American Mineralogist, Volume 83, pages 1419–1425, 1998

Alteration of microbially precipitated iron oxides and hydroxides

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Abstract

Iron oxide and hydroxides can be precipitated from solution with both Fe^{2+} and Fe^{3+} states by a microbial consortium enriched from surface water draining a granitic batholith. The Fe^{2+}/Fe^{3+} ratio of the microbial precipitate is determined by both the initial environment and subsequent diagenesis. To evaluate the thermal aspects of diagenesis, biological precipitates, either largely Fe^{2+} or equally divided between Fe^{2+} and Fe^{3+} states, were heated at 80 °C for 12 weeks, under various redox conditions and compared to samples maintained under the same conditions at 4 °C. Mössbauer spectroscopy showed the iron oxide and hydroxides precipitated as Fe^{2+} to be more stable than that as Fe^{3+}. Only under air at 80 °C are the ferrous minerals altered to hematite, while the more labile ferric minerals are altered to $Fe(OH)_2$ at 4 °C and to hematite at 80 °C. In contrast, chemically precipitated Fe compounds, when incubated with the consortium, only form Fe^{3+} compounds, mainly fine-grained hematite. When no microbes are present, goethite is formed during diagenesis. Fe speciation in sediments may reflect a combination of microbial mediation that causes the initial precipitation of iron oxides and hydroxides and the subsequent conditions of the diagenetic processes characteristic of that particular depositional environment.

Introduction

Microorganisms play an important role in the natural environment by determining the speciation of Fe; they can also cause considerable Fe accumulation through biomineralization. For heterotrophic bacteria to grow they must have access to organic matter, and even in oligotrophic waters containing few nutrients, some microbial oxidation of organic material will take place. Such organisms obtain their energy by electron transfer from a reduced species to an oxidized one. Oxygen is the preferential electron acceptor, but where it is not available, inorganic compounds are utilized instead (Stumm and Morgan 1981). The most common electron acceptor in the natural environment is Fe, and because of its widespread abundance, groundwaters are generally reduced due to the activity of the Fe-reducing bacteria.

Ehrenberg (1836) was the first to put forward the suggestion that biological processes were important in the deposition of Fe-rich sediments. In 1888, Winogradsky showed that a bacterium, *Leptothrix,* was only able to live and grow where Fe^{2+} was present in solution. Harder and Chamberlin (1915) suggested that, although the precipitation of FeOOH from solution during the deposition of Fe-formations could be purely chemical, it was much more likely to have been caused by "well-known iron bacteria." However, Fe precipitation was regarded by most geologists as a chemical process, so at that time the idea that it could be due to biological processes was not generally accepted (Starkey and Halvorson 1927), and even when microorganisms were thought to be involved, their action was considered to be non-specific (Lovley 1995). It has never been demonstrated that the widespread reduction of Fe^{3+} to Fe^{2+} in nature could be mediated by chemical means alone. Indeed, Lovley (1991) has shown that actual contact with the bacterial cell is required for enzymatic reduction to occur. More importantly, it has become apparent that most, if not all, of the Fe reduction occurring in natural waters is microbially mediated (Schwertmann and Taylor 1989; Lovley 1991, 1995). Microbial metabolic reactions therefore have a widespread influence on the geochemistry of natural waters.

In oligotrophic terrestrial waters bacteria generally live as consortia that form biofilms preferentially at rock/water interfaces. A biofilm is a layer of slime that comprises extracellular polymeric substances (EPS), often polysaccharides, that are excreted by the consortium (Costerton et al. 1994). Individuals species of the consortium are arranged so that each one can contribute most efficiently to the biofilm ecosystem and, as a result, few free-living microorganisms are found in oligotrophic waters. Both the cell walls and the EPS in the biofilm are negatively charged so that positively charged metal ions, such as Fe^{2+} and Fe^{3+}, are adsorbed onto their surfaces. These metal ions may then act as nucleation sites for the deposition of further minerals from solution (Beveridge and Fyfe 1985).

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0003–004X/98/1112–1419$05.00

Although Fe is an essential element for all life, the amount required as a nutrient is relatively small. The biological accumulation of large amounts of Fe in the environment is the result of biomineralization. This biomineralization can take place through direct reactions that are metabolically mediated including assimilation for nutrition, but a very much larger volume is involved in the production of energy for microbial growth by Fe-reducing bacteria that use Fe^{3+} as an electron acceptor. Indirect reactions are passive physicochemical sorption of Fe^{3+} and Fe^{2+} ions onto the negatively charged microbial and EPS surfaces, as well as the influence on environmental pH and Eh of the local solution chemistry (McLean et al. 1996).

Fe-rich sediments contain Fe^{2+} and Fe^{3+} compounds in various oxide and hydroxide minerals. When Fe^{2+} is liberated from silicate minerals in the ionic state into natural waters, it is rapidly oxidized under aerobic conditions to the Fe^{3+} state; this is then precipitated as ferrihydrite ($5Fe_2O_3 \cdot 9H_2O$), hematite, or goethite (Schwertmann and Taylor 1989). These compounds undergo diagenesis, which is the process of physical and chemical change that occurs in sediments, both during and after deposition, without introduction of great heat or pressure (Fairbridge 1983). For instance, Southam and Beveridge (1994) have shown that fine-grained gold colloids immobilized within bacteria can be altered during diagenesis at 60 °C to coalesce and form gold crystals. There is little experimental data available on low-temperature diagenesis of Fe minerals, although hematite crystallites have been reported to develop through the redistribution of vacancies within the transient phase after 130 h at 92 °C (Combes et al. 1990). Because thermophilic Fe-reducing bacteria, able to grow up to a temperature of 75 °C, recently have been reported (Zhang et al. 1997), we chose a temperature of 80 °C for our diagenesis experiments; as a temperature above that at which bacteria might be active but well below that where metamorphism is generally considered to commence (Fairbridge 1983). Here we report the results of experiments on diagenetic change comparing biologically precipitated iron oxides and hydroxides with others that are inorganically precipitated.

In previous experiments, we had observed that the ratio of C to Fe in the media could determine the valence state of the biologically precipitated iron compounds. In nature, the initial Fe precipitate formed by microbial reactions would be the result of substrate availability, so that in an environment, where little C is present, the Fe will more likely be as Fe^{2+}. Because the state of the Fe precipitate could be so easily altered by differences in metabolism and/or Fe concentration, it was considered important to investigate whether, after the biological activity had ceased, the state of the environment would continue to affect the valance state. We discuss here the effect of environmental redox conditions during low temperature diagenesis on different forms of Fe minerals precipitated by this microbial consortium.

Using the consortium we have prepared biological precipitates where the Fe has various valances. For comparative experiments we also precipitated Fe compounds entirely by inorganic chemical means. The samples were heated at 80 °C for 12 weeks under three different redox conditions and compared with those obtained at 4 °C. Mössbauer spectroscopy was used to investigate the thermal effect on the precipitates.

Materials and methods

Bacterial cultures

The consortium used for these experiments was obtained from an enrichment of surface water draining the granitic Lac du Bonnet Batholith in the Canadian Shield of southeastern Manitoba (Brown et al. 1997). This consortium has similar metabolic reactions to that of the original microbial consortium taken from a depth of 400 m at the Underground Research Laboratory (URL) excavated by Atomic Energy of Canada Ltd. in Manitoba, Canada (Brown et al. 1994). The consortium is maintained in a laboratory bioreactor on media containing 5 g ferric ammonium citrate (FAC), 0.5 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, and 0.01 g $CaCl_2 \cdot 2H_2O$ in 1 L of deionized water, with an initial pH of 7.0 (Brown and Hamon 1993).

Cultures to form the microbially produced precipitates were grown for 7 to 10 d in 50 mL of media in 250 mL Erlenmeyer laboratory flasks. The media were similar to that above except that ammonium citrate and $FeCl_2$ were substituted for FAC so the ratio of Fe to C could be varied. The concentrations of Fe ranged between 2.5 and 7.5 m*M* and that for citrate from 15 to 20 m*M*. The ratios of Fe to citrate varied between 1:1.5 and 1:10. When the ratio of Fe to C was 1:5 or greater the precipitate largely had Fe^{2+} and was designated White; when the ratio was smaller than this, the precipitate, designated Red, contained both Fe^{3+} and Fe^{2+} states.

Cultures were centrifuged after growth, and the supernatant removed until only sufficient liquid was left to cover the solids. Approximately 25 ml of the concentrated White or Red precipitates were transferred to 100 mL Wheaton vials and sealed. Some vials were maintained with a head space of air, whereas in others the air was extracted and replaced either by a mixture of 80% N_2 with 20% CO_2, or by pure H_2. The head space was maintained by flushing at four weekly intervals. The vials were incubated at 80 °C (353 K) for 12 weeks, while the controls were maintained at 4 °C (277 K).

For the comparative experiments, a chemical precipitate was prepared by neutralizing 0.4 *M* $FeCl_3$ with NaOH. This precipitate was then used in experiments similar to those with the biological precipitate, but with ammonium citrate instead of FAC in the media; controls were sterile media.

For simplicity in experiment nomenclature, (W) represents the white biological precipitates and (R) the red; the head space gases are designated (A) for air, (N) for N_2/CO_2 and (H) for H_2; the diagenesis temperatures are (4) at 4 °C and (80) at 80 °C. For the chemically precip-

itated Fe compounds, (I) is used for the cultures that were inoculated and (C) for the sterile controls.

Mössbauer spectroscopy

To maintain the state of the Fe^{2+} component, the precipitates needed to be kept wet, so the samples were stored in plastic vials before analysis by Mössbauer spectroscopy. Some specimens, where the precipitate had settled, were measured directly through the vial, whereas others, where the precipitate was more dispersed, were placed on thick filter paper and wrapped in paraffin foil to maintain moisture. Mössbauer spectra of 14.4 kV γ-rays of ^{57}Fe were collected at room temperature using a 30 mCi ^{57}Co source in Rh matrix and a Kr-CO_2 proportional counter. The spectra were fitted by a least-squares iterative procedure. A superposition of doublets and sextuplets was assumed, with proper relationships between line positions and intensities that are given by interaction between ^{57}Fe nuclei and extra nuclear electric and magnetic fields in an unpolarized, untextured absorber. The standard reference absorber is α-Fe. Conventionally, the velocity scale in the spectra and the spectral parameters are given in units of millimeters per second (mm/s).

Results

Microbial Fe precipitation

Fe^{3+} is only soluble at a low pH, so chelation is required for it to remain in solution at the higher pH (7.5 to 8.5) found in Shield waters. Citrate traditionally has been used as a chelator for environmental microbial studies, because it also provides organic carbon for cell growth. Inoculation with our consortium into FAC media produced, within 24 h, a large orange/red precipitate, mainly as a colloidal Fe^{3+} gel. This precipitate was slowly reduced over a period of a week and the color turned almost black. The reduction of the precipitate was accompanied by a rise in pH to greater than 8.5, and a reduction of Eh, in some cases to below −300 mV. If the precipitate was left in the flask for several more weeks, it was slowly reoxygenated by atmospheric oxygen until the original red color returned. The changes in color gave a good initial indication of the state of the Fe precipitate as it was first reduced and then reoxidized.

We have been unable to obtain any Fe precipitation in the absence of this consortium. No precipitate was produced in the sterile FAC medium even when the pH was raised above 8.5 and the Eh reduced to below −300 mV by chemical means. Adding dead cells to provide point sources for nucleation of the precipitate also had no effect, but when this amended media was inoculated with a live culture, a precipitate was formed within 24 h. Active microbial metabolism therefore appears to be essential in this system to remove the citrate chelation and precipitate Fe ions.

The Mössbauer spectrum of the original biofilm from the URL has three different components (Fig. 1 and Table 1). The first is an Fe^{2+} doublet with a large isomer shift (IS ~ 1.2 mm/s) and large quadrupole splitting (QS > 2.8 mm/s); the second is an Fe^{3+} doublet with a small isomer shift (IS ~ 0.4 mm/s) and small quadrupole splitting (QS ~ 0.7 mm/s); whereas the third with only a small relative intensity, is a magnetically split sextuplet due to Fe^{3+}. The second and third components are both hematite, αFe_2O_3, but the particle size is smaller than 10 nm in the second component and larger in the third. In our laboratory-grown biofilms, there is a fine-grained precipitate with a mixture of Fe^{2+} and Fe^{3+} states with some larger hematite particles (Fig. 1, Table 1). Incubations with various ratios of citrate to Fe in the media produce several different Fe components in the precipitate. When the ratio of Fe to C is 1:5 or greater, ~70% of the Fe is precipitated in the Fe^{2+} state; this precipitate is initially whitish in color but becomes greener over a period of several weeks (White in Fig. 1 and Table 1). Where the ratio of Fe to C is below 1:5 the Fe precipitate in incubations is initially ~50% in the Fe^{3+} state. This precipitate is a very fine-

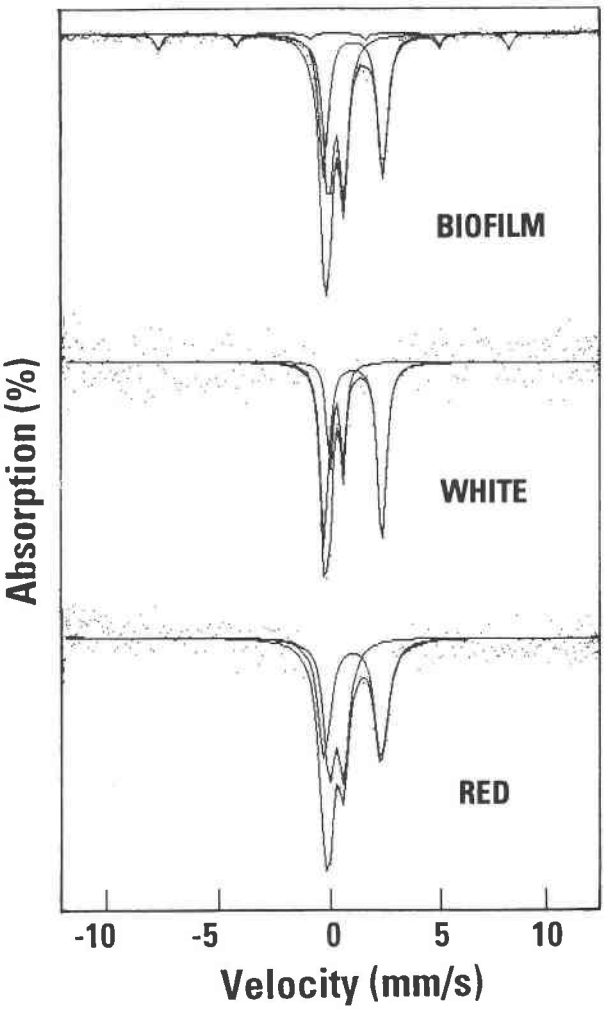

Figure 1. Mössbauer transmission spectra obtained at room temperature from a biofilm, and from White and Red precipitates obtained from laboratory cultures.

TABLE 1. Mössbauer parameters for biological precipitates

Sample	IS (mm/s)	QS (mm/s)	B_{hf} (T)	FWHM (mm/s)	A_i	Description
Biofilm	1.18 (1)	2.76 (1)	—	0.50 (2)	44 (1)	Fe^{2+}
	0.39 (1)	0.74 (1)	—	0.50 (1)	49 (1)	Fe^{3+}
	0.35 (2)	−0.21	51.3 (2)	0.36 (5)	7 (1)	Fe^{3+} α-Fe_2O_3 [>10 nm]
WHITE	1.18 (1)	2.70 (3)	—	0.43 (6)	71 (4)	Fe^{2+}
	0.40 (3)	0.64 (5)	—	0.48 (10)	29 (4)	Fe^{3+}
RED	1.16 (2)	2.67 (4)	—	0.72 (4)	53 (4)	Fe^{2+}
	0.41 (3)	0.69 (4)	—	0.62	47 (4)	Fe^{3+}

Notes: Data from ^{57}Fe for an original biofilm and WHITE and RED biologically produced precipitates obtained from flask incubations. IS = the isomer shift with respect to ^{57}Fe in metallic α-Fe; QS = the electric quadrupole splitting; B_{hf} = the hyperfine magnetic field; FWHM = the full line width at half maximum; and A_i = the relative spectral area of particular components. Last digit(s) statistical errors of parameters are given in parentheses, values given without errors were fixed during fitting.

grained colloidal gel that is first red in color (RED in Fig. 1 and Table 1), but which, during incubation, becomes nearly black as the Fe^{3+} is reduced to only ~20% of the total. The Mössbauer spectra for the WHITE and RED precipitates are very similar except the variation in the percentage of Fe^{2+} and Fe^{3+}.

Diagenesis at 80 °C of Fe precipitates

In the experimental procedure for diagenesis, both the WHITE and RED precipitates from the microbial cultures were kept at 80 °C for 12 weeks under each of the three head space gases with different reducing conditions. The Mössbauer spectra obtained after this diagenesis are shown in Figures 2 and 3. The results for the control samples kept at 4 °C are in the left panel in each figure, whereas the right panel shows results from samples that underwent diagenesis at 80 °C. The parameters for these spectra are listed in Table 2.

After diagenesis all but one of the WHITE Fe^{2+} samples show the Fe to be predominantly in the Fe^{2+} form with a small quantity of Fe^{3+} indicated by the small doublets for the samples under air at 4 °C (W4A) and under hydrogen at 80 °C (W80H). The one exception to this is the precipitate under air at 80 °C (W80A), where all the Fe occurs as Fe^{3+}. None of these spectra indicate any hyperfine magnetic interaction. The magnitude of the isomer shift for Fe^{2+} (IS = 1.17 to 1.24 mm/s) is typical of high-spin Fe^{2+} ions, but the quadrupole splitting (QS = 2.79 to 2.83 mm/s) is too large for the Fe^{2+} to be attributed to Fe^{2+} hydroxide, $Fe(OH)_2$, which has a considerably smaller quadrupole splitting (QS ~2.2 mm/s). The spectra for the RED Fe^{3+} precipitate show a superposition of Fe^{2+} and Fe^{3+} doublets. The Fe^{2+} doublet is probably $Fe(OH)_2$, although the spectrum of the reduced phase is not identical to that of $Fe(OH)_2$ in bulk (Stratmann and Hoffmann 1989).

The Fe^{3+} parameters from both RED and WHITE precipitates (IS = 0.21 to 0.37 mm/s and QS = 0.70 to 0.84

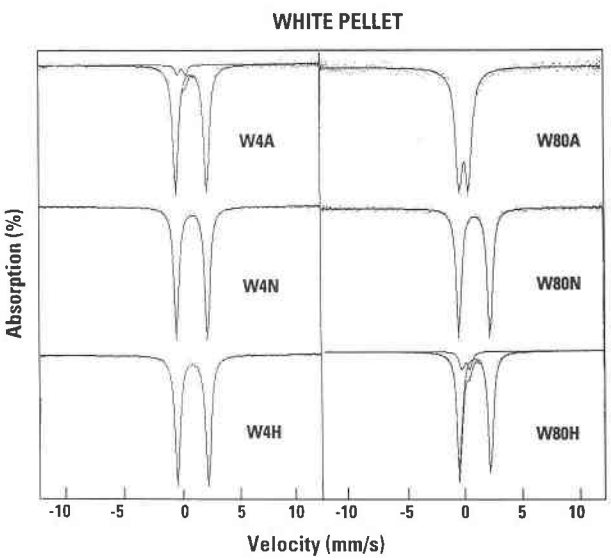

FIGURE 2. Mössbauer transmission spectra obtained at room temperature using a white (W) precipitate incubated at 4 °C (4) and 80 °C (80), under air (A), nitrogen/carbon dioxide (N) and hydrogen (H), for 12 weeks.

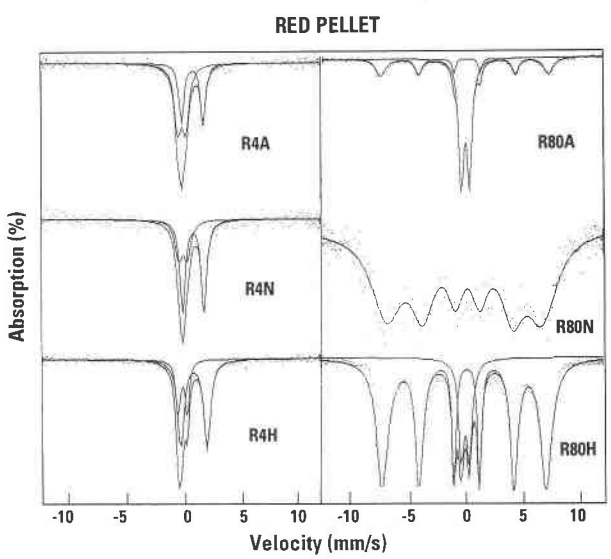

FIGURE 3. Mössbauer transmission spectra obtained at room temperature with a red (R) precipitate incubated at 4 °C (4) and 80 °C (80), under air (A), nitrogen/carbon dioxide (N) and hydrogen (H), for 12 weeks.

TABLE 2. Mössbauer parameters after 80 °C diagenesis

Sample	IS (mm/s)	QS (mm/s)	B_{hf} (T)	FWHM (mm/s)	A_i (%)	Description
W4A	1.21 (1)	2.80 (1)	—	0.56 (1)	95 (1)	Fe^{2+}
	0.37 (3)	0.76 (5)	—	0.38 (8)	5 (1)	Fe^{3+} α-Fe_2O_3 [<10 nm]
W4N	1.22 (2)	2.81 (1)	—	0.55 (1)	100	Fe^{2+}
W4H	1.22 (1)	2.79 (1)	—	0.55 (1)	100	Fe^{2+}
W80A	0.37 (1)	0.84 (1)	—	0.73 (2)	100	Fe^{3+} α-Fe_2O_3 [<10 nm]
W80N	1.22 (1)	2.80 (1)	—	0.52 (1)	100	Fe^{2+}
W80H	1.21 (1)	2.83 (1)	—	0.51 (1)	88 (1)	Fe^{2+}
	0.36 (1)	0.72 (4)	—	0.47 (5)	12 (1)	Fe^{3+} α-Fe_2O_3 [>10 nm]
R4A	1.17 (1)	1.95 (2)	—	0.52 (2)	42 (2)	Fe^{2+} $Fe(OH)_2$
	0.29 (1)	0.70 (2)	—	0.89 (3)	58 (2)	Fe^{3+}
R4N	1.24 (1)	1.95 (2)	—	0.57 (2)	65 (3)	Fe^{2+} $Fe(OH)_2$
	0.30 (3)	0.78 (6)	—	0.77 (6)	35 (3)	Fe^{3+}
R4H	1.23 (1)	2.38 (1)	—	0.68 (1)	64 (1)	Fe^{2+} $Fe(OH)_2$
	0.21 (1)	0.75 (2)	—	0.52 (2)	36 (1)	Fe^{3+}
R80A	0.40 (4)	−0.21 (2)	47.1 (1)	1.05 (4)	35 (1)	Fe^{3+} α-Fe_2O_3 [>10 nm]
	0.36 (1)	0.76 (1)	—	0.53 (1)	65 (1)	Fe^{3+} α-Fe_2O_3 [<10 nm]
R80N	0.38 (4)	−0.28 (6)	43.6 (3)	3.32 (15)	100	Fe^{3+} α-Fe_2O_3 [>10 nm]
R80H	0.38 (1)	−0.19 (1)	46.0 (1)	1.25 (2)	85 (1)	Fe^{3+} α-Fe_2O_3 [>10 nm]
	0.36 (2)	0.75 (2)	—	0.62 (3)	15 (1)	Fe^{3+} α-Fe_2O_3 [<10 nm]

Notes: Spectra of ^{57}Fe for white (W) and red (R) biological precipitates under air (A), nitrogen/carbon dioxide (N) and hydrogen (H), incubated at 4 °C (4) and 80 °C (80) for 12 weeks. IS = the isomer shift with respect to ^{57}Fe in metallic α-Fe; QS = the electric quadrupole splitting; B_{hf} = the hyperfine magnetic field; FWHM = the full line width at half maximum; and A_i = the relative spectral area of particular components. Last digit(s) statistical errors of parameters are given in parentheses, values given without errors were fixed during fitting.

mm/s) are typical of high-spin octahedrally coordinated Fe^{3+} ions and are compatible with ferric oxyhydroxide, ferrihydrite, and monocrystalline hematite. Quadrupole-split doublets seen in samples kept at 4 °C are characteristic of particles of hematite <10 nm and indicate super paramagnetic behavior due to the increasing relaxation time. At 298 K, a paramagnetic doublet is only seen for particles less than 10 nm. For a crystallite size of hematite or goethite of approximately 10 nm, fluctuations of the magnetic field result in a field distribution that is shifted to lower values than those of bulk crystal values. When these particles are greater than 10 nm, Mössbauer spectra at 298 K indicate they are magnetically split, whereas the smaller particles appear paramagnetic (Kündig et al. 1966). This would explain the magnetic splitting into sextuplets seen in the spectra of all the red precipitates under diagenesis at 80 °C, no matter what the head space gas.

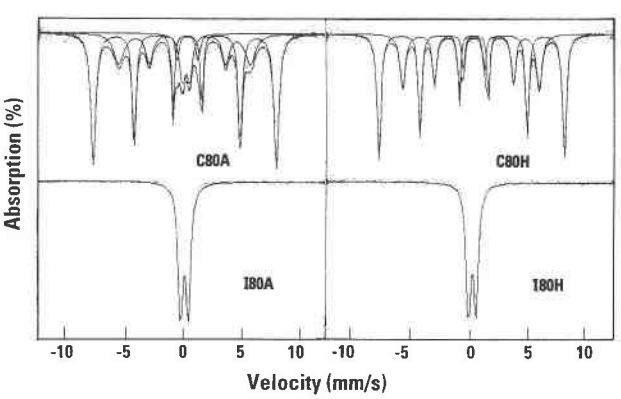

FIGURE 4. Mössbauer transmission spectra obtained at room temperature for sterile chemically precipitated Fe compounds (C) and incubated (I) at 4 °C (4) and 80 °C (80), under air (A), and hydrogen (H), for 12 weeks.

Chemically precipitated iron compounds

To investigate whether there was any difference between the behavior of biological and chemical precipitates, a chemical precipitate was incubated in ammonium citrate medium with the same microbial consortium (I in Fig. 4, Table 3). Control experiments contained the same chemical precipitate but were not inoculated (C in Fig. 4, Table 3). Only fine-grained hematite was formed when the chemical precipitate was incubated for a week, and this did not change during diagenesis (I80A and I80H). In the case of samples C4A and I4A, the Mössbauer spectra showed only Fe^{3+}, and there was no apparent difference between the sterile and inoculated samples at 4 °C. The spectra from this experiment do not, therefore, resemble the spectra of the Fe^{2+}/Fe^{3+} mixture produced from using the biological precipitate (Fig. 1). Diagenesis at 80 °C of the chemically precipitated Fe compounds again show only Fe^{3+}, but here 28% under air (C80A) and 39% under hydrogen (C80H) are in the form of goethite (α-FeOOH) with the remainder being hematite (α-Fe_2O_3).

DISCUSSION

We have shown previously that our microbial consortium exhibits active biomineralization through the precipitation of Fe in several different forms, the reduction of Fe^{3+} to Fe^{2+}, leaching of Fe from minerals such as magnetite present in a granitic host rock, as well as partial alteration of magnetite to hematite over a period of several weeks (Brown et al. 1994, 1997).

We have not yet defined the microbiology of the con-

TABLE 3. Mössbauer parameters for chemical precipitates.

Sample	IS (mm/s)	QS (mm/s)	B_{hf} (T)	FWHM (mm/s)	A_i (%)	Description
C4A	0.35 (1)	0.71 (1)	—	0.55 (1)		Fe^{3+}
C80A	0.37 (1)	−0.21 (1)	50.4 (1)	0.52 (1)	61 (2)	Fe^{3+} α-Fe_2O_3
	0.36 (2)	0.63 (3)	—	0.57 (4)	11 (1)	Fe^{3+} α-Fe_2O_3 [<10 nm]
	0.38	−0.28	36.2 (2)	1.07 (6)	28 (1)	Fe^{3+} α-FeOOH
C80H	0.37 (1)	−0.20 (1)	50.9 (1)	0.42 (1)	61 (1)	Fe^{3+} α-Fe_2O_3 [>10 nm]
	0.37 (1)	−0.27 (2)	37.5 (1)	0.59 (3)	39 (1)	Fe^{3+} α-FeOOH
I4A	0.31 (2)	0.76 (3)	—	0.59 (6)		Fe^{3+}
I80A	0.35 (1)	0.69 (1)	—	0.49 (1)		Fe^{3+}
I80H	0.35 (1)	0.68 (1)	—	0.48 (1)		Fe^{3+}

Notes: Spectra of ^{57}Fe for precipitates from chemically precipitated iron oxide sterile (C) and incubated (I), during diagenesis at 80 °C (80) and 4 °C (4), under air (A) and hydrogen (H). IS = the isomer shift with respect to ^{57}Fe in metallic α-Fe; QS = the electric quadrupole splitting; B_{hf} = the hyperfine magnetic field; FWHM = the full line width at half maximum; and A_i = the relative spectral area of particular components. Last digit(s) statistical errors of parameters are given in parentheses, values given without errors were fixed during fitting.

sortium, but initial results from phospholipid fatty acid analysis indicate that the majority of the bacteria are Gram negative with a few that are Gram positive. The profile also suggests *Shewanella* sp., which are known dissimilatory Fe-reducing bacteria, as well as the sulphate reducer *Desulfovibrio* sp. There are indications that *Pseudomonas* and *Actinomycetes* are also present. Transmission electron microscopy has confirmed the presence of a curved flagellated rod similar to *Desulfovibrio* sp., as well as many other bacteria with distinctive morphological features that are mainly Gram negative (Brown et al. 1998).

Biofilms that contain this consortium in the natural environment form siderite from Fe^{2+}, whereas hematite is produced from Fe^{3+} (Brown et al. 1994). These two minerals are deposited in close proximity because the biofilms are typically less than 10 mm thick. This implies that the very different stability fields required for the formation of these minerals must be produced on a micro scale by microbial activity within the biofilm itself. Therefore, at environmental temperatures (20 °C), it seems that the presence of microorganisms determines the state of the Fe precipitate, which can contain both Fe^{3+} and Fe^{2+} compounds.

Under our diagenetic thermal conditions the white precipitate was only completely oxidized when oxygen was present at 80 °C; in all other cases it was almost wholly in the Fe^{2+} state, indicating that further reduction probably takes place during diagenesis. The Fe^{3+} precipitates are much finer-grained than the Fe^{2+}, and may therefore be considerably more susceptible to a faster rate of alteration. When kept at 4 °C the red precipitate remained in the mixed Fe^{2+}/Fe^{3+} state, but during diagenesis at 80 °C, under all experimental redox conditions, the Fe was always oxidized to hematite.

The hyperfine parameters measured by Mössbauer spectroscopy of the colloidal particles of the biological precipitates are poorly defined and may differ significantly from those determined for the corresponding Fe compounds in the bulk crystal form. The spectra discussed in this work were obtained under ambient conditions (298 K). Much better resolution and information of the hyperfine interactions and nature of the particles could be obtained by measuring the spectra at low temperatures, but this was beyond the scope of out present study. In particular, the measurements at low temperatures would have allowed us to identify the distinct hyperfine magnetic patterns of Fe^{2+} hydroxide, similar to the identification we were able to make for siderite (Sawicki et al. 1995).

In general, the Mössbauer spectra we have obtained show two forms of high-spin Fe, Fe^{2+}, and Fe^{3+}. Broadening of the spectral lines can be credited to the poor definition of extra nuclear electric and magnetic fields in fine grained colloidal particles. Although the valence state of Fe can be defined with a good degree of certainty, the actual attribution of a specific compound to each spectral component requires great caution.

This was the first time we have encountered the formation of goethite in our work, but this was also the first time that we had investigated chemically precipitated Fe compounds in our media. After incubation the iron was still Fe^{3+} (Fig. 4, I4A), and the spectrum is quite distinct from the typical Fe^{2+}/Fe^{3+} mixture from the biological precipitates (Fig. 1). Even following diagenesis this spectrum did not alter (I80A). The formation of goethite only occurred after diagenesis using the chemical precipitate in the absence of the microbial consortium, mainly under a hydrogen head space but also to some extent under air.

Baltpurvins et al. (1996) suggest hematite is preferentially formed under neutral to alkaline pH conditions, whereas goethite will form at more extreme values of pH. Incubations of our chemical precipitates varied between pH 7 and 8, so that hematite should have been formed. The formation of hematite also depends on which anions are present; there is considerable organic carbon available in our incubations, particularly in the biofilm EPS with ample negative charges. As well, the Fe is precipitated within the biofilm matrix and this may alter the surface structure of the precipitate. Langmuir (1971) proposed that once hematite is formed it cannot rehydrate to form geothite, but our results indicate that although biofilm me-

diation is conducive to hematite formation, hematite can only be altered to goethite when no biomass is present.

Conclusion

The ultimate fate of precipitates such as these may be their incorporation into Fe-rich sediments. The oxidation state within sediments is often varied and may be controlled by the differing local environments during sedimentation and lithification. This could be a possible explanation for the variation in oxidation states of Fe that we have measured in banded Fe formations (Sawicki and Brown 1998), where our results supported the idea that the color of the band is determined to a large extent by the Fe^{2+}/Fe^{3+} ratio. The range of different Fe species that we have obtained, both from the microbially mediated precipitation and from low temperature diagenesis experiments, indicates that these could be the source of various compounds that are found in Fe-rich sediments.

Acknowledgments

This work was supported by NSERC through a Collaborative Grant to B.L.S., T.J. Beveridge, and M.B. McNeil, as well as a University Research Fellowship and a Research Grant to B.L.S. We thank the Associate Editor Jill Banfield and two anonymous reviewers for their comments, which have considerably improved the manuscript.

References cited

Baltpurvins, K.A., Burns, R.C., and Lawrance, G.A. (1996) Effect of pH and anion type on the aging of freshly precipitated iron(III) hydroxide sludges. Environmental Science and Technology, 30, 939–944.

Beveridge, T.J. and Fyfe, W.S. (1985) Metal fixation by bacterial cell walls. Canadian Journal of Earth Science, 22, 1893–1898.

Brown, D.A. and Hamon, C. (1994) Initial investigation of groundwater microbiology at AECL's Underground Research Laboratory. Atomic Energy of Canada Technical Record TR-608.

Brown, D.A., Kamineni, D.C., Sawicki, J.A., and Beveridge, T.J. (1994) Minerals associated with biofilms occurring on exposed rock in a granitic Underground Research Laboratory. Applied and Environmental Microbiology, 60, 3182–3191.

Brown, D.A., Sherriff, B.L., and Sawicki, J.A. (1997) Microbial transformation of magnetite to hematite. Geochimica et Cosmochimica Acta, 61, 3341–3348.

Brown, D.A, Beveridge, T.J., Keevil, C.W., and Sherriff, B.L. (1998) Evaluation of microscopic techniques to observe iron precipitation in a natural microbial biofilm. FEMS Microbiology Ecology in press.

Combes, J.M., Manceau, A., and Calas, G. (1990) Formation of Fe^{3+} oxides from aqueous solutions: A polyhedral approach by X-ray Absorption Spectroscopy: II. Hematite formation from Fe^{3+} gels. Geochimica et Cosmochimica Acta, 54, 1083–1091.

Costerton, J.W., Lewandowdki, Z., DeBeer, D., Caldwell, D., Korber, D., and James, G. (1994) Biofilms, the customized microniche. Journal of Bacteriology 176, 2137–2142.

Ehrenberg, D.C.G. (1836) Vorläufige Mittheilungen über das wirkliche Vorkommen fossiler Infusorien und ihre grosse Verbreitung. Annalen der Physik und Chemie, 38, 213–227.

Fairbridge, R.W. (1983) Syndiagenesis-anadiagenesis-epidiagenesis: phases in lithogenesis. In G. Larsen and G.V. Chilingar, Eds., Developments in Sedimentology, p. 17–113. Elsevier Science Publication Company, Amsterdam.

Harder, E.C. and Chamberlain, R.T. (1915) Geology of central Minas Gerais, Brasil. Journal of Geology, 23, 341–378.

Kündig, W, Bömmel, H., Constabaris, G., and Lindquist, R.H. (1966) Some properties of supported small-Fe_2O_3 particles determined with the Mössbauer effect. Physical Review 142, 327–333.

Langmuir, D. (1971) Particle size effect on the reaction goethite + hematite + water. American Journal of Science, 271, 147–156.

Lovley, D.R. (1991) Dissimulatory Fe^{3+} and Mn(IV) reduction. Microbiological Reviews, 55, 259–297.

——— (1995) Microbial reduction of iron, manganese and other metals. Advances in Agronomy, 54, 175–231.

McLean, R.J.C., Fortin, D., and Brown, D.A. (1996) Microbial metal-binding mechanisms and their relation to nuclear waste disposal. Canadian Journal of Microbiology, 42, 392–400.

Sawicki, J.A., Brown, D.A., and Beveridge, T.J. (1995) Microbial precipitation of siderite and protoferrihydrite in a biofilm. Canadian Mineralogist, 33, 1–6.

Sawicki, J.A. and Brown, D.A. (1998) Investigation of microbial-mineral interactions by Mössbauer spectroscopy. Hyperfine Interactions, in press.

Schwertmann, U. and Taylor, R.M. (1989) Iron Oxides. In J. Boris and S.B. Weed, Eds., Minerals in Soil Environments, Soil Science Society of America, p. 379–438. Madison, Wisconsin.

Starkey, R.L. and Halvorson, H.O. (1927) Studies on the transformation of iron in nature. Soil Science, 24, 381–402.

Southam, G. and Beveridge, T.J. (1994) The in vitro formation of placer gold by bacteria. Geochimica et Cosmochimica Acta, 58, 4527–4530.

Stratmann, M. and Hoffmann, K. (1989) In situ Mössbauer spectroscopic study of reactions within rust layers. Corrosion Science, 29, 1329–1352.

Stumm, W. and Morgan, J.J. (1981) Aquatic Chemistry. Wiley, New York.

Winogradsky, S. (1888) Ueber Eisenbacterian. Bontanishe Zeitung, 46, 261–270.

Zhang, C., Liu, S., Phelps, T.J., Cole, D.R., Horita, J., Fortier, S.M., Elless, M., and Valley, J.W. (1997) Physiochemical, mineralogical, and isotopic characterization of magnetite-rich iron oxides formed by thermophilic iron-reducing bacteria. Geochimica et Cosmochimica Acta, 61, 4621–4632.

Manuscript received February 16, 1998
Manuscript accepted August 10, 1998
Paper handled by Jillian F. Banfield