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Toni A. M. Bridge, D. Barrie Johnson
[a] School of Biological Sciences University of Wales Bangor, Gwynedd, United Kingdom
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Reductive Dissolution of Ferric Iron Minerals by *Acidiphilium* SJH

TONI A. M. BRIDGE
D. BARRIE JOHNSON

School of Biological Sciences
University of Wales
Bangor, Gwynedd, United Kingdom

The acidophilic heterotrophic eubacterium Acidiphilium *SJH was shown to catalyze the reductive dissolution of a wide range of ferric iron–containing minerals (akageneite, goethite, jarosite, natrojarosite, and amorphous ferric hydroxide) and of the mixed ferrous/ferric mineral magnetite. The specific rates of dissolution varied with the structural stabilities of the minerals, such that amorphous ferric hydroxide was the most rapid and jarosite and akageneite were the slowest of the minerals tested. The reductive dissolution of both amorphous ferric hydroxide and magnetite was faster in pH 2.0 than in higher pH (2.8–3.0) cultures, even though* Acidiphilium *SJH has a pH optimum close to pH 3. Contact between bacteria and ferric mineral was not necessary for reductive dissolution to occur. Adding EDTA or diethylenetriamine pentaacetic acid to bacterial cultures accelerated the solubilization of goethite and amorphous ferric hydroxide. Although cell-free spent media and heat-killed* Acidiphilium *SJH also appeared to enhance mineral dissolution (indicated by formation of soluble* Fe^{3+}*), this was far less extensive than that in active bacterial cultures, and no iron reduction was observed in the absence of viable cells. Experimental results suggested that* Acidiphilium *SJH accelerates the reductive dissolution of ferric iron minerals by way of an indirect mechanism, in which bacterial reduction of soluble ferric iron causes a shift in equilibrium between solid phase (mineral) and soluble ferric iron, thereby causing further dissolution of the mineral.*

Keywords *Acidiphilium*, acidophilic bacteria, iron minerals, iron-reducing bacteria, magnetite

In most environments, ferric iron is highly insoluble and occurs in a variety of amorphous and crystalline mineral forms (Nealson 1983; Johnson 1995). The major exception to this are extremely acidic environments (pH < 2.5) where appreciable concentrations of soluble ferric iron may occur, such as in streams draining coal and metal mines, and some geothermal areas. The redox potential ($E°$) of the ferrous/ferric couple also varies with pH; for example, at pH 2 the $E°$ is +0.77 V, while at pH 7 (in bicarbonate-containing environments) the $E°$ is +~0.2 V (Ehrenreich and Widdel 1994). In thermodynamic terms, ferric iron is therefore an attractive electron sink to oxygen, particularly in environments that are extremely acidic.

The ability of neutrophilic bacteria to reduce ferric iron to ferrous has been known for some time. Some fermentative iron-reducing bacteria use ferric iron as a minor (though

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Address correspondence to Dr. D. Barrie Johnson, School of Biological Sciences, University of Wales, Bangor, Gwynedd, LL57 2UW, UK. E-mail: d.b.johnson@bangor.ac.uk

variable) electron sink during fermentation; for example, *Bacillus* spp., *Vibrio* sp., and the strict anaerobe *Pelobacter carbinolicus* (Ottow and Glathe 1971; Jones et al. 1984; Lovley et al. 1995). Iron-reducing bacteria that can couple the oxidation of hydrogen or organic substrates exclusively to the reduction of ferric iron have also been described, including the facultative anaerobe *Shewanella putrefaciens* (Nealson and Myers 1992) and the strict anaerobe *Geobacter metallireducens* (Lovley et al. 1993). Neutrophilic iron-reducing bacteria have also been shown to cause the reductive dissolution of some ferric iron minerals. *Shewanella* spp. can reduce both amorphous and crystalline solid-phase ferric iron minerals (Arnold et al. 1988; Roden and Zachara 1996), and *S. putrefaciens* can also cause the reductive dissolution of the mixed-valence mineral magnetite (Kosta and Nealson 1995). Cummings et al. (1999) recently reported the isolation and preliminary characterization of a novel iron-reducing bacterium (*Ferribacterium limneticum*) isolated from a freshwater lake sediment contaminated with mineral tailings. This prokaryote is also obligately anaerobic.

Microbiological studies of extremely acidic environments have tended to focus predominantly on the microbial dissimilatory oxidation of iron and sulfur, principally because of the central importance of these reactions in the bioprocessing of metal ores (Rawlings 1997). However, there have been several reports of iron reduction brought about by acidophilic microorganisms. Brock and Gustafson (1976) reported that the mesophilic chemolithotrophic bacteria *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*, and the thermophilic archaean *Sulfolobus acidocaldarius*, could all couple the oxidation of elemental sulfur to the reduction of ferric iron; however, whether or not this was an energy-transducing reaction that could support the growth of these acidophiles was not confirmed. Later work by Pronk et al. (1992) confirmed that *T. ferrooxidans* was capable of growth under anaerobic conditions, using ferric iron as sole electron sink. The ability to reduce ferric iron to ferrous has also been reported for moderately thermophilic iron-oxidizing bacteria; *Sulfobacillus* spp. and *Acidimicrobium ferrooxidans* can couple the oxidation of organic substrates to the reduction of ferric iron under strictly anoxic conditions, reaction that supports the growth of these eubacteria in the absence of oxygen (Bridge and Johnson 1998).

Other microorganisms found in extremely acidic, metal-rich environments include mesophilic, heterotrophic bacteria, for example, *Acidiphilium*, *Acidocella*, and *Acidomonas* spp. (Johnson 1998). Johnson and McGinness (1991) demonstrated that the ability to reduce soluble ferric iron to ferrous was widespread among heterotrophic acidophiles, although the propensity for iron reduction varied greatly between the different isolates examined. One of the most efficient of the iron-reducing heterotrophs tested was an *Acidiphilium*-like isolate coded SJH. Ferric iron can act as sole electron sink for this isolate, supporting its growth in strictly anoxic conditions, although that growth rate was much less than the rate observed under aerobic conditions. Reduction of ferric iron and growth of *Acidiphilium* SJH have been found to be closely correlated ($r = 0.99$) under microaerobic conditions (Bridge 1995). Preliminary experiments by Johnson and McGinness (1991) indicated that this acidophile could reduce some solid-phase ferric iron compounds, and Johnson and Bridge (1997) later showed that *Acidiphilium* SJH could bring about the reductive dissolution of "ochre" deposits from streams impacted by acid mine drainage.

This paper describes the reductive dissolution of a variety of ferric iron–containing minerals by *Acidiphilium* SJH and reports the differential rates of reductive mineral dissolution brought about by this acidophile under laboratory conditions. In addition, experimental work aimed at elucidating the mechanism of mineral dissolution by *Acidiphilium* SJH is described.

Methods

Bacterial Cultures

Acidiphilium SJH was originally isolated from a subterranean, acidic (pH 2.3), metal-rich stream located within a derelict pyrite mine (Cae Coch) in north Wales (Johnson et al. 1979). Phylogenetically (from base sequence analysis of its 16S rRNA gene) the isolate is most closely related (among sequenced bacteria) to *Acidiphilium cryptum*, with which it shares 97.8% sequence similarity (F. F. Roberto, Idaho National Engineering and Environmental Laboratory, Idaho Falls; personal communication); *Acidiphilium* SJH also has many physiological characteristics in common with other *Acidiphilium* spp. (Johnson, unpubl. data). Unless specified otherwise, *Acidiphilium* SJH was grown in liquid medium containing the following concentrations (g/L) of components: $(NH_4)_2SO_4$, 0.15; $MgSO_4$, 0.50; KCl, 0.05; KH_2PO_4, 0.05; $Ca(NO_3)_2$, 0.01; yeast extract, 0.2. Glycerol (at 10 mM) was added as carbon and energy source, and the medium was adjusted to pH 2.0 with 25% (v/v) sulfuric acid. For all experiments (except those in which specific iron reduction rates were measured) *Acidiphilium* SJH was grown in 250-ml conical flasks containing 100 ml of medium and was then incubated, unshaken, at 30°C.

Preparation of Ferric Iron Minerals

Samples of amorphous ferric hydroxide, goethite (α-FeOOH) and akageneite (β-FeOOH) were prepared by alkali precipitation from a ferric chloride solution, using the methods described by Atkinson et al. (1977) and Lovley and Phillips (1986). The basic iron sulfate minerals jarosite $[KFe_2(SO_4)_2(OH)_6]$ and natrojarosite $[NaFe_2(SO_4)_2(OH)_6]$ were produced biologically by using the type strain of *T. ferrooxidans* (Lazaroff et al. 1982). Hematite was obtained from a geological museum sample (provided by Dr. David Jenkins, University of Wales, Bangor) and ground to a fine powder by using a Heiko vibrating mill. Finally, the mixed ferrous/ferric iron mineral, magnetite, was purchased from Merck UK Ltd. Each compound was dried, ground, analyzed by X-ray diffraction analysis and compared with published data (Brindley and Brown 1984) to confirm its identity.

Determination of Specific Rates of Ferric Iron Reduction and Reductive Dissolution

Specific rates of reduction of soluble ferric iron and reductive dissolution of ferric iron–containing minerals were determined by using cell suspensions of cultures grown to early stationary phase. *Acidiphilium* SJH was grown in liquid medium containing 25 mM ferric sulfate; the cells were harvested by centrifugation, washed in iron-free liquid medium, and re-centrifuged. The bacteria were then redispersed in a small volume of liquid medium, and the protein concentration of the cell suspension was measured. The washed cells were finally put into 30-ml universal bottles and iron-free liquid medium was added. Ferric sulfate was added to some of the bottles, to give a final concentration of 25 mM soluble ferric iron. Ferric iron–containing minerals were added to other bottles to give a final concentration of 0.5% (w/v) in each case. At the start of the experiment each universal bottle contained 20 ml of cell suspension with a protein concentration of 0.5 mg/ml. The bacteria/mineral suspensions were incubated at 30°C, and soluble ferrous iron concentrations were measured at regular intervals.

Effect of Cell Contact on the Dissolution of Amorphous Ferric Hydroxide by Acidiphilium *SJH*

Acidiphilium SJH was grown in batch cultures in which the bacteria and amorphous ferric hydroxide were physically separated. Visking dialysis tubing (24 mm diameter; Medicell Ltd., London, UK) was boiled for 5 min in a solution containing 0.5% (w/v) ethylenediamine tetraacetic acid (EDTA) and 2% (w/v) sodium bicarbonate, which was followed by a second 5-min boiling in distilled water to remove metal ions and ensure a uniform pore size. The tube was then filled with 10 ml of a 2% suspension of amorphous ferric hydroxide in liquid medium, the ends were tied, and the sac was placed in 250-ml conical flasks containing 90 ml of liquid medium. Culture media were heat-sterilized, inoculated with *Acidiphilium* SJH, and incubated without shaking at 30°C for as long as 12 days. Changes in both total iron and ferrous iron concentrations were recorded.

Effect of Culture pH on the Dissolution of Amorphous Ferric Hydroxide and Magnetite

Acidiphilium SJH was grown in batch culture in liquid medium containing 0.2% (w/v) amorphous ferric hydroxide, adjusted to either pH 2.0 or 2.8 with sulfuric acid. Changes in culture pH and concentrations of total iron and ferrous iron were measured over a period of 12 days. *Acidiphilium* SJH was also grown in batch cultures of liquid media containing 0.1% (w/v) magnetite, with pH adjusted initially to either pH 2.0 or 3.0. Cultures were incubated for as long as 22 days, and the ferrous iron and total iron concentrations were measured at regular intervals.

Dissolution of Amorphous Ferric Hydroxide by Killed Bacterial Biomass and Cell-Free Spent Medium

Cultures of *Acidiphilium* SJH were grown in liquid medium containing 25 mM soluble ferric sulfate for 6 days, by which time most of the iron had been reduced to the ferrous form. One culture was then sterilized by heat treatment (120°C for 20 min), whereas the cells were removed from the other by membrane filtration (0.2-μm pore size, cellulose nitrate filters; Millipore Ltd., Bedford, MA). Both treated cultures were then supplemented with 0.2% (w/v) amorphous ferric hydroxide and incubated for another 10 days at 30°C, during which the concentrations of total iron and ferrous iron were monitored. A separate sterile control of fresh liquid medium supplemented with 25 mM ferrous sulfate was also incubated.

Effect of Chelating Agents on the Reductive Dissolution of Ferric Iron Minerals by Acidiphilium *SJH*

The effects of the metal chelators EDTA and diethylenetriamine pentaacetic acid (DPTA) on the reductive dissolution of goethite by *Acidiphilium* SJH was assessed in unshaken batch cultures. Liquid medium containing 0.2% (w/v) goethite and either EDTA (final concentration 5 mM) or DPTA (final concentration 2.5 mM) were inoculated with acid-washed (10 mM sulfuric acid) *Acidiphilium* SJH grown to early stationary phase. Control cultures containing no metal-chelating agents were also prepared. All cultures were adjusted to pH 2.0 with sulfuric acid and were incubated at 30°C for as long as 48 days. Concentrations of total soluble iron were measured at regular intervals during this time. The effect of 5 mM EDTA on the specific rate of reductive dissolution of amorphous ferric hydroxide was also determined, using the method described above. Cell suspensions of stationary

phase cells were washed, concentrated, resuspended in liquid media, and amended with 0.5% (w/v) amorphous ferric hydroxide and 5 mM EDTA. The bacterial suspensions were then incubated at 30°C and the total soluble iron concentrations were measured at regular intervals.

Analytical Methods

Ferrous iron concentrations were measured with the ferrozine reagent (Lovley and Phillips 1987); total iron concentrations were measured by atomic absorption spectrophotometry. Protein concentrations were determined by the Folin–Lowry method (Lowry et al. 1951), using bovine serum albumin as standard. The pH values of cultures and media were recorded with a pHase Rapid Renew glass electrode (Merck Ltd.) fitted to an EIL pH meter. X-ray diffraction analysis of finely ground mineral samples was carried out with equipment supplied by Philips Ltd., scanning over the range 2–50° at a rate of 1° 2θ/min.

Results

Specific Rates of Reduction of Soluble and Solid-Phase Ferric Iron by Acidiphilium *SJH*

Acidiphilium SJH was able to catalyze the reductive dissolution of all of the ferric iron–containing minerals tested (except hematite), although the rates of solubilization varied considerably (Figure 1). Under the experimental conditions used, ferrous iron production was linear over a period of at least 5.25 h, and because the concentrations of protein in each of the bacteria/mineral mixtures were identical and did not change during this time (data not shown), the line graphs in Figure 1 give a direct comparison of the specific rates of iron reduction with the different minerals. The reductive dissolution of amorphous ferric hydroxide was much more rapid than all of the crystalline iron minerals and, as anticipated, the fastest rates of iron reduction were found with soluble ferric iron rather than solid-phase ferric minerals. Calculations of the specific rates of iron reduction by *Acidiphilium* SJH are given in Table 1.

TABLE 1 Specific rates of reduction of soluble ferric iron and ferric iron–containing minerals by *Acidiphilium* SJH (mean ± SD)

Iron form	Specific rate of Fe^{3+} reduction ($\mu g \cdot min^{-1} \cdot mg^{-1}$ protein)
Soluble Fe^{3+}	1.46 ± 0.46
Amorphous $Fe(OH)_3$	0.76 ± 0.10
Magnetite	0.33 ± 0.04
Goethite	0.17 ± 0.05
Natrojarosite	0.17 ± 0.01
Akaganeite	0.08 ± 0.05
Jarosite	0.06 ± 0.02
Hematite	no significant dissolution

$n = 4$, except for natrojarosite, where $n = 2$.

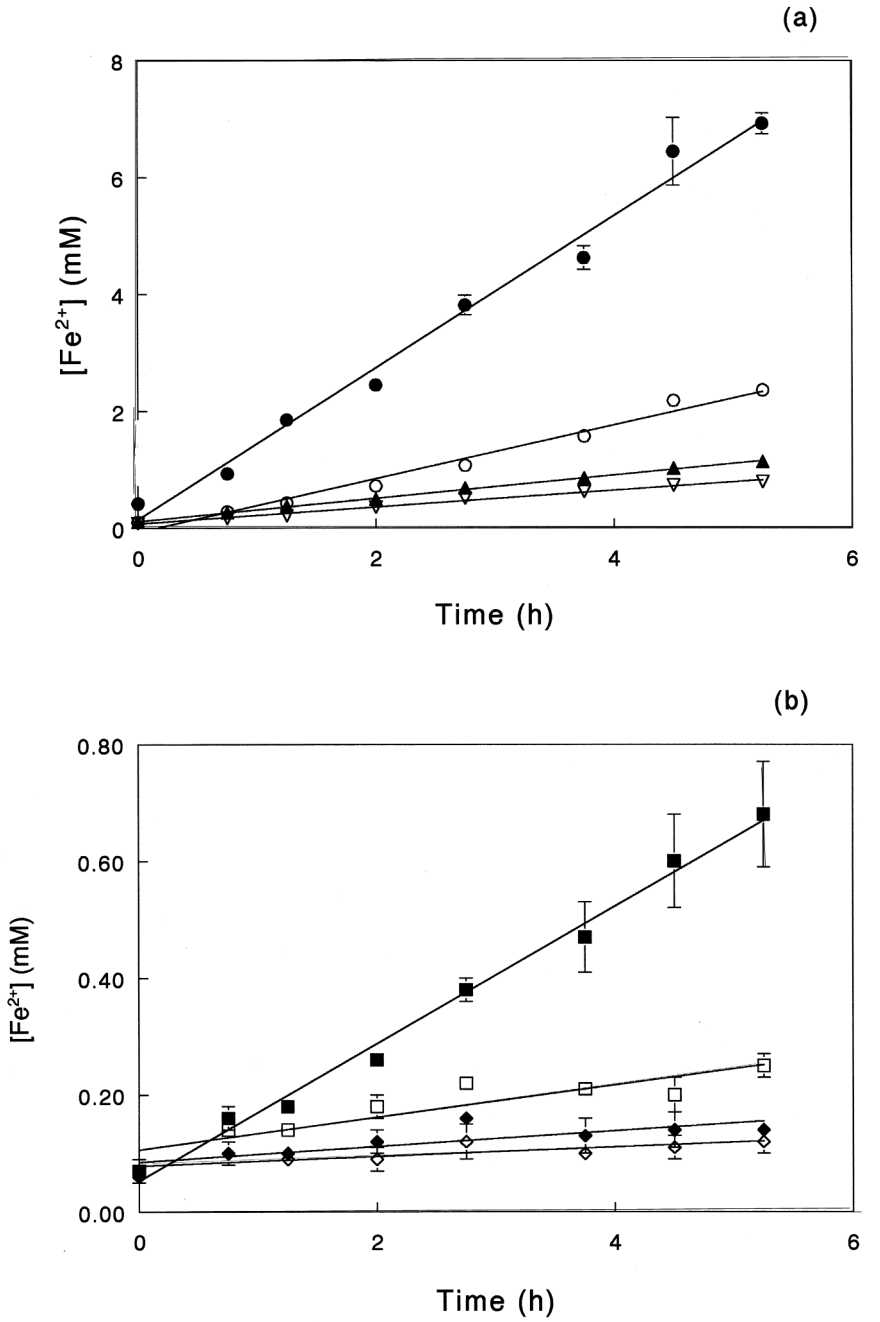

FIGURE 1 Reduction of (a) soluble ferric iron (●), amorphous ferric hydroxide (○), magnetite (▲), goethite (▽), (b) natrojarosite (■), jarosite (□), akaganeite (◆) and hematite (◇) by *Acidiphilium* SJH. Cells were grown to early stationary phase, and harvested suspensions (containing identical protein concentrations) were incubated in the presence of 25 mM ferric iron or 0.5% (w/v) iron mineral at 30°C. Glycerol (10 mM) was added as electron donor, and changes in ferrous iron concentrations measured with the ferrozine reagent. Each point represents the mean of four cultures (two in the case of natrojarosite), and error bars show standard deviations.

Mechanism of Reductive Dissolution of Amorphous Ferric Hydroxide by Acidiphilium *SJH*

Several experiments were carried out to elucidate the mechanism whereby *Acidiphilium* SJH accelerated the reductive dissolution of ferric iron minerals. Because the fastest rate of reduction was found with amorphous ferric hydroxide (Figure 1), we used this compound for the majority of subsequent experiments. Preventing direct contact between the bacteria and solid-phase ferric hydroxide (by containing the latter in a dialysis sac) caused the rate of mineral dissolution to be lower than in those where contact was allowed, although solubilization of ferric hydroxide was still considerably greater in the former cultures than in sterile controls (Figure 2). The data in Figure 2 show changes in total soluble iron concentrations with time in inoculated and control cultures. Measurements of ferrous iron concentrations (not shown) showed that all of the soluble iron in inoculated cultures was present in the ferrous form, whereas in the uninoculated cultures it was exclusively ferric iron.

A key parameter in determining rates of dissolution of amorphous and crystalline ferric iron minerals by *Acidiphilium* SJH was culture pH (Figure 3). In cultures adjusted initially to pH 2.8, no dissolution of ferric hydroxide was observed in sterile cultures, and (at this pH) the addition of *Acidiphilium* SJH resulted in an additional 2 mM soluble iron during 12 days of culture incubation (Figure 3a). In contrast, although the mean concentrations of soluble iron in bacteria-free cultures set at pH 2.0 were 1.5 mM greater after 12 days than at the

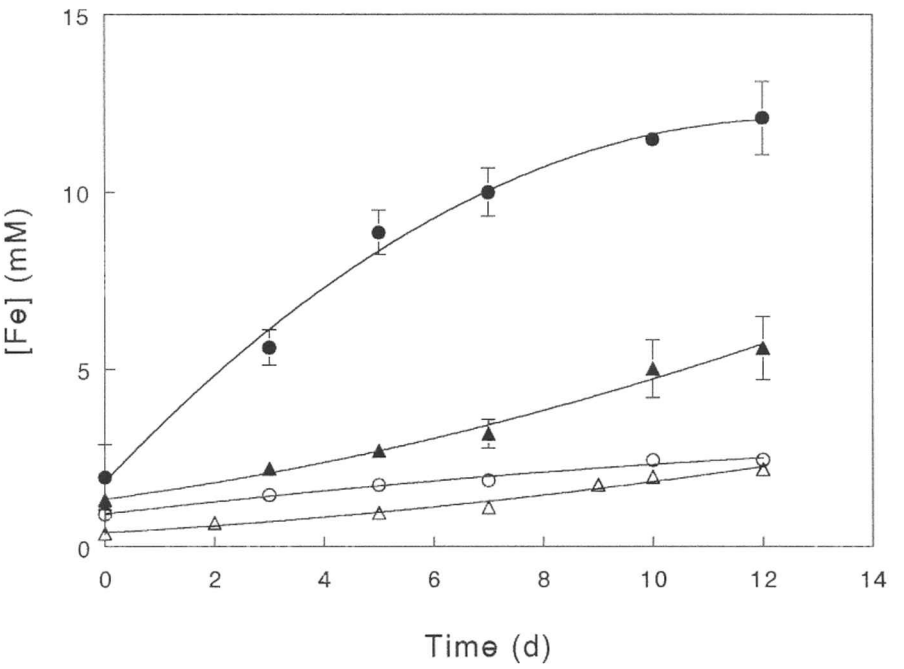

FIGURE 2 The effect of bacterial/mineral contact on the dissolution of amorphous ferric hydroxide by batch cultures of *Acidiphilium* SJH. Bacteria were grown in unshaken flasks containing ferric hydroxide that was held in dialysis sacs ("no contact") or free in the culture suspension ("contact"). (●) + *Acidiphilium* SJH/contact; (▲) + *Acidiphilium* SJH/no contact; hollow symbols (○, △) are from corresponding sterile control cultures. Each point represents the mean value of triplicate cultures, and error bars are standard deviations.

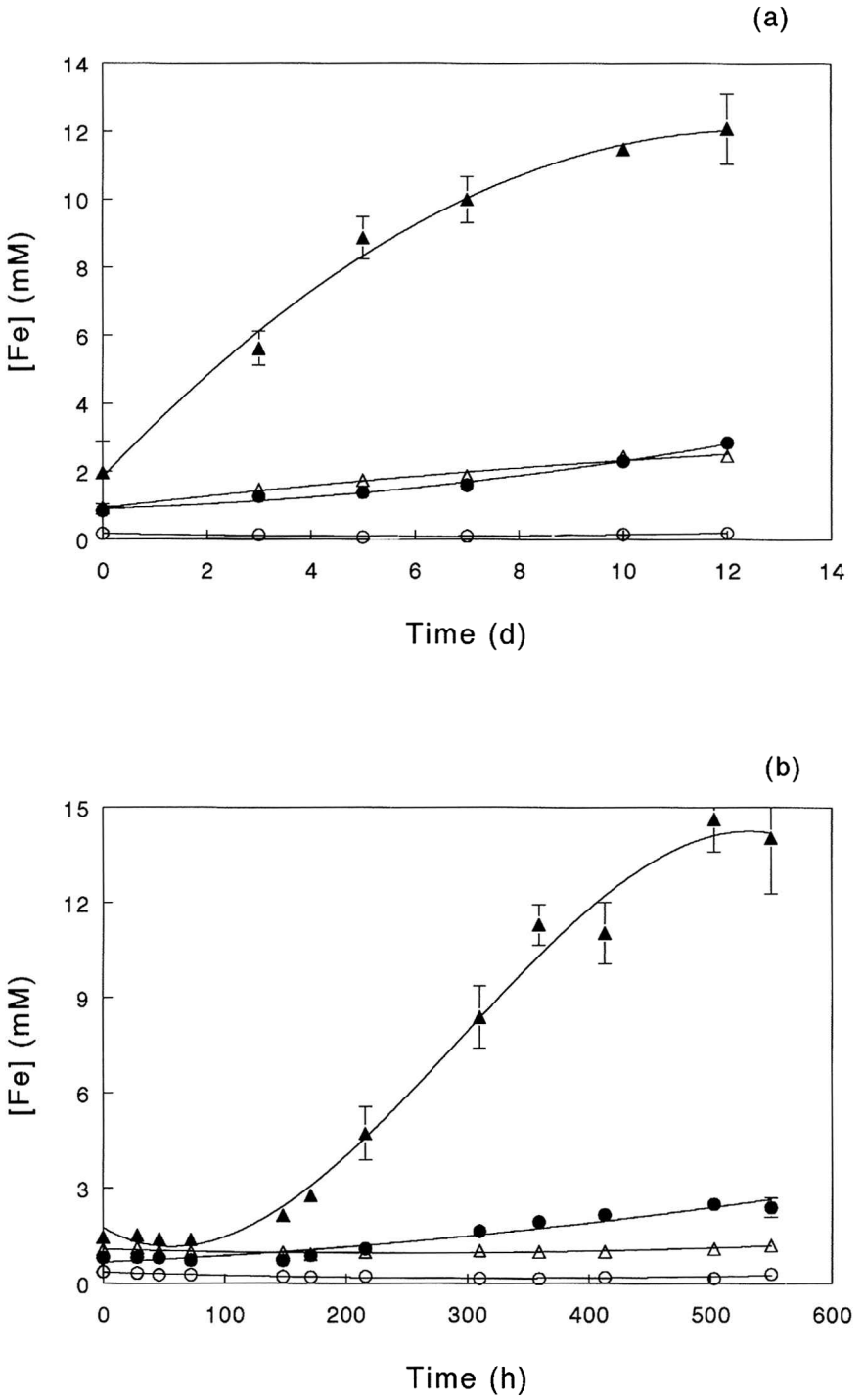

FIGURE 3 The effect of medium pH on the dissolution of (a) ferric hydroxide and (b) magnetite by batch cultures of *Acidiphilium* SJH. (▲) Cultures adjusted initially to pH 2.0; (●) cultures adjusted initially to pH 2.8 [$Fe(OH)_3$] or 3.0 (magnetite); hollow symbols (○, △) are from corresponding sterile control cultures. Each point represents the mean value of triplicate cultures, and error bars are standard deviations.

start of the experiment, those in cultures inoculated with *Acidiphilium* SJH had increased by ~10 mM. As with the dialysis sac experiment, soluble iron in inoculated cultures was exclusively ferrous iron, whereas that in the sterile cultures was in exclusively ferric iron. Similar trends were observed in the case of magnetite (Figure 3b), for which the reductive dissolution after 23 days of incubation, determined from differences in concentrations of soluble iron, was approximately eightfold greater in cultures adjusted initially to pH 2.0 than in those set at 3.0. Because of the poor buffering capacity of the medium used, the reductive dissolution of ferric iron minerals often resulted in increased pH values; for example, those in the magnetite-containing cultures set originally at pH 2.0 rose to a mean value of pH 2.7 over the 22-day incubation period.

Whether the reductive dissolution of ferric iron minerals by *Acidiphilium* SJH was enzymatic, catalyzed by the production of extracellular products (which may again be enzymes), or both, was tested by comparing the solubilization of ferric hydroxide by heat-killed cultures and cell-free spent media with a control containing basal salts and an equivalent amount of ferrous sulfate. The results, shown in Figure 4, indicate that mineral dissolution was enhanced by both heat-killed cultures and cell-free medium, and in both cases rates were similar to those observed when using live batch cultures of *Acidiphilium* SJH (Figure 2). The data in Figure 4 have been adjusted to account for the initial soluble iron present in each case. In contrast to cultures containing viable cells, the soluble iron produced from the dissolution of ferric hydroxide by sterile spent *Acidiphilium* SJH medium was exclusively ferric iron; there was no evidence of any reduction of iron in these cultures.

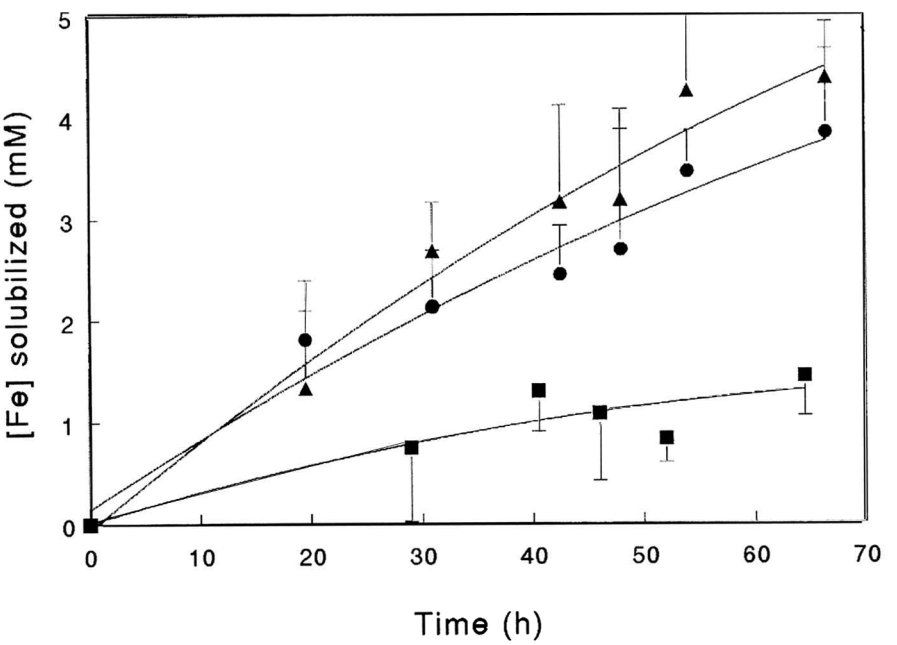

FIGURE 4 Dissolution of amorphous ferric hydroxide in filtered (0.2-μm pores; ●) and autoclaved (120°C, 15 min; ▲) spent media of *Acidiphilium* SJH, grown to stationary phase. Control flasks (■) contained sterile liquid medium to which 23 mM Fe(II) was added. The graph shows the increase in total soluble iron, as measured by atomic absorption spectrophotometry, with time. Each point represents the mean value from duplicate flasks, and error bars are standard deviations.

Effect of Chelating Agents on Mineral Dissolution by Acidiphilium *SJH*

The comparative effects of 5 mM EDTA and 2.5 mM DPTA on the dissolution of goethite by *Acidiphilium* SJH are shown in Figure 5. After 48 days of incubation, the dissolution of goethite in cultures containing both *Acidiphilium* SJH and EDTA was about twice that in those containing EDTA alone and this, in turn, was ~50% more than in cultures containing *Acidiphilium* SJH and no chelating agent. Enhanced goethite dissolution by *Acidiphilium* SJH was also observed when DPTA was added to cultures, though the effect was less pronounced, possibly because of the lower concentration of DPTA used. In this case, mineral dissolution by *Acidiphilium* SJH (DPTA-free cultures) was found to be superior to that brought about by the chelating agent alone. The effect of EDTA on the dissolution of amorphous ferric hydroxide by *Acidiphilium* SJH was less pronounced than with goethite (Table 2). As with the other experiments using harvested cell suspensions (Figure 1), the increase in soluble iron concentrations was linear during the relatively short (4-h) incubation period, so specific rates of mineral dissolution could be readily evaluated.

Analysis of ferrous iron using the ferrozine reagent was not possible in these experiments; the chelating agents interfered with the assay (data not shown) so the speciation of iron could not be established. Attempts using other colorimetric (e.g., phenanthroline) and titrimetric (e.g., ceric sulfate) reagents also proved ineffective (data not shown). Microscopic examination of cultures indicated that *Acidiphilium* SJH was not adversely affected by the concentrations of EDTA and DPTA used in these experiments.

Discussion

The acidophilic heterotrophic bacterium *Acidiphilium* SJH is able to catalyze the reductive dissolution of a wide range of ferric iron–containing minerals. The relative rates of mineral dissolution appear to correlate with the structural stability of the mineral. For example, the standard free energy of formation (ΔG°) of ferric hydroxide is −91 kJ/mol, whereas values for goethite and hematite are −489 and −739 kJ/mol, respectively (Arnold et al. 1986; Lide 1995). Similar trends have been noted with pure cultures of the fermentative neutrophile *Clostridium butyricum* (Munch and Ottow 1980) and in mixed populations in constructed wetlands (Tarutis and Unz 1995). Neutrophilic *Shewanella* spp. have been shown to cause the reductive dissolution of both goethite and hematite (Arnold et al. 1988; Roden and Zachara 1996).

Data from the experiments described in this report suggest that *Acidiphilium* SJH accelerates mineral dissolution by way of an indirect mechanism. In contrast to the neutrophilic

TABLE 2 Effect of 5 mM EDTA on the dissolution of amorphous ferric hydroxide by *Acidiphilium* SJH (mean ± SD)

Treatment	Specific rate of mineral dissolution
Inoculated, +EDTA	0.73 ± 0.09^a
Inoculated, −EDTA	0.63 ± 0.03^a
Uninoculated, +EDTA	0.68 ± 0.10^b
Uninoculated, −EDTA	0.08 ± 0.01^b

$n = 3$ for all cultures.
[a]Fe solubilized, $\mu g \cdot min^{-1} \cdot mg^{-1}$ protein.
[b]Fe solubilized, $\mu g \cdot min^{-1}$.

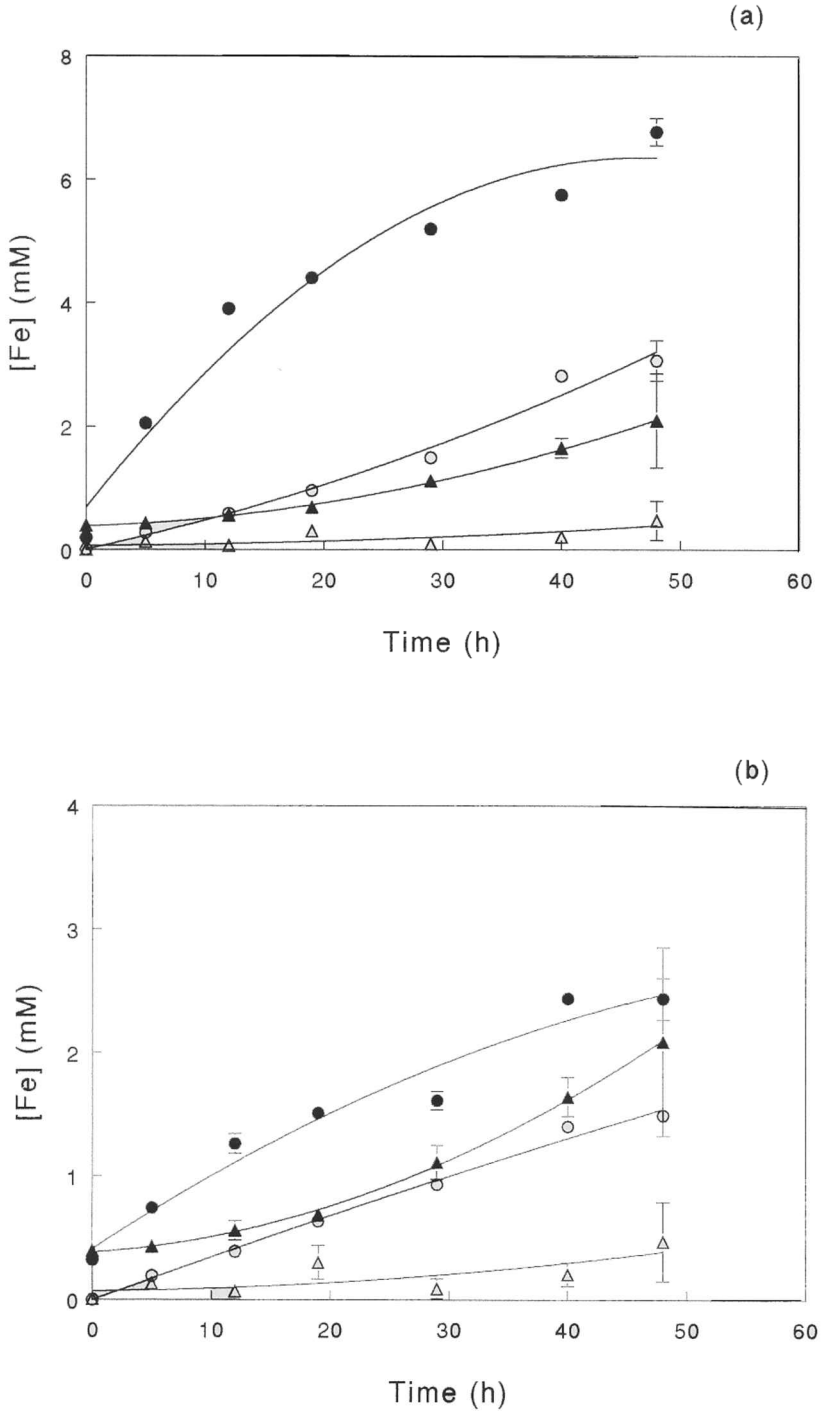

FIGURE 5 The effect of (a) 5.0 mM EDTA and (b) 2.5 mM DPTA on the dissolution of goethite by *Acidiphilium* SJH. (●) Inoculated cultures containing chelating agent; (○) uninoculated cultures containing chelating agent; (▲) inoculated cultures without chelating agent; (△) uninoculated cultures without chelating agent. Each point represents the mean value of triplicate cultures, and error bars are standard deviations.

bacteria *C. butyricum*, *Bacillus polymyxa*, and *S. putrefaciens*, contact between bacteria and ferric mineral was not necessary for mineral dissolution to occur. The fact that ferric hydroxide was solubilized by *Acidiphilium* SJH at a slower rate when the two were physically separated could have resulted from the dialysis sac decreasing the diffusion of chemically solubilized ferric iron from the sac to the outer bacteria-containing solution. Culture pH had a major impact on ferric mineral dissolution by *Acidiphilium* SJH, the cultures at very low pH (2.0) being far superior to those at pH 2.8–3.0. This was not attributable to a direct effect of pH of the growth and activity of *Acidiphilium* SJH, the pH optimum of which is ~3.0 (D. B. Johnson, unpubl. data). Chemical dissolution of amorphous ferric hydroxide was also more pronounced at pH 2.0 than at 2.8, though the soluble iron produced was exclusively in the ferric form. Soluble ferric iron is very readily reduced to ferrous iron by *Acidiphilium* SJH under conditions of limited aeration. This would cause a shift in equilibrium between solid-phase and soluble ferric iron, resulting in more chemical dissolution of the mineral, as follows:

$$Fe(OH)_3 \leftrightarrow Fe^{3+}_{soluble} \ (+\textit{Acidiphilium}\ \text{SJH}) \rightarrow Fe^{2+}_{soluble}$$

A similar scenario of slightly enhanced chemical dissolution and greatly enhanced biological dissolution was observed with magnetite, though the time course involved was far greater than with amorphous ferric hydroxide, for reasons described above. Ferric iron minerals are known to be more soluble in low-pH than in near-neutral solutions, because of the adsorption of protons to the mineral surface, which causes ferric iron to be released from the lattice structure (Wieland et al. 1988). The rate-limiting step in the reductive dissolution of iron minerals is that of $Fe^{3+}_{solid\ phase} \rightarrow Fe^{3+}_{soluble}$, a reaction that can be accelerated by adding iron-chelating compounds such as EDTA and DPTA. Addition of these chelating agent to cultures of *Acidiphilium* SJH enhanced goethite dissolution compared with that in cultures containing either bacteria or chelating agent alone, as would be predicted from the proposed mechanism of mineral dissolution by *Acidiphilium* SJH. Iron-complexing agents such as EDTA and nitrilotriacetic acid have been used frequently in the study of iron reduction by neutrophilic bacteria. Arnold et al. (1988) found that the specific rates of dissolution of goethite and hematite by *S. putrefaciens* increased ~15- and 6-fold, respectively, when 1.86 mM EDTA was added. A much smaller effect of accentuated goethite dissolution by EDTA was found with *Acidiphilium* SJH; however, dissolution of ferric iron minerals by chelating agents is known to be slower in acidic solutions because the chelate is more protonated at low pH values (Laitinen and Harris 1975).

Iron reduction by *Acidiphilium* SJH appears to be mediated by an enzyme system associated with the bacterial cells. No iron reduction activity was found in heat-killed cells or in cell-free spent media. The location of this "iron reductase" is not known at present, though logically (given the insolubility of ferric iron at the cytoplasmic pH of *Acidiphilium* spp.) it would be outside of the cell membrane, for example, in the periplasmic space of these Gram-negative bacteria. An intriguing finding was that *Acidiphilium* SJH appears to produce a heat-stable extracellular compound that accelerates the dissolution of ferric iron–containing minerals but does not catalyze iron reduction. A variety of organic acids (e.g., citric, oxalic, ascorbic) can act as nonspecific chelators of iron, whereas other materials (most notably siderophores) are very specific, high-affinity chelators of the metal. However, extensive analyses of spent media of *Acidiphilium* SJH have failed to identify a metabolite, such as an organic acid, that might be responsible for the enhanced dissolution of iron mineral.

The reductive dissolution of magnetite by *Acidiphilium* SJH is particularly interesting. Magnetite (which, in contrast to the other iron minerals used, contains a mixture of both

ferrous and ferric ions) is an end product of dissimilatory iron reduction by *Geobacter metallireducens* and some other neutrophilic bacteria (Lovley 1991) and was once considered not to be further reduced by microorganisms. However, *S. putrefaciens* is known to bring about the reductive dissolution of magnetite in mildly acidic (pH 5–6) cultures (Kosta and Nealson 1995). Abiotic dissolution of magnetite by sulfuric acid is congruent at pH < 1, with ferrous and ferric iron being released in the ratio 2:1. However, at pH > 1, abiotic dissolution of this mineral is incongruent; iron is predominantly released as ferrous, and a layer of maghemite (γ-Fe_2O_3) forms on the mineral surface. Maghemite is thermodynamically more stable than magnetite, and its formation results in a decrease in the rate of mineral dissolution (White et al. 1994). The amount of ferrous iron present in magnetite-containing cultures of *Acidiphilium* SJH at the end of the incubation period was greater than that originally present in the mineral itself (>90% of the mineral iron was by then present as soluble ferrous iron), indicating that the dissolution of this mineral by the bacteria again involved iron reduction. The reduction of ferric iron released from the magnetite would have precluded the formation of surface coatings of maghemite, thereby facilitating congruent reductive dissolution of the mineral rather than the partial incongruent dissolution that occurs in abiotic solutions.

References

Arnold RG, DiChristina TJ, Hoffmann MR. 1988. Reductive dissolution of Fe(III) oxides by *Pseudomonas* sp. 200. Biotechnol Bioeng 32:1081–1096.

Arnold RG, Olson TM, Hoffmann MR. 1986. Kinetics and mechanism of dissimilative Fe(III) reduction by *Pseudomonas* sp. 200. Biotechnol Bioeng 28:1657–1671.

Atkinson RJ, Posner AM, Quirk JP. 1977. Crystal nucleation and growth in hydrolysing iron (III) chloride solutions. Clays Clay Min 25:49–56.

Bridge TAM. 1995. Iron reduction by acidophilic bacteria. PhD Thesis, University of Wales, UK.

Bridge TAM, Johnson DB. 1998. Reduction of soluble iron and reductive dissolution of ferric iron-containing minerals by moderately thermophilic iron-oxidising bacteria. Appl Environ Microbiol 64:2181–2186.

Brindley GW, Brown G. 1984. Crystal structures of clay minerals and their X-ray identification. London Mineralogical Soc Monogr 5.

Brock TD, Gustafson J. 1976. Ferric iron reduction by sulfur- and iron-oxidising bacteria. Appl Environ Microbiol 32:567–571.

Cummings DE, Caccavo F Jr, Spring S, Rosenzweig RF. 1999. *Ferribacterium limneticum*, gen. *nov*., sp. *nov*., an Fe(III)-reducing microorganism isolated from mining-impacted freshwater lake sediments. Arch Microbiol 171:183–188.

Ehrenreich A, Widdel F. 1994. Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. Appl Environ Microbiol 60:4517–4526.

Johnson DB. 1995. The role of "iron bacteria" in the biodegradation of minerals. Biodeterior Abst 9:1–7.

Johnson DB. 1998. Biodiversity and ecology of acidophilic micro-organisms. FEMS Microbiol Ecol 27:307–317.

Johnson DB, Bridge TAM. 1997. The role of microbial dissimilatory reduction processes in the bioremediation of metal-rich, acidic drainage waters. In: Verachtert H, Verstraete W, editors. Environmental Biotechnology: Part 1. Proceedings of the International Symposium. Oostende, Belgium: Technologisch Instituut, p 183–186.

Johnson DB, McGinness S. 1991. Ferric iron reduction by acidophilic heterotrophic bacteria. Appl Environ Microbiol 57:207–211.

Johnson DB, Kelso WI, Jenkins DA. 1979. Bacterial streamer growth in a disused pyrite mine. Environ Pollut 18:107–118.

Jones JG, Gardner S, Simon BM. 1984. Reduction of ferric iron by heterotrophic bacteria in lake sediments. J Gen Microbiol 130:45–51.

Kosta JE, Nealson KH. 1995. Dissolution and reduction of magnetite by bacteria. Environ Sci Technol 29:2535–2540.

Laitinen HA, Harris WE, editors. 1975. Chemical analysis. New York: McGraw-Hill, 611p.

Lazaroff N, Sigal W, Wasserman A. 1982. Iron oxidation and precipitation of ferric hydroxysylfates by resting *Thiobacillus ferrooxidans* cells. Appl Environ Microbiol 43:924–938.

Lide DR, editor. 1995. CRC handbook of chemistry and physics, 76th edition. London: CRC Press, 2417p.

Lovley DR. 1991. Dissimilatory Fe(III) and Mn(IV) reduction. Microbiol Rev 55:259–287.

Lovley DR, Phillips EJP. 1986. Organic matter mineralization with reduction of ferric iron in anaerobic sediments. Appl Environ Microbiol 51:683–689.

Lovley DR, Phillips EJP. 1987. Rapid assay for microbially reducible ferric iron in aquatic sediments. Appl Environ Microbiol 53:1536–1540.

Lovley DR, Giovannoni SJ, White DC, Champine JE, Phillips EJP, Gorby YA, Goodwin S. 1993. *Geobacter metallireducens* gen. *nov*. sp. *nov*., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. Arch Microbiol 159:336–344.

Lovley DR, Phillips EJP, Lonergan DJ, Widman PK. 1995. Fe(III) and S° reduction by *Pelobacter carbinolicus*. Appl Environ Microbiol 61:2132–2138.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275.

Munch JC, Ottow JCG. 1980. Preferential reduction of amorphous to crystalline iron oxides by bacterial activity. Soil Sci 129:15–21.

Nealson KH. 1983. The microbial iron cycle. In: Krumbein WE, editor. Microbial geochemistry. Oxford, UK: Blackwell Scientific Publications, p 159–190.

Nealson KH, Myers CR. 1992. Microbial reduction of manganese and iron: new approaches to carbon cycling. Appl Environ Microbiol 58:439–443.

Ottow JCG, Glathe H. 1971. Isolation and identification of iron-reducing bacteria from gley soils. Soil Biol Biochem 3:43–55.

Pronk JT, de Bruyn JC, Bos P, Kuenen JG. 1992. Anaerobic growth of *Thiobacillus ferrooxidans*. Appl Environ Microbiol 58:2227–2230.

Rawlings DE, editor. 1997. Biomining: theory, microbes and industrial processes. Berlin: Springer-Verlag, 302p.

Roden EE, Zachara JM. 1996. Microbial reduction of crystalline Fe(III) oxides: influence of oxide surface area and potential for cell growth. Environ Sci Technol 30:1618–1628.

Tarutis WJ, Unz RF. 1995. Iron and manganese release in coal-mine drainage wetland microcosms. Water Sci Technol 32:187–192.

Weiland E, Wehrli B, Stumm W. 1988. The coordination chemistry of weathering: III. A generalization on the dissolution rates of minerals. Geochim Cosmochim Acta 52:1969–1981.

White AF, Peterson ML, Hochella MF Jr. 1994. Electrochemistry and dissolution kinetics of magnetite and ilmenite. Geochim Cosmochim Acta 58:1859–1875.