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BIOGEOCHEMICAL AND ENVIRONMENTAL FACTORS IN Fe BIOMINERALIZATION: MAGNETITE AND SIDERITE FORMATION

Y. ROH[1,*], C.-L. ZHANG[2], H. VALI[3], R. J. LAUF[4], J. ZHOU[1] AND T. J. PHELPS[1]

[1] Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA
[2] Department of Geological Sciences, University of Missouri-Columbia, Columbia, MO 65211, USA
[3] Electron Microscopy Center, McGill University, Montreal, Quebec H3A 2B2, Canada
[4] Metal and Ceramics Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

Abstract—The formation of siderite and magnetite by Fe(III)-reducing bacteria may play an important role in C and Fe geochemistry in subsurface and ocean sediments. The objective of this study was to identify environmental factors that control the formation of siderite ($FeCO_3$) and magnetite (Fe_3O_4) by Fe(III)-reducing bacteria. Psychrotolerant (<20ºC), mesophilic (20–35ºC) and thermophilic (>45ºC) Fe(III)-reducing bacteria were used to examine the reduction of a poorly crystalline iron oxide, akaganeite (β-FeOOH), without a soluble electron shuttle, anthraquinone disulfuonate (AQDS), in the presence of N_2, N_2-CO_2(80:20, V:V), H_2 and H_2-CO_2 (80:20, V:V) headspace gases as well as in HCO_3^--buffered medium (30–210 mM) under a N_2 atmosphere. Iron biomineralization was also examined under different growth conditions such as salinity, pH, incubation time, incubation temperature and electron donors. Magnetite formation was dominant under a N_2 and a H_2 atmosphere. Siderite formation was dominant under a H_2-CO_2 atmosphere. A mixture of magnetite and siderite was formed in the presence of a N_2-CO_2 headspace. Akaganeite was reduced and transformed to siderite and magnetite in a HCO_3^--buffered medium (>120 mM) with lactate as an electron donor in the presence of a N_2 atmosphere. Biogeochemical and environmental factors controlling the phases of the secondary mineral suite include medium pH, salinity, electron donors, atmospheric composition and incubation time. These results indicate that microbial Fe(III) reduction may play an important role in Fe and C biogeochemistry as well as C sequestration in natural environments.

Key Words—Biomineralization, Carbon Cycles, Fe(III)-reducing Bacteria, Magnetite, Siderite.

INTRODUCTION

Microbes participate in a variety of biogeochemical processes such as weathering and formation of minerals (Lovley *et al.*, 1987; Ferris *et al.*, 1994; Zhang *et al.*, 1997, 1998; Fredricksen *et al.*, 1998), formation of iron ore deposits (Nealson and Myers, 1990; Juniper *et al.*, 1995), and cycling of organic matter (Lovley, 1991; Nealson and Saffarini, 1994). Microbial metal reduction and mineral formation/dissolution not only plays an important role in cycling of metals, carbon, nitrogen, phosphate and sulfur in natural and contaminated subsurface environments (Lovley, 1991, 1993; Nealson and Saffarini, 1994), but also impacts on the speciation and the fate of a variety of trace metals and nutrients in anoxic subsurface environments (Lovley, 1995; Fredrickson *et al.*, 2001; Roh *et al.*, 2001).

The Fe(II) ion and Fe(II)-containing minerals generated by the Fe(III)-reducing bacteria can chemically reduce multivalent metals such as U(VI), Cr(VI) and Tc(VII). Nitroaromatics and chlorinated solvents can be abiotically reduced by microbially formed Fe(II)-containing minerals (Heijman *et al.*, 1993, 1995). The use of Fe(III) and other metals by certain microbial groups as terminal electron acceptors for anaerobic respiration is of particular relevance to bioremediation and natural attenuation of heavy metals and radionuclides (Lovley, 1995; Zhang *et al.*, 1996).

Many species of microorganisms, mainly anaerobic bacteria, are capable of reducing crystalline and amorphous Fe(III) oxides (Lovley, 1993; Roden and Zachara, 1996; Zhang *et al.*, 1997, 1998; Roh *et al.*, 2001). Anaerobic Fe(III)-reducing bacteria precipitate or transform these iron oxides into crystalline Fe(II)-containing phases such as magnetite (Fe_3O_4), siderite ($FeCO_3$), vivianite [$Fe_3(PO_4)\cdot 2H_2O$], and iron sulfide (FeS) (Postma, 1981; Zhang *et al.*, 1997, 1998; Fredrickson *et al.*, 1998). Biogenic Fe minerals may serve as physical indicators for previous biological activities in modern and ancient geological settings (Liu *et al.*, 1997; Zhang *et al.*, 1997, 1998).

Iron biomineralization has been commonly divided into two modes: biologically controlled mineralization in which bacteria genetically control the mineralization process (Frankel and Blakemore, 1990), and biologically facilitated mineralization in which bacteria facilitate mineral formation by creating external chemical environments suitable for precipitation. The formation of magnetite by magnetotactic bacteria represent biologically controlled mineralization in which the particles are formed inside bacterial cells templated by a specific internal membrane (Blakemore, 1982; Bazylinki *et al.*, 1988; Frankel and Blakemore, 1990). In contrast, the

* E-mail address of corresponding author:
rohy@ornl.gov
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formation of magnetite by Fe(III)-reducing bacteria, such as a thermophilic bacterium (*Thermoanerobacter ethanolicus*, TOR-39) (Zhang *et al.*, 1998) or a mesophilic bacterium (*Geobacter metallireducens*, GS-15) (Lovley *et al.*, 1987), represents biologically facilitated mineralization in which the particles are formed extracellularly as a byproduct of microbial Fe(III) respiration.

The formation of intra- and extra-cellular magnetite requires suitable regulation of redox, pH and Fe chemistry (Mann *et al.*, 1990; Schwertmann and Fitzpatrick, 1992). Schwertmann and Fitzpatrick (1992) suggested that microbial magnetite formation is the result of a suitable pH/Eh, a favorable rate of Fe(II) and Fe(III) supply to the growing crystal and a buffering capacity of the system. Further, for any mineral to precipitate, the solution should be supersaturated with respect to the mineral (Schwertmann and Fitzpatrick, 1992; Fredrickson *et al.*, 1998). It is well recognized that the metabolism of anaerobic bacteria promotes the formation of magnetite and siderite during microbial Fe(III) reduction and that the relative distribution of these phases is, in part, a function of pH and Eh (Bell *et al.*, 1987).

Both amorphous hydrous ferric oxide and crystalline iron oxide (goethite, magnetite and hematite) are reducible by Fe(III)-reducing bacteria (Liu *et al.*, 1997; Rodon and Zachara, 1996; Fredrickson *et al.*, 1998, 2001; Dong *et al.*, 2000; Liu *et al.*, 2001; Kukkadapu *et al.*, 2001; Zachara *et al.*, 2002). Fredrickson *et al.* (1998) showed that media composition, iron oxide properties, electron shuttle and electron acceptors influenced Fe(III) reduction and biomineralization of amorphous hydrous ferric oxide. The composition of aqueous media in which microbial Fe(III) reduction occurred impacted strongly on the rate and extent of Fe reduction and the nature of the reduced solids (Fredrickson *et al.*, 1998). Other factors including the process of nucleation for crystal growth may also influence the formation of magnetite and siderite by bacteria (Schwertmann and Cornell, 1991) because bacteria can serve as nucleation sites for mineral precipitation (Fortin *et al.*, 1997).

Microbial formation of magnetite and siderite using amorphous hydrous ferric oxide (Fredrickson *et al.*, 1998) and crystalline Fe oxides (Roden and Zachara, 1996; Dong *et al.*, 2000; Liu *et al.*, 2001; Kukkadapu *et al.*, 2001; Zachara *et al.*, 2002) in the presence of exogeneous electron carrier substances such as humic acids (*i.e.* anthraquinone-2,6-disulfonate, a humic acid analog) have been examined extensively. However, little is known about the microbial formation of magnetite and siderite using a crystalline Fe(III) oxide without an exogeneous electron carrier. The objective of this study was to identify environmental factors (*i.e.* pH, chemical milieu, atmospheric composition) that control microbial formation of siderite and magnetite without an exogeneous electron carrier using a crystalline Fe(III)oxide, akaganeite, as an electron acceptor and various Fe(III)-reducing bacteria including psychrotolerant, mesophilic and thermophilic bacteria isolated from various environmental conditions.

MATERIALS AND METHODS

Source of organisms

In this study, we examined the microbial formation of Fe minerals using thermophilic (*Thermoanaerobacter ethanolicus,* TOR-39) (Liu *et al.*, 1997), mesophilic (*Shewanella alga,* BrY) (Rossellomora *et al.*, 1994), and psychrotolerant (*Shewanella alga,* NV-1*; Shewanella pealeana*, W3-7-1) bacteria (Stapleton *et al.*, 2002). These Fe(III)-reducing bacteria were isolated from sediments and water collected from a variety of environments, including deep subsurface sediments (TOR-39), estuary sediments (BrY), deep Pacific Ocean sediments (W3-7-1), and the water column (NV-1) near a hydrothermal vent off the coast of Hawaii (Table 1). These Fe(III)-reducing bacteria (Table 1) were used to examine Fe mineral formation under various geochemical and environmental conditions.

Growth conditions

The culture medium contained the following ingredients (g/L): 2.5 $NaHCO_3$, 0.08 $CaCl_2{\cdot}2H_2O$, 1.0 NH_4Cl, 0.2 $MgCl_2{\cdot}6H_2O$, 10 NaCl, 0.4 $K_2HPO_4{\cdot}3H_2O$, 7.2

Table 1. Source of Fe-reducing bacteria and experimental conditions.

Strains	Source	Incubation temperature (ºC)	Electron donor	Headspace
Thermoanaerobacter ethanolicus (TOR-39)	Subsurface sediments	65	Formate, pyruvate, lactate, acetate	N_2, N_2/CO_2
Shewanella alga (BrY)	Estuary sediments	25	Formate, pyruvate, lactate, acetate, H_2	N_2, N_2/CO_2 H_2, H_2/CO_2
Shewanella pealeana (W3-7-1)	Oceanic sediments	14, 25	Formate, pyruvate, lactate, acetate, H_2	N_2, N_2/CO_2 H_2, H_2/CO_2
Shewanella alga (NV-1)	Seawater near hydrothermal vent	14, 25	Formate, pyruvate, lactate, acetate, H_2	N_2, N_2/CO_2 H_2, H_2/CO_2

HEPES (hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 1.0 rasazurin (0.01%), 0.5 yeast extract, and 10 trace minerals and 1 vitamin solution (Phelps *et al.*, 1989). No exogeneous electron carrier substance (*i.e.* anthraquinone disulfonate) or reducing agent (*i.e.* cysteine) was added to the medium. The trace mineral solution contained (g/L): 1500 nitrilotriacetic acid, 200 $FeCl_2{\cdot}4H_2O$, 100 $MgCl_2{\cdot}6H_2O$, 20 sodium tungstate, 100 $MnCl_2{\cdot}4H_2O$, 100 $CoCl_2{\cdot}6H_2O$, 1000 $CaCl_2{\cdot}2H_2O$, 50 $ZnCl_2$, 2 $CuCl_2{\cdot}2H_2O$, $5H_3BO_3$ 10 sodium molybdate, 1000 NaCl, 17 Na_2SeO_3, 24 $NiCl_2{\cdot}6H_2O$. The vitamin solution contained (g/L): 0.02 biotin, 0.02 folic acid, 0.1 B6 (pyridoxine) HCl, 0.05 B1 (thiamine) HCl, 0.05 B_2 (riboflavin), 0.05 nicotinic acid (niacin), 0.05 pantothenic acid, 0.001 B12 (cyanobalamine) crystalline, 0.05 PABA (P-aminobenzoic acid), 0.05 lipoic acid (thioctic). The initial pH of the medium ranged from 7.8 to 8.0.

Microbial reduction of the crystalline Fe(III) oxide, akaganeite (β-FeOOH), was investigated. The akaganeite was prepared as follows: NaOH solution (10 M) was slowly added into a $FeCl_2{\cdot}6H_2O$ solution (0.4 M) to precipitate $Fe(OH)_2$ by gravity only and with rapid stirring at pH 7.0 (Roh *et al.*, 2001). The suspension was aerated overnight by magnetic stirring, ensuring homogeneous oxidation. The Fe(III) phases formed were washed three times in deionized water and centrifuged after each washing. A final solution of ~0.7 M FeOOH was flushed with N_2 and then autoclaved for microbiological use. X-ray diffraction (XRD) analysis showed that the autoclaved Fe(III) phase was mainly poorly crystalline akaganeite (β-FeOOH) (Figure 1).

Hydrogen (100% or 80% H_2-20% CO_2), formate (10 mM), pyruvate (10 mM), acetate (10 mM), and lactate (10 mM) served as electron donors (Table 1) and an akaganeite (~70 mM) was used as an electron acceptor to examine the effect of electron donors on Fe biomineralization. To examine the effect of gas composition on the secondary mineral suite, Fe biomineralization using akaganeite as an electron acceptor was examined in the presence of different gas compositions including N_2(100% N_2), N_2-CO_2 (80% N_2-20% CO_2), H_2(100%), and H_2-CO_2 (80% H_2-20% CO_2) headspace gases (Table 1). In addition, experiments with CO_2 pressure (0.05%CO_2) close to atmospheric pressure were also examined to see the influence of low CO_2 on Fe biomineralization. The effect of a bicarbonate buffer concentration (30–210 mM) on Fe biomineralization was also examined using the Fe(III)-reducing bacteria with lactate (10 mM) as an electron donor and akaganeite (70 mM) as an electron acceptor. Experiments were performed at 14 or 25ºC for psychrotolerant cultures (*Shewanella pealeana,* W3-7-1; *Shewanella alga,* NV-1), at 25ºC for the mesophilic culture (*Shewanella alga*, BrY), and at 65ºC for the thermophilic culture (*Thermoanaerobacter ethanolicus*, TOR-39) (Table 1). Experiments were terminated after 30 days of incubation for psychrotolerant and mesophilic bacteria and after 22 days for thermophilic bacteria.

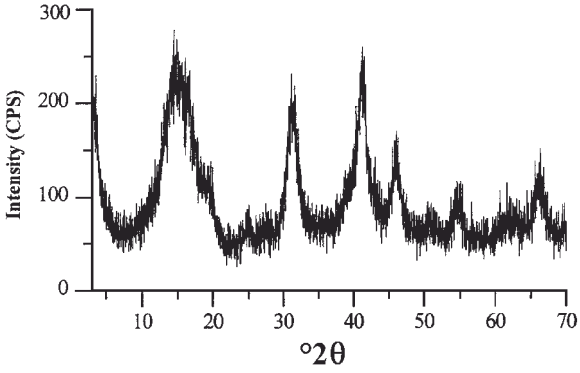

Figure 1. XRD pattern of akaganeite used as an electron acceptor.

To examine inorganic growth of magnetite after biological induction of magnetite nuclei, anaerobic gluderaldehyde (final concentration 2.5%) was added to culture tubes at certain time points (Zhang *et al.*, 1998): when magnetite was first detected at day 1 for *Thermoanaerobacter ethanolicus* (TOR-39) and at day 6 for *Shewanella alga (*NV-1). These tubes were continuously incubated until the end of experiment (22 days for *Thermoanaerobacter* and 30 days for *Shewanella*). Abiotic controls without cells also accompanied each bacterial magnetite formation experiment.

Chemical and mineralogical characterization

The redox potential (Eh) and pH values in bacterial cultures at the beginning and end of the experiments were measured at room temperature in an anaerobic chamber. The pH measurements used a combination of pH electrode and an ORION EA 920 expandable ion analyzer (Orion Research, Beverly, MA), standardized with pH buffer 7 and the appropriate buffer of either pH 4 or 10 (Roh *et al.*, 2001). Eh values were measured using platinum micro-electrodes (Microelectrodes, Inc., Londonderry, NH) (Roh *et al.*, 2001). The probe was placed directly into the sample tube and equilibrated for at least 5 min before recording the value. Fe(II) concentrations in enrichment cultures and in abiotic controls were determined by the ferrozine method (Zhang *et al.*, 1997). In this method, 0.1 mL of sample was added to 2 mL of anaerobic 0.5 M HCl solution. After 15 min, 0.1 mL of the mixture was added to 3 mL of ferrozine (1 g/L) in 50 mM HEPES (N-2-hydroxyethylpiperazine acid) buffer at pH 7. The sample was mixed, filtered through a Whatman syringe-filter (13 mm filter diameter, 0.2 µm pore diameter), and measured for maximum absorbance at 562 nm. Standards for the ferrozine assay were prepared with ferrousethylenediammonium sulfate dissolved in 0.5 M HCl. A JEOL JSM-35CF (JEOL LTD, Tokyo, Japan) scanning electron microscope (SEM) with energy dispersive X-ray analysis (EDX) was used for the analysis of morphology, mineralogy, chemistry of the precipitated

Table 2. Changes in solution chemistry during Fe(III) reduction by *Shewanella* (W3-7-1 and NV-1). Incubation temperature was 25ºC.

Measurement	Atmosphere					
	Lactate (100% N_2)		Lactate (80%N_2-20%CO_2)		Hydrogen (80%H_2-20%CO_2)	
	Initial[1]	Final[2]	Initial	Final	Initial	Final
pH	7.8–8.0	7.3–7.8	7.8–8.0	6.8–7.5	7.8–8.0	6.5–7.3
Eh (mV)	−60 to −80	−190 to −280	−6 to −-80	−280 to −360	−60 to −80	−340 to −450
Fe(II) (mM)[3]	0.04–0.08	1.5–4.1	0.04–0.08	5.6–20.2	0.04–0.08	16.4–40.2

[1] Initial time = within 1 h of inoculation of *Shewanella*
[2] Final time = end of experiments (30 days of incubation)
[3] Initial Fe(II) resulting from abiotic reduction of Fe(III) before incubation

or transformed phases by the Fe(III)-reducing bacteria. The mineralogical composition of the precipitated or transformed phases was determined using XRD. All XRD analyses were performed on a Scintag (Scintag, Inc., Sunnyvale, CA) XDS 2000 diffractometer (40 kV, 35 mV) using CoKα radiation (λ = 0.17889 nm) and a scan rate of 2º2θ/min. Mineralogical characterization of the precipitates was also performed by transmission electron microscopy (TEM) to study the mineral morphology of the precipitated crystalline Fe minerals (Zhang *et al.*, 1998).

RESULTS

Solution chemistry

Table 2 summarizes the ranges of pH, Eh and Fe(II) concentrations for the experiments performed in this study. The measurement of Eh and pH values were plotted on Eh-pH stability fields for lepidocrocite, magnetite and siderite in the Fe-water-CO_2 system at 25ºC and 1 atm total pressure (Figure 2). The final pH varied less (6.8–7.8) in the lactate-enriched cultures in the N_2 and N_2-CO_2 atmospheres than in the H_2-CO_2-enriched cultures (6.5–7.5). The microbial utilization of hydrogen under a H_2-CO_2 atmosphere resulted in significantly lower Eh values (−340 to −450 mV) than lactate utilization under a N_2 (−190 to −280 mV) and a N_2-CO_2 (−280 to −360 mV) atmosphere (Figure 2, Table 2), as expected from thermodynamics of H_2 oxidation. Microbial H_2-CO_2 utilization resulted in significantly higher soluble Fe(II) concentration (16.4–40.2 mM) than the lactate utilization in the N_2 (1.5–4.1 mM) and N_2-CO_2 (5.6–20.2 mM) atmospheres, suggesting greater extent of microbial reduction of Fe(II) in association with H_2 oxidation. Maximum concentration of Fe(II) ranged from 0.5 to 2.3 mM in the

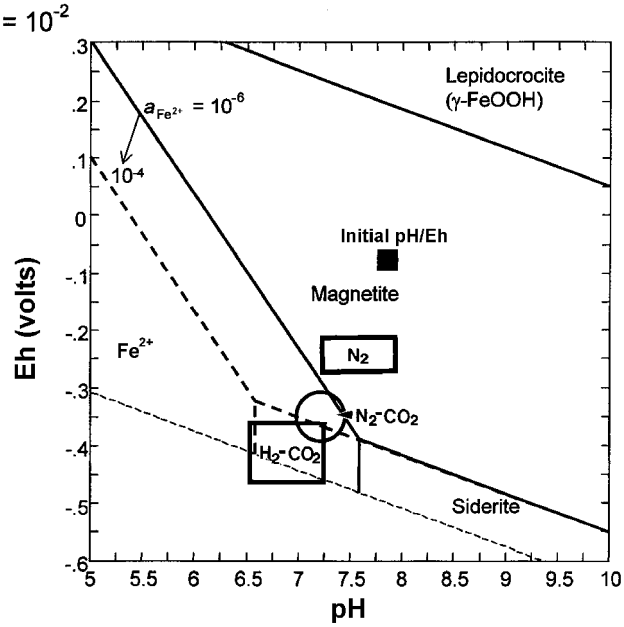

Figure 2. Eh-pH stability fields for lepidocrocite, magnetite and siderite in the water-Fe-CO_2 system at 25ºC and 1 atm total pressure. The CO_2 partial pressure was fixed at 10^{-2} atm whereas the activity of Fe(II) was allowed to vary between 10^{-6} and 10^{-4} M. Measured Eh and pH values were also plotted.

controls and resulted from abiotic reduction of Fe(III) by organic nutrients such as yeast extract. Similarly, lactate and akaganeite under a higher bicarbonate buffer (140–210 mM) establish lower Eh values than with a lower bicarbonate buffer (30–70 mM) (data not shown), suggesting greater microbial reduction of Fe(III) in association with the increased bicarbonate buffering capacity.

The observation of microbial siderite formation using akaganeite in a higher bicarbonate buffer (210 mM) and under a H_2-CO_2 atmosphere was consistent with the Eh measurement. This study indicated that the presence of a H_2-CO_2 atmosphere and the high bicarbonate buffer (210 mM) provided more bicarbonate and significant buffering capacity, allowing the siderite formation, than did the N_2/N_2-CO_2 atmosphere and low bicarbonate buffer (30–140 mM).

Effect of salinity and pH

Microbial Fe(III) reduction and Fe mineral formation by *Shewanella* (W3-7-1 and NV-1) occurred at a salinity range of 0.05–5% NaCl (wt./v) at 14ºC using lactate (10 mM) as an electron donor and akaganeite as an electron acceptor, but was not detected at 10% NaCl. Fe(III) reduction and iron mineral formation by *Thermoanerobacter* (TOR-39) also occurred at a salinity range of 0.05–5% NaCl (wt./v) using lactate as an electron donor and akaganeite as an electron acceptor, but not at 6% NaCl or higher. This result is consistent with other observations (Boone *et al*., 1995) that the thermophilic metal-reducing bacteria from Taylorsville Basin have an optimum growth at 3% NaCl (wt.%), but they did not grow at salinities >5% NaCl or <0.5% NaCl.

Microbial transformation of akaganeite to magnetite by all of the Fe(III)-reducing bacteria occurred at pH 7.5 to 8.5 (Figure 3). The bacteria crystallized magnetite at a slightly alkaline pH (>7.5) and a low Eh value (<–200 mV) using akaganeite as an electron acceptor and lactate as an electron donor. These results showed that microbial magnetite formation is favored by neutral to slightly alkaline conditions (Lovley, 1990; Bell *et al*., 1987; Pye *et al*., 1990).

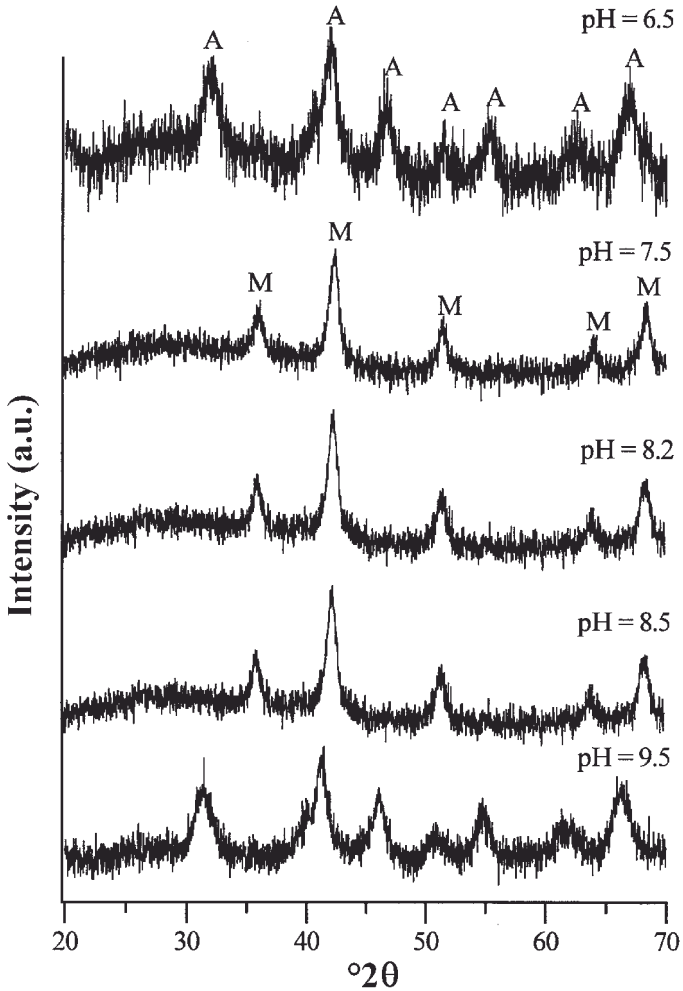

Figure 3. XRD patterns of Fe minerals formed by the *Shewanella* (NV-1) under different pH. A: akaganeite; M: magnetite.

Effect of incubation time

The rates of magnetite formation by *Shewanella* (NV-1 and W3-7-1) were generally slower than those of *Thermoanerobacter* (TOR-39). X-ray diffraction analyses of Fe minerals formed by *Thermoanerobacter* (TOR-39) using akaganeite as a precursor at 65ºC showed that a magnetite peak appeared at ~42º2θ using CoKα radiation after a day of incubation,. This first evidence of a magnetite peak agreed with the visual observation of the enrichment culture in which the reddish magnetite precursor turned black. After 3 days of incubation, the precipitates became completely black, and concurrently magnetite peaks were dominant in the diffraction pattern. At the end of the experiments (25 days), major magnetite peaks became prevalent. It took more than five days at 25ºC and 10 days at 14ºC for magnetite formation by *Shewanella alga* (NV-1). X-ray diffraction analyses of Fe minerals formed in *Shewanella alga* (NV-1) at different incubation times at 25ºC showed that the initial akaganeite revealed the first evidence of a magnetite peak within 6 days. No magnetite formation occurred in control tubes. More importantly, no progress of magnetite formation was observed in *Thermoanerbacter* and *Schwanella* cultures in which bacterial cells were killed by gluderaldehyde after starting crystal formation. This experiment indicates that magnetite is not readily formed by a purely inorganic mechanism under the conditions examined, even with the presence of highly reactive seed material, akaganeite. These results further suggest that bacterial activity played a governing role in the kinetics of magnetite formation under the experimental conditions.

Effect of electron donors

Table 3 shows XRD analysis data of Fe minerals formed by the Fe(III)-reducing bacteria using various electron donors. *Thermoanerobacter* (TOR-39) reduced Fe(III) and formed magnetite using lactate, formate or acetate, but not pyruvate and hydrogen, to reduce Fe(III) under a N_2 atmosphere. The XRD analysis showed that Fe minerals formed by this culture using lactate, formate and acetate as an electron donor under a N_2 atmosphere were mainly magnetite. *Shewanella* (BrY, W3-7-1, and NV-1) utilized H_2 and formed magnetite. The minerals precipitated by *Shewanella* (BrY, W3-7-1, NV-1) under a H_2 (100%) atmosphere were predominantly magnetite. These cultures can use lactate and formate as an electron donor under a N_2 atmosphere and formed mainly magnetite. However, these *Shewanella* (BrY, W3-7-1, NV-1) cannot use acetate or pyruvate as an electron donor to reduce akaganeite. These results indicate that these *Shewanella* (BrY, W3-7-1, NV-1) incompletely oxidize their electron donors, such as lactate, to acetate. Magnetite did not form in control tubes containing akaganeite and the same electron donors without cells (Table 3).

Effect of different atmospheric composition and bicarbonate concentration

The Fe(III)-reducing bacteria formed a mixture of magnetite and siderite using a medium buffered with $NaHCO_3$ (30–210 mM) under a N_2 atmosphere (Figure 5). The XRD analysis based on peak intensity showed that siderite precipitation increased with increasing bicarbonate concentration. Iron reduction under a N_2 atmosphere predominantly formed magnetite in all bacteria cultures (Figure 4, Table 3). Iron minerals formed by *Shewanella* (BrY, W3-7-1, NV-1) under a H_2 (100%) atmosphere were also predominantly magnetite (Figure 4, Table 3). Iron minerals formed by *Shewanella* (BrY, W3-7-1 and NV-1) under a CO_2 pressure close to atmosphere (0.05% CO_2 atmosphere) were also predominantly magnetite. A mixture of siderite and magnetite was seen in the *Thermoanerobacter* (TOR-39) and *Shewanella* (BrY, W3-7-1 and NV-1) cultures in the presence of a N_2-CO_2 headspace (Figure 5). *Shewanella* cultures formed predominantly siderite under a H_2-CO_2 atmosphere (Figure 4). No magnetite or siderite was detected in abiotic controls (Figures 4,5).

Under a N_2-CO_2 and a H_2-CO_2 atmosphere, dissolved bicarbonate from the headspace reacted with akaganeite present in the culture environment (*e.g.* the growth

Table 3. Effect of electron donors on Fe mineral formation using akaganeite as an electron acceptor.

Organisms	Incubation temp. (ºC)	Electron donor				
		Lactate N_2	Pyruvate N_2	Hydrogen H_2	Formate N_2	Acetate N_2
Thermoanaerobacter ethanolicus (TOR-39)	65	Magnetite	Akaganeite (no growth)	No growth	Magnetite	Magnetite
Shewanella alga (BrY)	25	Magnetite	Akaganeite (no growth)	Magnetite	Magnetite	Akaganeite (no growth)
Shewanella pealeana (W3-7-1)	14, 25	Magnetite	Akaganeite (no growth)	Magnetite	Magnetite	Akaganeite (no growth)
Shewanella alga (NV-1)	14, 25	Magnetite	Akaganeite (no growth)	Magnetite	Magnetite	Akaganeite (no growth)

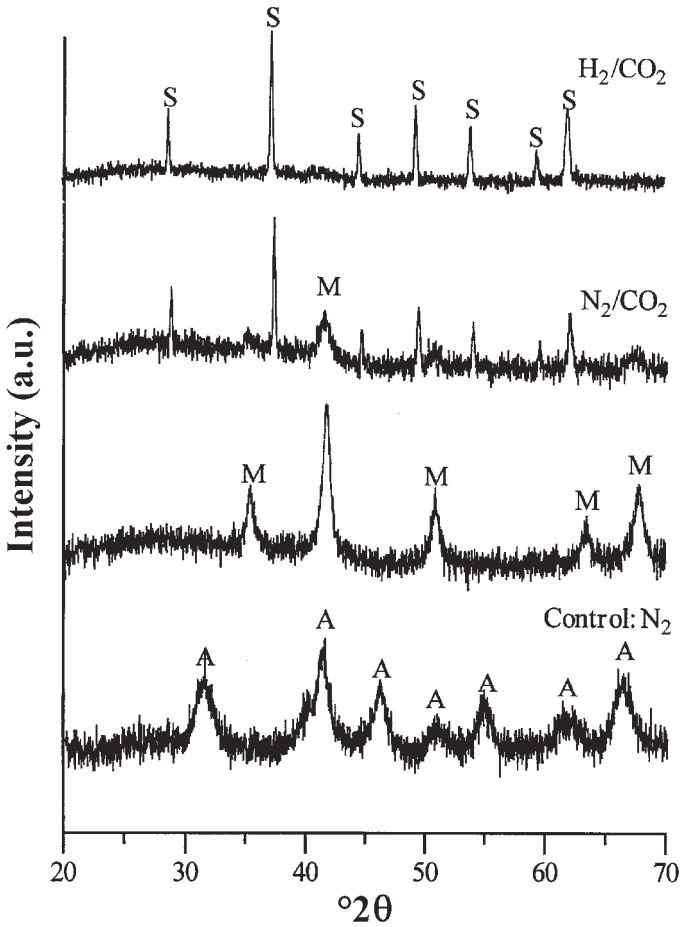

Figure 4. XRD patterns of Fe minerals formed by the *Shewanella* (NV-1) under different atmospheric composition. A: akaganeite; M: magnetite; S: siderite.

conditions) and bacteria facilitated siderite formation. In the presence of a N_2-CO_2 headspace, a mixture of magnetite and siderite was formed because siderite formation can compete with magnetite formation for Fe(II) (Lovley, 1990). The presence of a H_2-CO_2 atmosphere provides a lower redox potential, allowing the complete reduction of Fe(III), than the N_2 and N_2-CO_2 atmosphere. When hydrogen is added to the system, the redox potential shifts downwards to a point at which siderite is the stable phase (Figure 2). Although the medium typically contains PO_4 and HCO_3^- in molar concentration ($K_2HPO_4 = 2.8 \times 10^{-3}$ M and $NaHCO_3$ = 30–210 mM), vivianite [$Fe_3(PO_4)_2 \cdot 8H_2O$] and green rust I [$Fe^{2+}Fe^{3+}(OH)_{16}CO_3 \cdot 4H_2O$] had not been observed.

Magnetite and siderite morphology

Transmission electron microscopy of magnetite crystals formed by *Shewanella* (W3-7-1) at 14°C showed aggregates of small magnetite crystals ranging in size from 20 to 40 nm (Figure 6a). Most of the particles are of superparamagnetic size range (<35 nm) and similar to particles formed by *Geobacter metallireducens* (GS-15) (Sparks *et al.*, 1990). However, *Thermoanerobacter* (TOR-39) formed sharp, octahedral crystals (Figure 6b). These crystals are generally in the single-domain size range (Zhang *et al.*, 1998).

The TEM replica showed that siderite particles formed by *Thermoanerobacter* (TOR-39) using a medium-buffered 90 mM $NaHCO_3$ were globules with diameters between 3 and 5 μm (Figure 7d,e). The magnetite nanoparticles formed by *Thermoanerobacter* (TOR-39) coexisting with siderite have octahedral shapes with edge lengths <0.3 μm (Figure 7e). Siderite globules formed by TOR-39 showed that the surface structure appears to be composed of flakes of crystals (Figure 7e) rather than a single rhombohedral crystal formed by *Geobacter metallireducens* (GS-15) (Mortimer and Coleman, 1997). The TEM replica showed that siderite crystals formed by *Shewanella alga* (BrY) under a H_2-CO_2 atmosphere were disk-like crystals having diameters between 2 and 3 μm and thicknesses <0.4 μm (Figure 7c). The disk-like crystals formed by *Shewanella alga* (BrY) were also different from siderite formed by *Geobacter metallireducens* (Mortimer and Coleman, 1997). The SEM photograph

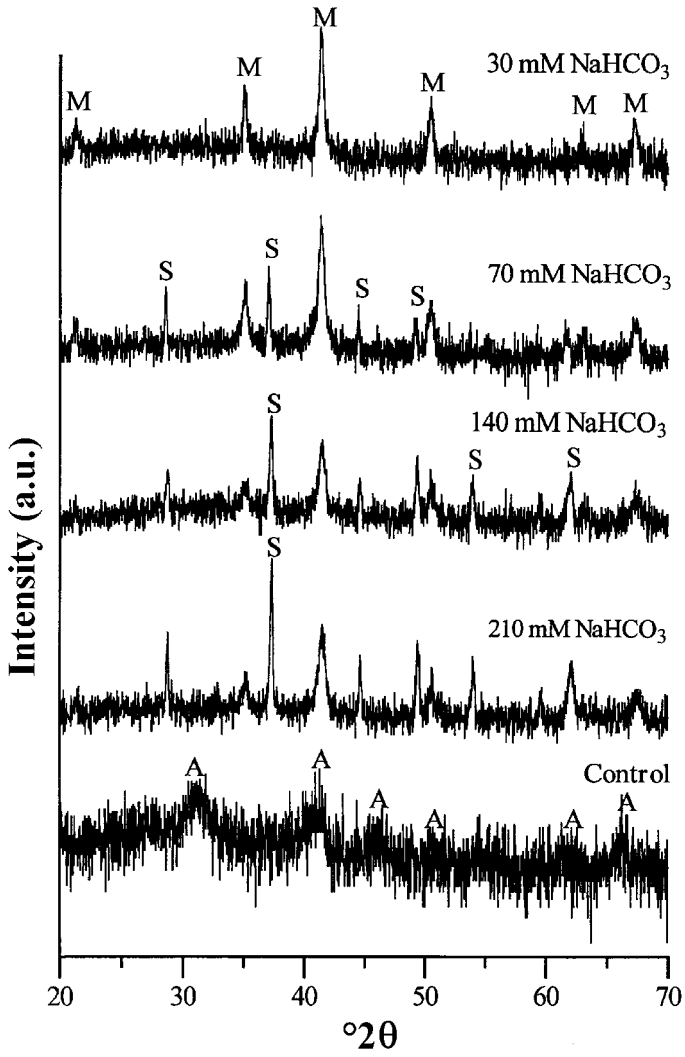

Figure 5. XRD patterns of Fe minerals formed by the *Shewanella* (NV-1) with different bicarbonate buffer concentrations under a N_2 atmosphere. A: akaganeite; M: magnetite; S: siderite.

showed that rhombohedral siderite crystals (Figure 7b) formed by *Shewanella alga* (NV-1) are similar to siderite formed by *Geobacter metallireducens* (GS-15) and found in natural samples. Some of the siderite crystals formed by *Shewanella alga* (NV-1) are linked as chains. However, a siderite globule (Figure 7a) formed by the *Shewanella* (W3-7-1) cultures was different from siderite formed by *Shewanella* (NV-1) and *Geobacter metallireducens* (GS-15).

DISCUSSION

Environmental factors in iron biomineralization

This study shows that geochemical and environmental factors in Fe biomineralization include medium pH, salinity, incubation time, electron donors, atmospheric composition and chemical milieu. Microbial transformation of akaganeite to magnetite by Fe(III)-reducing bacteria is favored in neutral to slightly alkaline conditions. Measured pH and Eh in the media are consistent with the thermodynamic stability of magnetite and siderite formation. Despite the existence of appropriate thermodynamic conditions for Fe mineral formation (*e.g.* Eh and pH), the color of the solids in the control bottles remained reddish and no magnetism was detected. This means that the formation of extracellular deposits of magnetite and siderite seems to be controlled by both solution chemistry and microbial activity (Zhang *et al.*, 1997, 1998; Roh *et al.*, 2001). It is well recognized that the metabolism of anaerobic bacteria promotes the formation of magnetite and siderite and that the relative distribution of these phases is, in part, a function of pH and Eh (Bell *et al.*, 1987).

The details of the biological reduction and mineralization process are not yet fully understood (Lovley, 1991, 1993; Zhang *et al.*, 1998). In particular, the mechanism by which the bacterium actually splits hydrogen or other substrates is not known with certainty

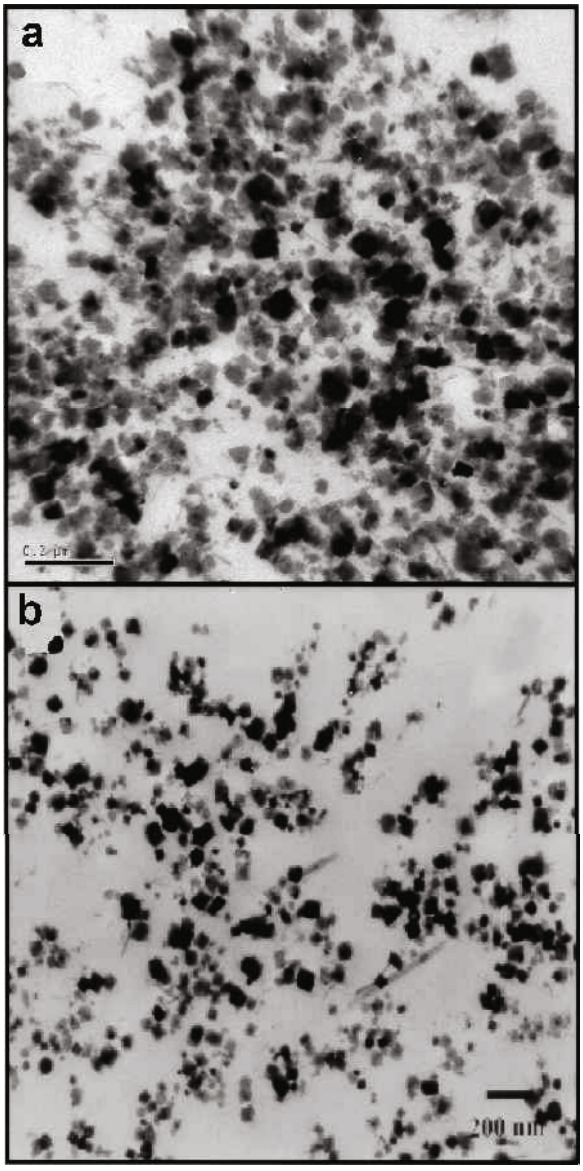

Figure 6. TEM images of magnetite formed by *Shewanella* W3-7-1 at 14ºC (a), and *Thermoanerobacter* (TOR-39) at 65ºC (b).

and the specific metabolic pathways of the resulting electrons have not been identified. However, by placing the overall mineralizing action into the context of the Eh-pH diagram (Figure 2) the effect of these processes on the external thermodynamics and kinetics can be appreciated. The Eh-pH diagram shows the fields of thermodynamic stability of various species under equilibrium conditions. Starting from a point of zero potential (−60 to −80 mV) and pH 7.8–8.0, hydrous trivalent iron oxide is the stable Fe(III)-containing phase. When hydrogen, for example, is added to the system, the potential shifts downwards to a point at which magnetite and siderite are the stable phase. Yet without the bacteria present the kinetics of this reaction are imperceptibly slow. So in one sense the bacteria may be thought of as a catalyst: by performing the crucial step of splitting the substrate, electrons are thereby made available so the reduction of Fe(III) can proceed. In contrast to a classical catalyst, however, the bacteria extract some of the electrochemical energy in order to live, after which the electrons are shed to their surroundings at a potential that is sufficient to precipitate magnetite and siderite. It is therefore perhaps most appropriate to think of the bacteria as an electrode that is substantially indifferent to the exact mix of metal ions present in the surrounding medium. This viewpoint is best illustrated by a previous study (Roh *et al.*, 2001) in which a divalent ion (*i.e.* Zn^{2+}, Ni^{2+}, Co^{2+}) was added to the medium with Fe(III) oxide. The resulting magnetic precipitate contained these divalent ions. Since these elements were divalent both in the aqueous solution and in the ferrite solid solution, it had clearly not been reduced by the bacteria, yet it became incorporated into the growing magnetite phase as thermodynamics would predict. Magnetite and siderite formation is thus the result of biologically-mediated mineralization; *i.e.* the organisms alter the local Eh and pH conditions which, in turn, shifts local mineral solubility equilibria, potentially also facilitating magnetite nucleation and formation of magnetite particles on or near the exterior surface of the cell (Zhang *et al.*, 1997, 1998). In other words, the formation of extracellular deposits of magnetite and siderite might be controlled by both solution electrochemistry and bacterial nucleation action (Zhang *et al.*, 1997). If nucleation effects are important, direct contact between cell and oxide surfaces might be necessary for efficient microbial respiration, metal reduction, and mineral formation (Lovley and Phillips, 1988).

This study showed that salt is required for microbial Fe(III) reduction and magnetite formation by *Shewanella* (W3-7-1 and NV-1) and *Thermoanerobacter* (TOR-39). The rates of Fe reduction and Fe mineral formation by *Shewanella* (W3-7-1 and NV-1) and *Thermoanerobacter* (TOR-39) showed that mineral formation took longer in the *Shewanella* culture (W3-7-1) than in the *Thermoanerobacter* (TOR-39). This further indicates that metabolic activity plays a governing role in biogenic Fe mineral formation and bacterial growth accelerates the precipitation kinetics of Fe minerals under the conditions examined (Zhang *et al.*, 1998).

This study also showed that microbial Fe(III) reduction can be an important process for organic matter oxidation in anaerobic subsurface environments. Previous studies have shown that organic compounds such as lactate, formate, acetate and pyruvate are potentially available in terrestrial subsurface environments (Walker, 1984; Lovley, 1991). The oxidation of organic compounds coupled with reduction of Fe(III) oxides can be expected to release Fe(II) ions in subsurface environments (Lovley, 1993; Fredrickson *et al.*, 1998). The *Shewanella* (W3-7-1, NV-1 and BrY)

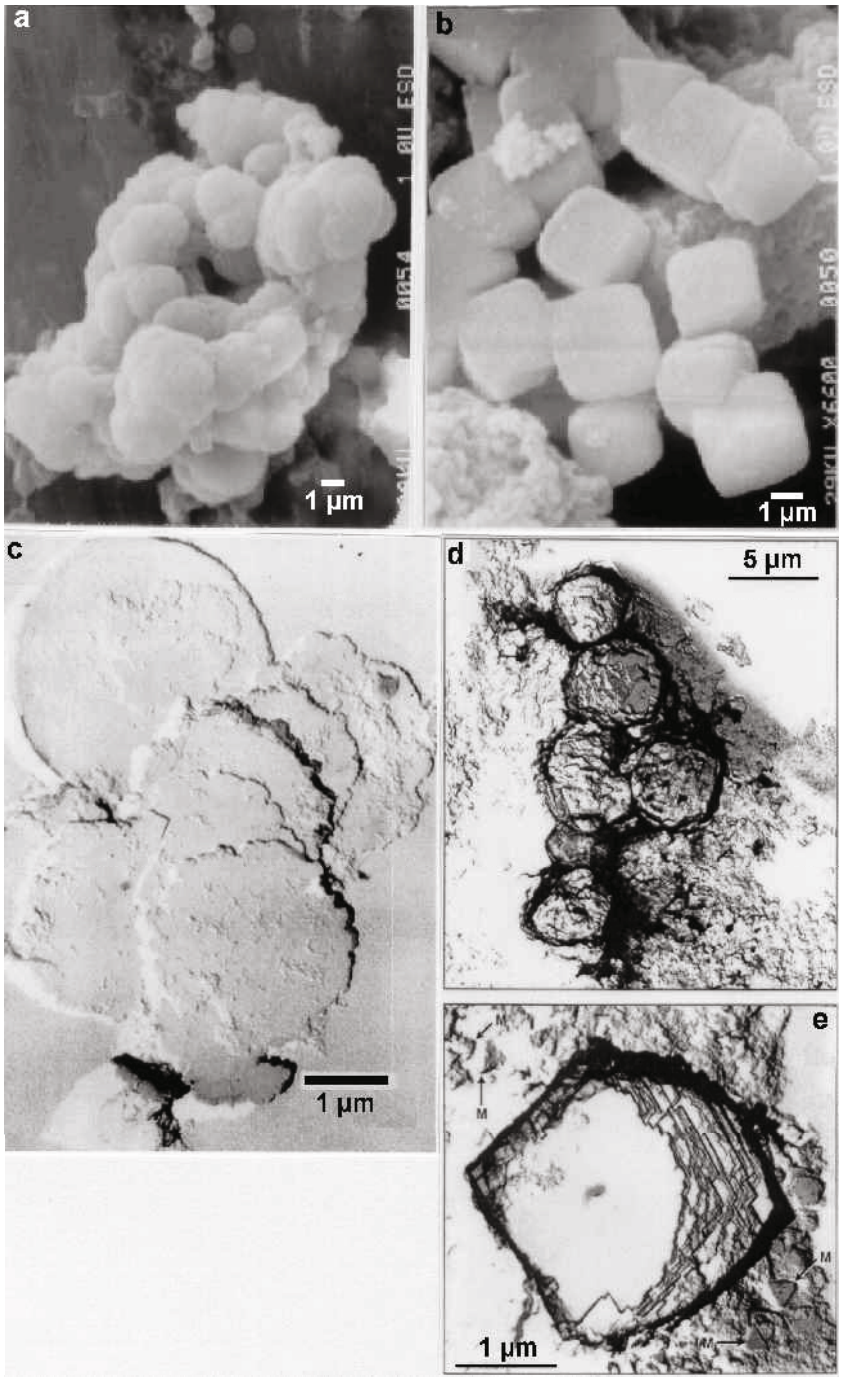

Figure 7. SEM images of magnetite and siderite formed by *Shewanella* and *Thermoanerobacter*: (a) siderite globule formed by *Shewanella* (W3-7-1) under a H_2/CO_2 atmosphere and (b) rhombohedral siderite formed by *Shewanella* (NV-1) under a H_2/CO_2 atmosphere. TEM replica of siderite formed by *Shewanella* (BrY) and thermophilic Fe-reducing bacteria: (c) disk-like siderite formed by *Shewanella* (BrY), (d) siderite globule formed by the *Thermoanerobacter* (TOR-39) using a medium buffered 90 mM $NaHCO_3$, and (e) siderite coexisting with magnetite formed by the *Thermoanerobacter* (TOR-39).

cultures can couple the oxidation of H_2 to the reduction of Fe(III).

Hydrogen gas is generated from anaerobic decomposition of organic matter as well as from geochemical processes in subsurface environments and probably constitutes a sustainable source of energy for subsurface biosphere ecosystems (Pedersen, 2000).

Atmospheric composition and bicarbonate buffer concentrations affected the mineralogical composition of Fe minerals formed by Fe(III)-reducing bacteria. High

bicarbonate concentration and Fe^{2+}, as promoted by bacterial activity, seem to favor siderite precipitation (Rajan *et al*., 1996; Mortimer and Coleman, 1997; Fredrickson *et al*., 1998). Siderite formation is generally associated with the bacterial respiration of organic matter coupled with dissimilatory Fe reduction (Suess, 1979; Pye *et al*., 1990); although the *in situ* geochemical conditions under which siderite forms are not well established (Pye *et al*., 1990).

Geochemical implications of iron biomineralization

This study suggests that the Fe(III)-reducing bacteria in subsurface environments may have the capacity to form magnetite (Fe_3O_4) and siderite ($FeCO_3$) when growing on short-chain fatty acids or hydrogen as an energy source in a variety of geochemical conditions. The biomineralization of Fe(II)-containing minerals as they undergo reduction by Fe(III)-reducing bacteria is a complex process influenced by multiple biological and chemical factors. The major factors controlling Fe biomineralization are the composition and concentration of cations and anions, medium pH, atmospheric composition and bacterial growth conditions (*i.e.* incubation temperature and time). Increasing bicarbonate concentration and p_{CO_2} in the media resulted in increased proportions of siderite relative to magnetite. In fact, the presence of the inorganic ligand, CO_3^{2-}, facilitated the reduction of akaganeite, probably by creating conditions where reduction was thermodynamically favored. This study showed that microbial magnetite formation is favored by neutral to slightly alkaline and reducing conditions (Lovley, 1990; Bell *et al*., 1987; Pye *et al*., 1990).

A crystalline Fe(III) oxide, hematite (α-FeOOH), was reducible by some Fe(III)-reducing microorganisms (*e.g. Shewanella*) with a soluble electron shuttle, AQDS (Roden and Zachara, 1996). However, this study showed that the biologically facilitated formation of magnetite and siderite using a crystalline Fe(III)-oxide, akaganeite, as an electron acceptor does not require the addition of exogenous electron carrier substances, humic acid (*e.g.* AQDS) or a reducing agent (cysteine). The ability of Fe(III)-reducing bacteria to reduce crystalline Fe(III) oxide, akaganeite, and to form magnetite and siderite has far-reaching implications for microbial processes in subsurface sediments where Fe(III) associated with crystalline Fe oxides may represent the largest mass of electron acceptor. Microbial formation of carbonate mineral and iron oxides may play an important role in trace metal immobilization because metals (Co, Cr, Ni) are readily incorporated into the magnetite and siderite crystal structure when the Fe(III)-reducing bacteria formed magnetite (Fredrickson *et al*., 2001; Roh *et al*., 2001). The ferrous iron and Fe(II)-containing minerals generated by the Fe(III)-reducing bacteria can chemically reduce multivalent metals such as U(VI), Cr(VI) and Tc(VII) and can abiotically reduce nitroaromatics and chlorinated solvents (Heijman *et al*., 1993, 1995).

Carbon sequestration implication of iron biomineralization

Siderite is a frequently observed diagenetic precipitate in recent aquatic and geologic sediments (Pye *et al*., 1990; Mortimer *et al*., 1997). Its formation is generally associated with the bacterial respiration of organic matter coupled with dissimilatory Fe(III) reduction (Suess, 1979; Pye *et al*., 1990). This study showed that the atmosphere and bicarbonate buffer concentration in conjunction with Fe biomineralization processes exhibited profound influences on Fe carbonate formation. Siderite formation with different concentrations of bicarbonate and under N_2-CO_2/H_2-CO_2 atmospheres indicates that the microbial siderite formation via reduction of Fe(III) oxides may occur naturally when such a ligand (HCO_3^-) and appropriate electron donors are in sufficient concentration.

This study indicates that siderite formation is generally associated with the bacterial respiration of organic matter or hydrogen coupled with microbial Fe(III) reduction with the conditions of reducing environment, CO_2 atmosphere, and high alkalinity (Suess, 1979; Pye *et al*., 1990; Fredrickson *et al*., 1998). Given the abundance of Fe in anaerobic sedimentary systems, the capacity of Fe(III)-reducing bacteria to precipitate siderite using iron oxides and dissolved Fe ion species could have a significant impact on carbon sequestration. In addition to precipitation of Fe carbonate minerals, the microbial utilization of organic matter and hydrogen may also contribute to direct or indirect precipitation of redox sensitive metals in subsurface environments.

CONCUSIONS

Geochemical and environmental factors in Fe biomineralization include atmospheric composition, bicarbonate buffer, pH, incubation temperature, incubation time, and species of bacteria. These parameters exhibit profound influences on the types of Fe minerals and morphology of siderite crystals. The *Shewanella* (W3-7-1, NV-1, and BrY) cultures under a H_2-CO_2 atmosphere reduced akaganeite and formed siderite in the absence of partially reduced magnetite. Magnetite was predominantly formed by Fe(III)-reducing bacteria using organic acids such as lactate and formate under a N_2 atmosphere. Mixtures of siderite and magnetite were significant components in Fe(III)-reducing cultures under a N_2-CO_2 atmosphere. The Fe(III)-reducing bacteria also formed magnetite and siderite using a medium buffered with $NaHCO_3$ (70–210 mM) and siderite precipitation increased with the increasing bicarbonate concentration.

The presence of high carbonate in the aqueous phase and a large reservoir of CO_2(g) to maintain aqueous bicarbonate concentration were important factors allowing the complete reduction of akaganeite and formation

of siderite in subsurface environments. Hence, the microbially directed formation of siderite may occur naturally when such a ligand and appropriate electron donors are in sufficient concentration. Given the abundance of Fe in anaerobic sedimentary systems, the sequestration of CO_2 by Fe biomineralization could have an equally significant impact. In addition to CO_2 sequestration, microbial reduction of akaganeite to siderite allows for greater oxidation of organic matter or contaminants than when magnetite is the end-product. Determining the potential importance of the microbial processes on carbonate mineral formation and gaining a fundamental understanding of the controlling factors, rate and extent of carbonate precipitation will significantly advance our understanding of carbon managements in the subsurface environments.

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