electron acceptor may be able to destroy these contaminants in the subsurface.

Immobilization of Contaminant Metals in the Subsurface Via Dissimilatory Metal Reduction

Many dissimilatory Fe(III)-reducing microorganisms have the ability to substitute various other metals and metalloids for Fe(III) as an electron acceptor. For example, a variety of Fe(III) reducers can also transfer electrons to U(VI) (Lovley et al. 1991; Lovley 1995). U(VI), which is highly soluble and therefore mobile in most groundwaters, is reduced to U(IV), which precipitates from most natural waters. An example of reductive precipitation of uranium in the subsurface is the formation of roll-front or sandstone-type uranium deposits (Lovley and Phillips 1992b). These deposits form when aerobic groundwater containing U(VI) in solution enters an anaerobic zone, where the U(VI) is reduced to U(IV) with the formation of the ore body. Although there is no direct evidence that U(VI)-reducing microorganisms are responsible for the reductive precipitation of uranium to form such uranium deposits, the U(VI) reduction in these environments is consistent with what is known about microbial U(VI) reduction.

Stimulation of microbial U(VI) reduction in the subsurface may be a strategy to prevent the further mobility of uranium in contaminated groundwaters. In laboratory incubations, microbial U(VI) reduction effectively removed dissolved U(VI) from contaminated waters, including uranium-contaminated groundwater (Lovley and Phillips 1992a). Fe(III) abiotically oxidizes U(IV) (Nevin and Lovley in press), and thus it is necessary to reduce Fe(III) in the subsurface in order to effectively precipitate U(IV). Therefore, strategies that promote Fe(III) reduction in the subsurface, such as the addition of electron donors and/or humics, are likely to be helpful for the immobilization of U(IV). However, the possibility that humics might chelate and thus solubilize U(IV) means that other

extracellular quinones, like AQDS, that do not have a significant chelation capacity might be preferable over humics for this application.

Both laboratory and field studies in which electron donors have been added to stimulate Fe(III) reduction in subsurface sediments have found that stimulation in this manner results in increased numbers of *Geobacter* species (Synoeyenbos-West et al. in press). Other commonly studied Fe(III)-reducing microorganisms, such as *Shewanella* species, were not detected with the highly sensitive and unbiased molecular approach that was employed. A similar predominance of *Geobacter* was observed when Fe(III) reduction was stimulated with the addition of humics or the humics analog AQDS.

In addition to U(VI), Fe(III)-reducing microorganisms can transfer electrons to a variety of other contaminant metals and metalloids (Lovley et al. 1993, 1997; Lovley 1995). For example, technetium, a contaminant associated with processing of nuclear materials, is soluble in the oxidized form Tc(VII). However, Fe(III)-reducing microorganisms can reduce Tc(VII) to Tc(IV), which is insoluble (Lloyd and Macaskie 1996). Fe(III) reducers can also reduce soluble and toxic Cr(VI) to less soluble, less toxic Cr(III) (Lovley 1993, 1995). Soluble Co(III)-EDTA can be reduced to Co(II), which is more likely to adsorb onto aquifer solids (Caccavo et al. 1994). Furthermore, some Fe(III)-reducing microorganisms can reduce soluble selenate to insoluble elemental selenium (Laverman et al. 1995).

In contrast to the extensive studies that have been conducted on degradation of aromatic hydrocarbons coupled to Fe(III) reduction, few investigations have been made into reduction of contaminant metals in subsurface environments. However, given the need for innovative strategies to remediate metal-contaminated subsurface environments, further study of the potential for microbial metal reduction to remediate subsurface environments is warranted.

Conclusions

Studies during the last decade have demonstrated that dissimilatory Fe(III)-reducing microorganisms have the potential to naturally aid in the remediation of subsurface environments contaminated with organic and/or metal pollutants. Furthermore, the activity of Fe(III)-reducing microorganisms can be stimulated in order to accelerate remediation. However, predictive modeling of natural attenuation or the development of engineered remediation strategies involving Fe(III)-reducing microorganisms requires more information on the microorganisms involved in Fe(III) reduction in the subsurface and the factors controlling the rate and extent of their metabolism. Preliminary studies indicate that members of the genus *Geobacter* are the predominant Fe(III)-reducing microorganisms in the

zone of active organic-contaminant degradation or when Fe(III) reduction is artificially stimulated. Similar studies need to be conducted in a wider diversity of aquifers to determine if this is a general phenomenon. If so, then current initiatives designed to better evaluate the physiology of *Geobacter* species through the sequencing of the *Geobacter* genome and biochemical studies may provide more insight into the mechanisms of contaminant remediation carried out by Fe(III)-reducing microorganisms.

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