Humic Substances as a Mediator for Microbially Catalyzed Metal Reduction

Huminstoffe als Vermittler bei der mikrobiell katalysierten Metallreduktion

**D. R. Lovley*, J. L. Fraga*,
E. L. Blunt-Harris*, L. A. Hayes*,
E. J. P. Phillips**, and J. D. Coates*****

Keywords: Humic Substances, Anaerobic, Iron Oxides, Quinones, Bioremediation

Summary: The potential for humic substances to serve as a terminal electron acceptor in microbial respiration and to function as an electron shuttle between Fe(III)-reducing microorganisms and insoluble Fe(III) oxides was investigated. The Fe(III)-reducing microorganism *Geobacter metallireducens* conserved energy to support growth from electron transport to humics as evidenced by continued oxidation of acetate to carbon dioxide after as many as nine transfers in a medium with acetate as the electron donor and soil humic acids as the electron acceptor. Growth of *G. metallireducens* with poorly crystalline Fe(III) oxide as the electron acceptor was greatly stimulated by the addition of as little as 100 μM of the humics analog, anthraquinone-2,6-disulfonate. Other quinones investigated, including lawsone, menadione, and anthraquinone-2-sulfonate, also stimulated Fe(III) oxide reduction. A wide phylogenetic diversity of microorganisms capable of Fe(III) reduction were also able to transfer electrons to humics. Microorganisms which can not reduce Fe(III) could not reduce humics. Humics stimulated the reduction of structural Fe(III) in clay and the crystalline Fe(III) forms, goethite and hematite. These results demonstrate that electron shuttling between Fe(III)-reducing microorganisms and Fe(III) via humics not only accelerates the microbial reduction of poorly crystalline Fe(III) oxide, but also can facilitate the reduction of Fe(III) forms that are not typically reduced by microorganisms in the absence of humics. Addition of humic substances to enhance electron shuttling between Fe(III)-reducing microorganisms and Fe(III) oxides may be a useful strategy to stimulate the remediation of soils and sediments contaminated with organic or metal pollutants.

Schlagwörter: Huminstoffe, anaerob, Eisenoxide, Chinone, biologische Sanierung

Zusammenfassung: Es wurde untersucht, inwieweit Huminstoffe als terminale Elektronenakzeptoren bei der mikrobiellen Atmung und als Vermittler bei der Elektronenübertragung zwischen Fe(III)-reduzierenden Mikroorganismen und unlöslichen Fe(III)-oxiden fungieren können. Das Fe(III)-reduzierende Bakterium *Geobacter metallireducens* gewinnt Energie zum Wachstum aus der Elektronenübertragung auf Huminstoffe. Das wurde offensichtlich, als nach 9 aufeinanderfolgenden Transfers des Bakteriums auf frisches Medium mit Acetat als Elektronendonor und Boden-Huminstoff als Elektronenakzeptor seine Fähigkeit zur Oxidation von Acetat zu CO_2 erhalten blieb. Das Wachstum von *G. metallireducens* mit niedrigkristallinem Fe(III)-oxid als Elektronenakzeptor konnte durch den Zusatz des Huminstoff-Analogen Anthrachinon-2,6-disulfonat bereits in Konzentrationen von 100 µmol/L deutlich stimuliert werden. Auch weitere untersuchte Chinone wie z. B. Lawson (2-Hydroxy-1,4-naphthochinon), Menadion (2-Methyl-1,4-naphthochinon) und Anthrachinon-2-sulfonat stimulierten die Fe(III)-oxid-Reduktion. Eine große Anzahl phylogenetisch unterschiedlicher Mikroorganismen, die zur Fe(III)-Reduktion befähigt sind, zeigten gleichzeitig die Fähigkeit zum Elektronentransfer auf Huminstoffe. Zur Fe(III)-Reduktion nicht befähigte Mikroorganismen konnten auch Huminstoffe nicht reduzieren. Durch Huminstoffe konnte die Reduktion von Fe(III) stimuliert werden, das in die Struktur von Tonmineralen und in kristalline Formen des Fe(III)-oxids, Goethit und Hämatit, eingebaut ist. Diese Ergebnisse zeigen, daß durch die vermittelnde Funktion der Huminstoffe bei der Elektronenübertragung zwischen Fe(III)-reduzierenden Mikroorganismen und Fe(III) nicht nur die mikrobielle Reduktion von niedrigkristallinem Fe(III)-oxid beschleunigt wird, sondern auch die Reduktion von solchen Formen des Fe(III) erleichtert wird, welche im allgemeinen in Abwesenheit von Huminstoffen durch Fe(III)-reduzierende Mikroorganismen nicht reduziert werden. Die Zugabe von Huminstoffen zur Verbesserung der Elektronenübertragung zwischen Fe(III)-reduzierenden Mikroorganismen und Fe(III)-oxiden könnte eine nützliche Strategie zur Stimulierung der Sanierung von mit organischen oder metallischen Kontaminanten verunreinigten Böden und Sedimenten sein.

---

* Derek. R. Lovley, Jocelyn L. Fraga, Elizabeth L. Blunt-Harris, Lory A. Hayes, Department of Microbiology, University of Massachusetts, Amherst, MA 01003, USA

** Elizabeth J. P. Phillips, Water Resources Division, Mail Stop 430, U. S. Geological Survey, Reston, VA 22092, USA

*** Dr. John D. Coates, Department of Microbiology, Southern Illinois University, Carbondale, IL 62901, USA

Correspondence to D. R. Lovley
E-mail: dlovley@microbio.umass.edu

0323–4320/98/0305–0152 $ 17.50+.50/0

1 Introduction

A recent study demonstrated that two microorganisms, *Geobacter metallireducens* and *Shewanella alga*, can use humic substances as electron acceptors to support anaerobic oxidation of organic compounds and/or H_2 [1]. If such metabolism is widespread among microorganisms this could have important implications for microbial metabolism in anaerobic environments such as flooded soils, aquatic sediments, and aquifers. It is generally considered that the most important electron acceptors for anaerobic metabolism in such environments are nitrate, Mn(IV), Fe(III), sulfate, and carbon dioxide [2–4]. However, given the abundance of humics in some soils and sediments, electron transfer to humics might also be important if a diversity of microorganisms are capable of this form of respiration.

Microbial electron transfer to humics might even be significant in environments in which there are low concentrations of humics, if these environments also contain Fe(III). Previous studies have demonstrated that the electrons that microorganisms transfer to humics can be quantitatively transferred from humics to Fe(III) [1]. The transfer of electrons from humics to Fe(III) is an abiotic process which can take place in the absence of microorganisms. Once oxidized by Fe(III), humics may again accept electrons from humics-reducing microorganisms (Fig. 1). Thus, in Fe(III)-containing environments, even small quantities of humics could be important in electron transfer as they could be recycled as electron acceptors numerous times [1].

To date, transfer of electrons from humics to Fe(III) has only been evaluated with poorly crystalline Fe(III) oxides. However, this Fe(III) form is just one of a variety of Fe(III) forms that could potentially serve as electron acceptors for microbial metabolism in soils and sediments. Furthermore, other metals, including important contaminants such as U(VI) and Cr(VI), that can also be microbially reduced [5–7] might be able to accept electrons from microbially reduced humics. Therefore, it is of interest to determine if humics might also stimulate the reduction of alternative Fe(III) forms and other metals.

The electron-accepting group(s) on humic substances are yet to be determined. Quinones are often used as humics analogs in abiotic electron transfer studies because it is considered that quinones are important electron-accepting groups on humics [8–10]. In previous studies with the humics analog, anthraquinone-2,6,-disulfonate (AQDS), both *G. metallireducens* and *S. alga* carried out a two electron transfer to AQDS, producing anthrahydroquinone-2,6-disulfonate (AHDS). AHDS could abiotically transfer electrons to Fe(III) with the regeneration of AQDS [1]. Both *G. metallireducens* and *S. alga* could grow in anaerobic medium with AQDS serving as the sole electron acceptor. This demonstrated that microorganisms could conserve energy to support growth from electron transport to AQDS. However, it is yet to be determined if extracellular quinones other than AQDS can also serve as electron acceptors for microbial metabolism.

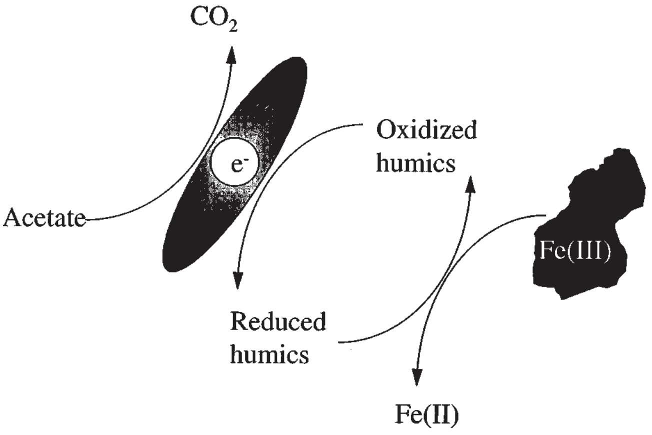

Fig. 1: Model for humics serving as an electron shuttle between Fe(III)-reducing microorganisms and insoluble Fe(III) oxides.

Modell für die Vermittlerfunktion von Huminstoffen bei der Elektronenübertragung zwischen Fe(III)-reduzierenden Mikroorganismen und unlöslichen Fe(III)-oxiden.

The purpose of the studies summarized here was to investigate the diversity of microorganisms that might be involved in humics reduction in soils and sediments; to determine if quinones other than AQDS could serve as electron acceptors for these organisms; and to determine if metals other than poorly crystalline Fe(III) oxides could accept electrons from microbially reduced humics.

2 Methods

All organisms came from our laboratory culture collection with the following exceptions (source in parentheses): *Desulfuromonas acetexigens* (DSM culture collection 1397), *Aeromonas hydrophila*, *Pseudomonas denitrificans*, and *Paracoccus denitrificans* (ATCC culture collection 7966, 13867, and 17741 respectively), *Azospirillum lipoferum* strain 4T (gift from Jacques Balandreau, Lyon-1 University), *Desulfomonile tiedjei* (gift from Joseph Suflita, University of Oklahoma), *Desulfitobacterium dehalogenans* (gift from Jürgen Wiegel, University of Georgia), and *Wolinella succinogenes* (gift from Steve Goodwin, University of Massachusetts).

Organisms were grown under strict anaerobic conditions using previously described procedures [11]. Studies on the growth of *G. metallireducens* with soil humic acids as the electron acceptor were carried out in the previously described medium [11] with the exception that Fe(III) was omitted and soil humic acids obtained from the International Humic Substances Society were added at 2 g/L. Acetate was provided as the electron donor at a concentration of 0.5 mM and this was supplemented with 1 µCi of [2-^{14}C]-acetate (44.5 mCi/mmol). Studies on the growth of *G. metallireducens* on Fe(III) oxide in the presence of AQDS were carried out in the same medium but with 100 mM poorly crystalline Fe(III) oxide [12] provided as the electron acceptor and 20 mM acetate provided as the electron donor. AQDS was added at 100 µM final concentration.

For studies with cell suspensions, the cells were grown anaerobically with electron donor and acceptor concentrations that are known to support their anaerobic growth. Studies on reduction of Fe(III), AQDS, and humics by cell suspensions were carried out as previously described [1, 13]. Poorly crystalline Fe(III) oxide, goethite, and hematite were prepared as previously described [12]. Ferruginous smectite (SWa-1) was obtained from the Clay Mineral Society. AQDS and soil humic acids were added at 100 µM and 2 g/L in the studies with these Fe(III) forms. For comparison of the ability of various quinones to serve as electron shuttles, the quinones were added at a final concentration of 500 µM.

Previously described methods were used for measuring the production of $^{14}CO_2$ [14], Fe(II) [15], AHDS [1], reduced humics [1], and cells [11].

3 Results and Discussion

3.1 Effect of Humics on Anaerobic Microbial Growth

Although previous studies [1] demonstrated that humics could serve as an electron acceptor for acetate oxidation by *G. metallireducens,* those studies did not determine if this electron transfer yielded energy to support growth. This was because the humics interfered with standard methods for monitoring growth such as protein determinations and direct cell counts [1]. In order to further assess the potential for growth of *G. metallireducens* on humics, *G. metallireducens* was inoculated into anaerobic medium with acetate as the sole electron donor and soil humic acids as the potential electron acceptor. There was a steady production of $^{14}CO_2$ from [2-^{14}C]-acetate in the humics-containing medium (Fig. 2a), but no production of $^{14}CO_2$ if humics were not provided as an electron acceptor. $^{14}CO_2$ continued when a 10 % inoculum of this culture was transferred into fresh acetate-humics medium. After nine such transfers, $^{14}CO_2$ production continued at rates comparable to that in the first transfer (Fig. 2b). This result could only be observed if *G. metallireducens* was capable of replicating in the acetate-humics media. In combination with the previous finding [1] that *S. alga* is capable of growing with humics serving as the sole electron acceptor, these results clearly demonstrate that humics respiration can yield energy to support cell growth.

As outlined in the Introduction, in many environments growth with humics as an electron acceptor is likely to be associated with a recycling of microbially reduced humics to the oxidized form by the humics reacting with Fe(III) (Fig. 1). In these instances Fe(III) functions as the terminal acceptor for the electrons from the oxidation of organics and/or H_2. In order to determine if humics and related compounds could stimulate the growth of Fe(III) reducers on Fe(III) oxide, the effect of the humics analog, AQDS, on growth with Fe(III) oxide was evaluated (Fig. 3). The humics analog was used rather than humics, because of the difficulties in monitoring cell numbers in the presence of humics, as noted above. Addition of as little as 100 µM AQDS greatly stimulated growth of *G. metallireducens* on Fe(III) oxide (Fig. 3). Stimulation of growth was accompanied by increased rates of Fe(III) reduction. These results suggest that quinone-containing compounds that can function as electron shuttles between Fe(III)-reducing microorganisms and insoluble Fe(III) oxides may have a long-term impact on the growth and activity of Fe(III) reducers in anaerobic Fe(III)-containing environments.

3.2 Diversity of Quinones Capable of Stimulating Fe(III) Reduction

The stimulation of Fe(III) reduction in the presence of AQDS clearly demonstrates that this particular quinone can serve as an electron shuttle between Fe(III)-reducing microorganisms and Fe(III) oxides. In order to determine if quinones in general could serve as an electron shuttle, rates of Fe(III) reduction by *G. metallireducens* were determined in the presence of a variety of quinones. The three additional quinones evaluated, menadione (1,2,3,4-tetrahydro-2-methyl-1,4-dioxo-2-naphthalene sulfonic acid), lawsone (2-hydroxy-1,4-naphthoquinone), and anthraquinone-2-sulfonate (AQ2S) all stimulated Fe(III) reduction by *G. metallireducens* (Fig. 4). These results suggest that a wide variety of quinone-containing compounds in soils and sediments might be involved in electron shuttling between Fe(III) reducers and Fe(III) oxides.

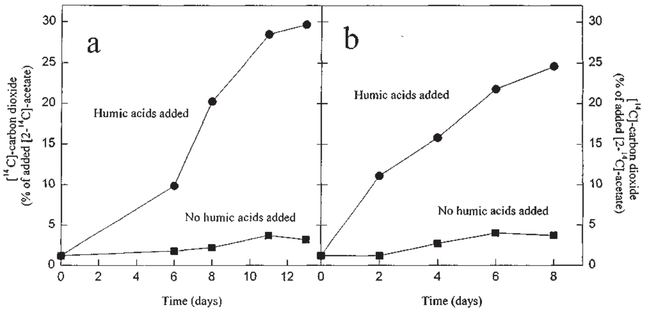

Fig. 2: Production of $^{14}CO_2$ from [2-^{14}C]-acetate when *Geobacter metallireducens* was inoculated into medium with acetate as the electron donor and humic acids as the potential electron acceptor. Panel a are the results from the first transfer into the medium. Panel b are the results after 9 successive transfers in this medium.

$^{14}CO_2$-Produktion aus [2-^{14}C]-Acetat in einem Medium mit Acetat als Elektronendonor und Huminsäuren als möglichen Elekronenakzeptoren nach Beimpfung mit *Geobacter metallireducens* nach dem ersten Transfer auf das Medium (Teilbild a) und nach 9 aufeinanderfolgenden Transfers (Teilbild b).

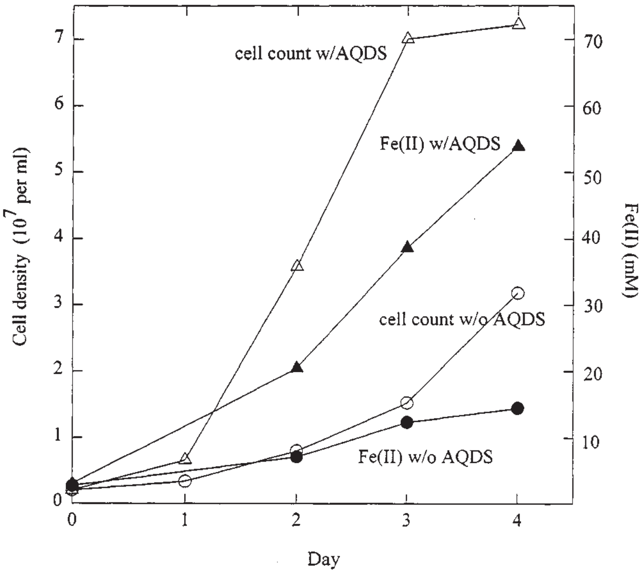

Fig. 3: Growth of *Geobacter metallireducens* in medium with acetate as the electron donor and poorly crystalline Fe(III) oxide as the electron acceptor in the presence (w) and absence (w/o) of 100 µM AQDS.

Wachstum von *Geobacter metallireducens* in einem Medium mit Acetat als Elektronendonor und niedrigkristallinem Fe(III)-oxid als Elektronenakzeptor in Gegenwart von Anthrachinon-2,6-disulfonat (AQDS) in einer Konzentration von 100 µmol/L (w) und in Abwesenheit von AQDS (w/o).

3.3 Diversity of Microorganisms Capable of Humics Reduction

Screening a diversity of microorganisms indicated that microorganisms which have the capacity to reduce Fe(III) also have the ability to reduce AQDS, whereas microorganisms that do not actively reduce Fe(III) oxides have little or no AQDS-reducing capability (Table 1). For example, of the organisms in the delta *Proteobacteria* that were examined, the Fe(III)-reducing *Geobacter* and *Desulfuromonas* species could reduce AQDS and those that were evaluated for humics reduction

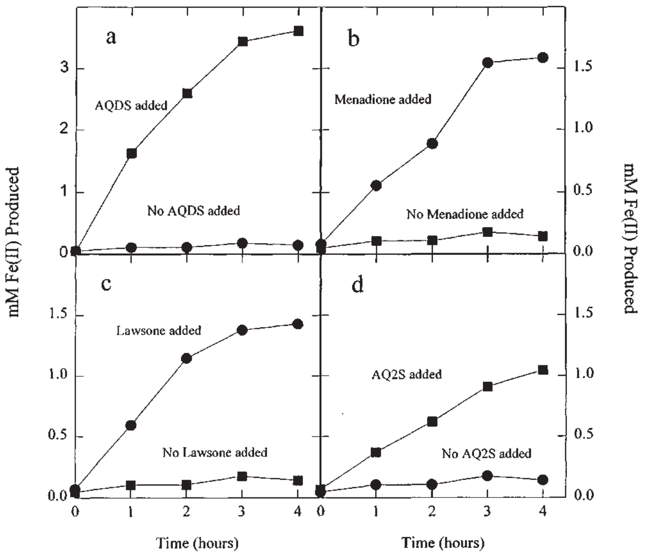

Fig. 4: Fe(II) production from poorly crystalline Fe(III) oxide in cell suspensions of *Geobacter metallireducens* in the presence and absence of 500 µM of various quinones.

Fe(II)-Produktion aus niedrigkristallinem Fe(III)-oxid in Zellsuspensionen von *Geobacter metallireducens* in Gegenwart (Konzentration: 500 µmol/L) und in Abwesenheit der Chinone Anthrachinon-2,6-disulfonat (AQDS), 2-Methyl-1,4-naphthochinon (Menadion), 2-Hydroxy-1,4-naphthochinon (Lawson) und Anthrachinon-2-sulfonat (AQS2).

Table 1: Reduction of Fe(III), anthraquinone-2,6-disulfonate (AQDS), and humics by various microorganisms.

Reduktion von Fe(III), Anthrachinon-2,6-disulfonat (AQDS) und Huminstoffen durch verschiedene Mikroorganismen.

Organism	Reduction of: Fe(III)	AQDS	Humics
Delta Proteobacteria			
Geobacter metallireducens	+[1]	+	+
Geobacter sulfurreducens	+	+	+
Geobacter humireducens	+	+	+
Desulfuromonas acetexigens	+	+	ND[2]
Desulfomonile tiedjei	–[3]	–	ND
Gamma Proteobacteria			
Shewanella alga	+	+	+
Shewanella putrefaciens	+	+	ND
Shewanella sacchrophila	+	+	ND
Aeromonas hydrophila	+	+	ND
Pseudomonas denitrificans	–	–	–
Gamma Proteobacteria			
Geospirillum barnseii	+	+	ND
Wolinella succinogenes	+	+	+
Alpha Proteobacteria			
Paracoccus denitrificans	–	–	–
Azospirillum lipoferum 4T	ND	–	ND
Gram Positive			
Desulfitobacterium dehalogenans	+	+	ND
Undefined Novel Bacterial Lineage			
Geothrix fermentens	+	+	ND

[1] +: reduction/Reduktion
[2] ND: not determined/nicht bestimmt
[3] –: no reduction/keine Reduktion

could also reduce the humics. However, the closely related *Desulfomonile teidjei* which is incapable of Fe(III) reduction also could not reduce AQDS.

All of the AQDS-reducing microorganisms that were tested for the ability to reduce humics were found to reduce humics (Table 1) which further demonstrates that AQDS reduction is a good predictor of the capacity for humics reduction. Similar results have recently been obtained in studies in which AQDS-reducing microorganisms were isolated from a variety of sediments and wetland soils [16]. In each instance the AQDS reducers that were isolated not only had the ability to reduce humics, but also had the ability to reduce Fe(III).

It is notable that *A. lipoferum* strain 4T was not able to reduce AQDS. Previous studies [17] had suggested that *A. lipoferum* strain 4T might be able to donate electrons to the free radicals or quinones generated when phenolic compounds were oxidized with oxygen or N_2O. If so, it might be expected that this organism might also be able to transfer electrons to AQDS. However, not only did *A. lipoferum* not reduce AQDS in cell suspensions, but no AQDS was reduced during growth under anaerobic conditions. Close inspection of previous studies [17] with *A. lipoferum* strain 4T indicates that the data supporting reduction of oxidized phenolics is only circumstantial.

3.4 Humics Electron Shuttling to Alternative Fe(III) Forms

Although poorly crystalline Fe(III) oxides are one of the most abundant potential electron acceptors in many soils and sediments [5, 18], there are other forms of Fe(III) that could potentially serve as electron acceptors for microbial metabolism. For example, a significant portion of the Fe(III) in soils and sediments may be in crystalline forms such as goethite and hematite which Fe(III)-reducing microorganisms may only poorly reduce, if at all [18, 19]. When goethite or hematite were incubated in cell suspensions of *G. metallireducens* there was little Fe(III) reduction (Fig. 5). However, if soil humic acids or AQDS was added then both crystalline forms of Fe(III) were significantly reduced. These results suggest that microbial reduction of humics with subsequent electron shuttling to crystalline Fe(III) may permit transfer of electrons onto crystalline Fe(III) forms which are not readily reduced in the absence of humics.

It has previously been suggested that structural Fe(III) in clays might also serve as electron acceptors for microbial respi-

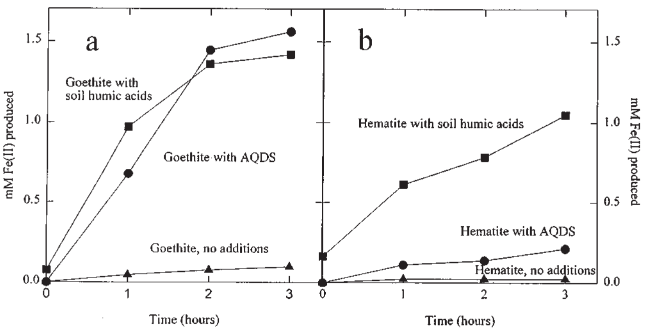

Fig. 5: Reduction of Fe(III) in goethite and hematite in cell suspensions of *Geobacter metallireducens* in the presence and absence of humic acids or AQDS.

Reduktion von Fe(III) in Goethit und Hämatit in Zellsuspensionen von *Geobacter metallireducens* in Gegenwart und in Abwesenheit von Huminsäuren oder Anthrachinon-2,6-disulfonat (AQDS).

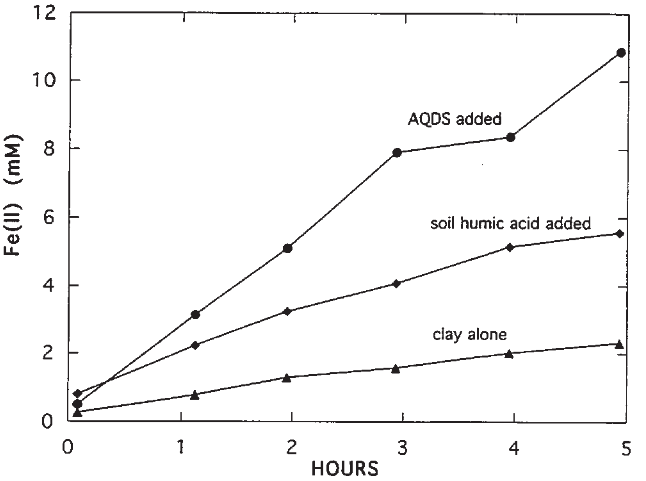

Fig. 6: Reduction of Fe(III) in ferruginous smectite in the presence and absence of humic acid or AQDS.

Reduktion von Fe(III) in eisenhaltigem Bentonit in Gegenwart und in Abwesenheit von Huminsäure oder Anthrachinon-2,6-disulfonat (AQDS).

ration [20, 21]. In order to determine if the presence of humics might stimulate the reduction of Fe(III) in clays, studies were conducted with the same type of ferruginous smectite used in previous studies of structural Fe(III) reduction. Both humic acids and AQDS significantly stimulated reduction of Fe(III) in the smectite by *G. metallireducens* (Fig. 6). Thus, the availability of this potentially important Fe(III) form is also increased by organics which can shuttle electrons from Fe(III)-reducing microorganisms.

One mechanism by which humics could stimulate reduction of structural Fe(III) in clay is that microbially reduced humics might be able to access Fe(III) within the clay particles that microorganisms can not access. Microorganisms are relatively large in comparison with clay and thus can only be expected to directly contact Fe(III) on the surface of the clay.

4 Conclusions

These studies demonstrate that a wide diversity of Fe(III)-reducing microorganisms also have the capacity to reduce humics and that microbial reduction of humics and related extracellular, quinone-containing compounds may greatly increase the rate and extent of Fe(III) reduction in soils and sediments. These findings impact on the understanding of how microorganisms transfer electrons to metals in natural pristine environments and have implications for potential remediation strategies for contaminant metals and organics in sedimentary environments.

Previous models for microbial Fe(III) reduction have suggested that microorganisms need to establish direct physical contact with insoluble Fe(III) oxides in order to reduce them [5, 18]. However, the finding that all bacteria that are known to be capable of reducing insoluble Fe(III) oxides can also reduce humics suggests that in environments that contain dissolved humics or related quinone-containing organics, Fe(III) reducers need not contact Fe(III) in order to reduce it. This may be true even if the concentrations of dissolved humics or related compounds are quite low as only catalytic amounts are required to act as electron shuttles between the Fe(III)-reducing microorganisms and the Fe(III) oxides. Not only can electron shuttling by humics increase the rate that microorganisms can reduce Fe(III) oxides that they would be able to slowly reduce in the absence of humics, but it could also make it possible for electrons to be transferred to Fe(III) that is not readily reducible and/or physically accessible to Fe(III)-reducing microorganisms in the absence of humics.

Electron shuttling by humics might be utilized in several types of environmental restoration strategies. For example, contamination of groundwater with benzene as the result of terrestrial oil spills is a major environmental problem. Most heavily contaminated aquifers are depleted of oxygen, the preferred electron acceptor for benzene degradation, but often contain significant quantities of Fe(III) [14, 18, 22, 23]. Benzene can be oxidized to carbon dioxide with the reduction of Fe(III), but this process is generally slow because the Fe(III) in the aquifer sediments is not readily available to the Fe(III)-reducing microorganisms [14]. The addition of humic substances to petroleum-contaminated aquifer sediments greatly stimulated anaerobic degradation of benzene [24], presumably as the result of humics serving as an electron shuttle to Fe(III) oxides. The addition of humics or humics analogs to anaerobic contaminated aquifers might stimulate the anaerobic degradation of organic contaminants other than benzene in a similar manner.

Fe(III) and Mn(IV) oxides strongly absorb a wide variety of toxic trace metals [18]. Thus, these trace metals can be difficult to extract from contaminated soils until the Fe(III) and Mn(IV) oxides have been removed. Microbial reduction of Fe(III) and Mn(IV) may be one mechanism for releasing such metals. This process might be enhanced with the addition of humics or related compounds, especially if tight pore spaces make significant quantities of the Fe(III) and Mn(IV) oxides inaccessible to Fe(III)- and Mn(IV)-reducing microorganisms (Fig. 7). Microbially reduced humics may be able to donate electrons to some contaminant metals such as Hg(II) [25] as well as U(VI) and Cr(VI) (unpublished data). Reduction of these metals may help remove them from the environment and/or convert them to less toxic forms [6]. Although these metal contaminants are generally soluble and thus easily accessible by microorganisms, humics might serve as an important electron shuttle to such metals if they are occluded in tight pore spaces that microorganisms can not enter (Fig. 7).

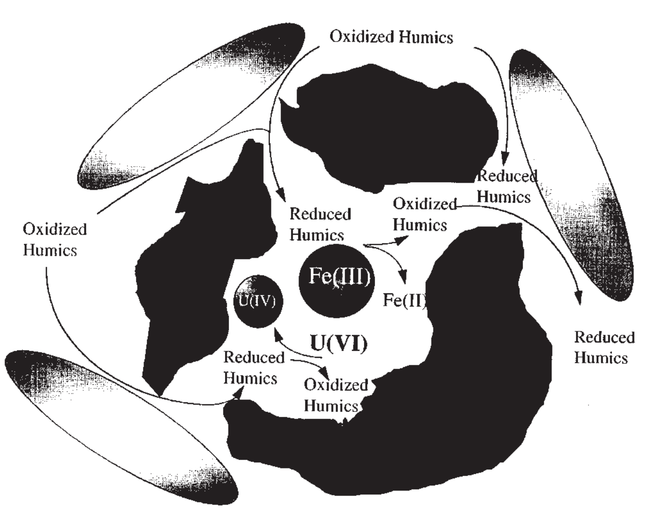

Fig. 7: Model of how humics might aid in the reduction of metals occluded in tight pore spaces in soils and sediments.

Modell, wie Huminstoffe als Hilfsstoffe bei der mikrobiellen Reduktion von Metallen in engen Poren von Böden und Sedimenten fungieren könnten.

From the present studies it is not possible to readily predict the relative importance of humics electron shuttling to the reduction of Fe(III) and other metals in various soils and sediments. However, it is now clear that the possibility of a two step process for microbial Fe(III) reduction, with humics serving as an electron shuttle between the Fe(III)-reducing microorganisms and the Fe(III) needs to be considered in future studies of microbial Fe(III) reduction.

Acknowledgement

We thank Jeanne Fontneau for technical assistance. This research was supported with grant N0014-96-1-0382 from the Office of Naval Research.

References

[1] *Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, E. J. P., Woodward, J. C.:* Humic substances as electron acceptors for microbial respiration. Nature *382*, 445-448 (1996).

[2] *Reeburgh, W. S.:* Rates of biogeochemical processes in anoxic sediments. Ann. Rev. Earth Planet. Sci. *11*, 269–298 (1983).

[3] *Ponnamperuma, F. N.:* The chemistry of submerged soils. Adv. Agron. *24*, 29–96 (1972).

[4] *Lovley, D. R., Chapelle, F. H.:* Deep subsurface microbial processes. Rev. Geophys. *33*, 365–381 (1995).

[5] *Lovley, D. R.:* Microbial reduction of iron, manganese, and other metals. Adv. Agron. *54*, 175–231 (1995).

[6] *Lovley, D. R.:* Bioremediation of organic and metal contaminants with dissimilatory metal reduction. J. Industr. Microbiol. *14*, 85–93 (1995).

[7] *Lovley, D. R., Coates, J. D., Saffarini, D. A., Lonergan, D. J.:* Dissimilatory Iron Reduction. In: *Winkelman G., Carrano C. J.* (Ed.): Iron and Related Transition Metals in Microbial Metabolism. Harwood Academic Publishers, Switzerland, 1997, pp. 187–215 .

[8] *Dunnivant, F. M., Schwarzenbach, R. P., Macalady, D. L.:* Reduction of substituted nitrobenzenes in aqueous solutions containing natural organic matter. Environ. Sci. Technol. *26*, 2133–2142 (1992).

[9] *Curtis, C. P., Reinhard, M.:* Reductive dehalogenation of hexachloroethane, carbon tetrachloride, and bromoform by anthrahydroquinone disulfonate and humic acid. Environ. Sci. Technol. *28*, 2393–2401 (1994).

[10] *Tratnyek, P. G., Macalady, D. L.:* Abiotic reduction of nitro aromatic pesticides in anaerobic laboratory systems. J. Agric. Food Chem. *37*, 248–254 (1989).

[11] *Lovley, D. R., Phillips, E. J. P.:* Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. Appl. Environ. Microbiol. *54*, 1472–1480 (1988).

[12] *Lovley, D. R., Phillips, E. J. P.:* Organic matter mineralization with reduction of ferric iron in anaerobic sediments. Appl. Environ. Microbiol. *51*, 683–689 (1986).

[13] *Lovley, D. R., Roden, E. E., Phillips, E. J. P., Woodward, J.C.:* Enzymatic iron and uranium reduction by sulfate-reducing bacteria. Mar. Geol. *113*, 41–53 (1993).

[14] *Lovley, D. R., Woodward, J. C., Chapelle, F. H.:* Stimulated anoxic biodegradation of aromatic hydrocarbons using Fe(III) ligands. Nature *370*, 128–131 (1994).

[15] *Lovley, D. R., Phillips, E. J. P.:* Availability of ferric iron for microbial reduction in bottom sediments of the freshwater tidal Potomac River. Appl. Environ. Microbiol. *52*, 751–757 (1986).

[16] *Coates, J. D., Ellis, D. J., Roden, R., Gaw, K., Blunt-Harris, E. L., Lovley, D. R.:* Recovery of humics-reducing bacteria from a diversity of sedimentary environments. Appl. Environ. Microbiol. *64*, 1504–1509 (1998).

[17] *Barkovskii, A., Bouillant, M.-L., Monrozier, L. J., Blandreau, J.: Azospirillum* strains use phenolic compounds as intermediates for electron transfer under oxygen-limiting conditions. Microb. Ecol. *29*, 99–114 (1995).

[18] *Lovley, D. R.:* Dissimilatory Fe(III) and Mn(IV) reduction. Microbiol. Rev. *55*, 259–287 (1991).

[19] *Phillips, E., Lovley, D. R., Roden, E. E.:* Composition of non-microbially reducible Fe(III) in aquatic sediments. Appl. Environ. Microbiol. *59*, 2727–2729 (1993).

[20] *Kostka, J. E., Stucki, J. W., Nealson, K. H., Wu, J.:* Reduction of structural Fe(III) in smectite by a pure culture of *Shewanella putrefaciens* strain MR-1. Clays Clay Miner. *44*, 522–529 (1996).

[21] *Stucki, J. W., Komadel, P., Wilkinson, H. T.:* Microbial reduction of structural iron(III) in smectites. Soil Sci. Soc. Am. J. *51*, 1663–1665 (1987).

[22] *Lovley, D. R., Baedecker, M. J., Lonergan, D. J., Cozzarelli, I. M., Phillips, E. J. P., Siegel, D. I.:* Oxidation of aromatic contaminants coupled to microbial iron reduction. Nature *339*, 297–299 (1989).

[23] *Lovley, D. R.:* Potential for anaerobic bioremediation of BTEX in petroleum-contaminated aquifers. J. Ind. Microbiol. *18*, 75–81 (1997).

[24] *Lovley, D. R., Woodward, J. C., Chapelle, F. H.:* Rapid anaerobic benzene oxidation with a variety of chelated Fe(III) forms. Appl. Environ. Microbiol. *62*, 288–291 (1996).

[25] *Alberts, J. J., Schindler, J. E., Miller, R. W., Nutter, D. E.:* Elemental mercury evolution mediated by humic acid. Science *184*, 895–897 (1974).

received 6 October 1997
accepted 8 January 1998