

The effect of microorganisms on Fe precipitation rates at neutral pH

Takeshi Kasama, Takashi Murakami *

Department of Earth and Planetary Science, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo (Sci. Bldg. 5), Bunkyo-ku, Tokyo 113-0033, Japan

Abstract

The effect of microorganisms on Fe precipitation rates at neutral pH in the field was examined. The studied area was a cave having Fe-stalactites composed mainly of ferrihydrite and associated microorganisms. The microorganisms were covered with ferrihydrite. Water associated with stalactites was slightly supersaturated with respect to ferrihydrite, and had a dissolved oxygen concentration of 2 ppm, a pH of 6, and an Fe concentration of approximately 14 ppm. Fe precipitation rates were estimated from decreases in Fe concentrations in water during flowing through the Fe-stalactites. The estimated Fe precipitation rate in the field ranges from 6.8×10^{-8} to 4.0×10^{-7} mol/l/s. These values are in good agreement with bulk estimates of Fe-stalactite growth rates derived from the length increase (1.3 cm) of one formation over 30 days. The estimated Fe precipitation rates are faster by about four orders of magnitude than expected inorganic precipitation rates. On-site Fe precipitation experiments with sterilized and unsterilized Fe-stalactites and without Fe-stalactites indicate that microorganisms are the controlling factor accelerating Fe precipitation rates at neutral pH. These results suggest that microbially accelerated Fe precipitation rates are more likely related to exopolysaccharides and microbial surface properties than metabolic precipitation mechanisms. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Fe-stalactites; Ferrihydrite; Precipitation

1. Introduction

Iron minerals occur widely in various geological environments at the Earth's surface, play an important role in Fe transport and distribution and are important adsorbents for various dissolved cations. Iron minerals can be formed by bacterial processes (e.g. Ferris et al., 1988; Ghiorse and Ehrlich, 1992; Mann et al., 1992; Brown et al., 1999). Iron(III) hydrolysis and precipitation proceed more rapidly

than Fe(II) oxidation at the Earth's surface, and therefore Fe(II) oxidation can be a rate-controlling influence on the formation of Fe minerals, especially ferrihydrite (Singer and Stumm, 1970; Brown et al., 1994; Kirby et al., 1999). Microorganisms such as *Thiobacillus ferrooxidans* catalyze the oxidation of Fe(II) in natural systems at low pH (Noike et al., 1983; Schrenk et al., 1998; Kirby et al., 1999), and thus accelerate the rate of Fe(II) oxidation (Singer and Stumm, 1970; Pesic et al., 1989; Kirby et al., 1999). Fe(II) oxidation rates are 10^5 to 10^6 times faster in acid mine drainage containing Fe-oxidizing bacteria such as *T. ferrooxidans* than abiotic laboratory rates under acidic pH conditions (Singer and Stumm, 1970; Noike et al., 1983; Williamson et al.,

* Corresponding author. Tel.: +81-3-5841-4541; fax: +81-3-3816-5714.

E-mail address: murakami@eps.s.u-tokyo.ac.jp (T. Murakami).

1992). However, the effect of Fe-oxidizing bacteria on Fe(II) oxidation rates has not been quantified adequately. For instance, Kirby et al. (1999) applied the rate law proposed by Pesic et al. (1989) to Fe oxidation rates in acid mine drainage with the presence of *T. ferrooxidans*, and found that the measured bacterial concentration is approximately 10^7 times lower than those required to show any significant bacterial effect on Fe(II) oxidation.

Barry et al. (1994) compared Fe(II) oxidation rates between untreated, gamma irradiated and autoclaved Fe(III) sediments in the laboratory at an initial Fe(II) concentration of 0.5 ppm and pH of ~ 5 ; Fe(II) oxidation rates decreased by a factor of 100 for gamma irradiated sediment and by ~ 2000 for autoclaved sediment, compared to untreated sediment. The differences in oxidation rate among the three sediments may be attributed to differences in pH and soil conditions (Barry et al., 1994).

Iron(II) oxidation is thought to occur predominantly inorganically at neutral pH, where oxidation rates obtained from field studies (Kirby and Elder Brady, 1998) are consistent with those calculated from the inorganic rate law of Stumm and Lee (1961). In these environments Fe-oxidizing bacteria such as *Gallionella* and *Leptothrix* are observed at groundwater seeps (Crerar et al., 1979; Lutters-Czekalla, 1990; Hallbeck et al., 1993; Emerson and Revsbech, 1994a; Ehrlich, 1996; Emerson and Moyer, 1997; Casanova et al., 1999). Hallbeck and Pedersen (1991) and Hallbeck et al. (1993) demonstrated that *G. ferruginea* grows autotrophically and mixotrophically with CO_2 , glucose, fructose and sucrose as carbon sources and Fe(II) as an electron donor. Brown et al. (1994) showed that Fe precipitates aerobically as ferrihydrite and anaerobically as siderite in neutral pH to weakly alkaline biofilms at depths of 400 m at the Underground Research Laboratory. Brown et al. (1998) indicated from the results of laboratory experiments at pH 7 that siderite (Brown et al., 1994) could occur only by the activity of microorganisms such as Fe-reducing bacteria. On the contrary, Ehrlich (1996) showed that there is no unequivocal evidence for Fe oxidation by *Gallionella*, and no clear evidence for enzymatic Fe oxidation by Fe-oxidizing bacteria at neutral pH. Thus, there is no definitive conclusion on the effect of microorganisms on Fe(II) oxidation at neutral pH.

Yoneyama (1998) found microorganisms in Fe-stalactites in a cave near the Seki hot spring, Niigata, Japan. This site is suitable for examining the role of microorganisms in the formation of Fe minerals at circumneutral pH. We investigated the mineralogy of Fe minerals occurring around the microorganisms present in the Fe-stalactites and measured Fe precipitation rates in the cave. We also did on-site Fe precipitation experiments with sterilized and unsterilized Fe-stalactites and without Fe-stalactites in the cave to examine the effect of microorganisms on Fe precipitation rates.

Fe-mineral precipitation includes reaction steps such as Fe(II) oxidation, Fe(III) hydrolysis and precipitation. Oxidation of Fe(II) is the rate controlling step at neutral pH (Singer and Stumm, 1970). Fe-mineral precipitation including Fe(II) oxidation is hereafter referred to as Fe precipitation in the present study to avoid unnecessary confusion.

2. Samples and methods

2.1. Samples

The Seki hot spring is located at the eastern foot of the Myoko Volcano in Niigata Prefecture, Japan. The rocks of this area are predominantly andesitic. Fe-stalactites (samples S1–S8) and initial water samples were collected from the ceiling at the entrance in the cave near the source of the hot spring. For the on-site Fe precipitation experiments mentioned below, we used another Fe-stalactite and water sample collected in the same way. The experimental procedures are given in Fig. 1 schematically.

2.2. Solid analysis

Solid-phase analyses (Fig. 1a) were carried out for Fe-stalactite samples S1–S4. Fe-stalactite samples were dried at room temperature for a week in advance for the solid analysis. Thin sections of the interiors of the Fe-stalactites were examined by light microscopy (LM). Density was determined by the Archimedes method; after a sample mass was measured in air, a mass of the sample in water, thinly coated with resin to avoid water penetration into the sample, was measured, yielding a volume estimate.

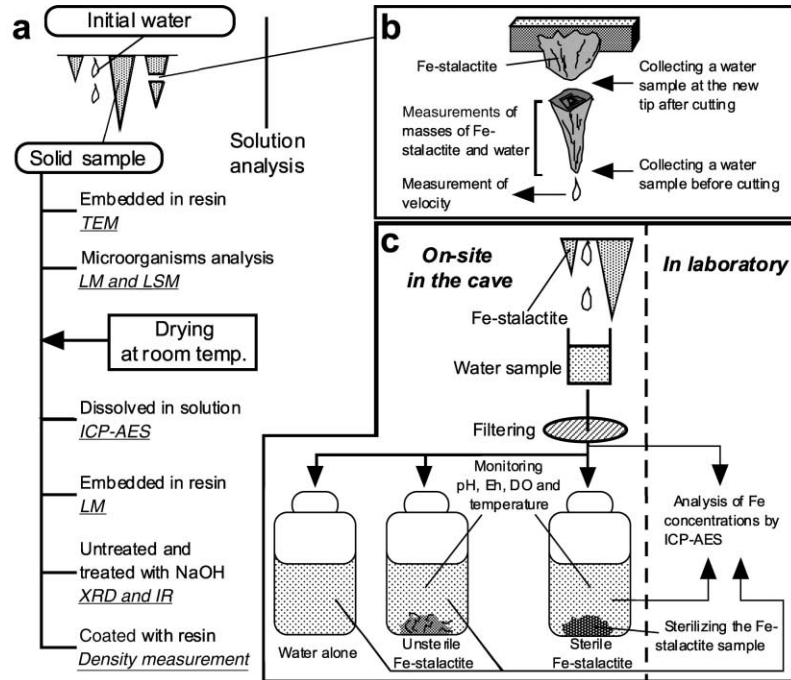


Fig. 1. Experimental procedures. (a) Flowchart of the procedures and methods, (b) illustration for the measurements of Fe precipitation rates in the field, and (c) illustration of the on-site Fe precipitation experiments with sterilized and unsterilized Fe-stalactites and without Fe-stalactite.

Chemical analysis of the Fe-stalactites was carried out by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Seiko Instruments, SPS7700). Twenty milligrams of sample was immersed into 100 ml of 1 M HCl solution and stirred with a magnetic stirrer at 80°C for 8 h. No precipitates were found in the acid digests and it was confirmed that all the materials were completely dissolved. The mass loss of the sample after drying at 110°C for 12 h was taken as mass of adsorbed water, $H_2O(-)$. The subsequent ignition at 1000°C for 4 h gave structural OH, $H_2O(+)$. The chemical compositions of the Fe-stalactites are given in Table 1.

Untreated and NaOH-treated samples of the Fe-stalactites were used for X-ray powder diffraction (XRD) and Fourier transform infrared (FT-IR) analysis. The treated sample was prepared as follows: an Fe-stalactite sample was immersed in 1 M NaOH solution at 80°C for 2 h and polymerized Si such as

biogenic opal and crystalline silica was extracted (Carlson and Schwertmann, 1981). XRD patterns were obtained by monochromatized $CuK\alpha$ radiation

Table 1
Chemical compositions of Fe-stalactites (wt.% for oven-dried material)

Elements	S1	S2	S3	S4
SiO ₂	17.29	17.28	16.03	17.31
Al ₂ O ₃	0.02	0.06	0.01	0.04
Fe ₂ O ₃	67.75	66.89	68.47	68.37
MnO	0.32	0.65	0.22	1.09
MgO	0.02	0.03	0.03	0.03
CaO	1.32	1.41	1.60	1.39
Na ₂ O	0.04	0.03	0.08	0.03
K ₂ O	0.02	0.01	0.02	0.01
P ₂ O ₅	0.19	0.28	0.24	0.26
H ₂ O(+)	8.23	8.98	8.16	8.59
Total	95.20	95.62	94.86	97.12
H ₂ O(-) ^a	16.75	15.96	16.88	15.88

^aBased on mass loss by heating at 110°C.

using X-ray diffractometer (Rigaku, RINT2000). For FT-IR analysis, a KBr pellet was made by homogenizing 200 mg KBr with 2 mg of sample. The FT-IR spectra were recorded using a JASCO FT/IR-350.

A sample of Fe-stalactite was dropped into a 2% liquid agar at 40–50°C, gently stirred, cooled with ice for 10 s, and fixed for 2 h with 2.5% glutaraldehyde in cacodylate buffer (pH 7.4), and then for 1.5 h in 1% OsO₄ in cacodylate buffer for transmission electron microscopy (TEM) examination. The sample was treated with ethanol, polypropylene oxide and resin by gradual substitution, and prepared as ultra thin sections (about 60 nm thick) by ultramicrotomy. TEM observations were carried out using a JEOL JEM2010 operating at 200 kV. Elemental analysis was made by energy dispersive X-ray spectrometry (EDS) attached to the TEM using a Kevex Sigma system software package.

2.3. Microorganism analysis

The presence of microorganisms in the Fe-stalactites was confirmed by laser scanning microscopy (LSM) (Olympus, FLUOVIEW) using ethidium bromide that stains DNA and RNA (Fisar et al., 1990). The morphologies of microorganisms in the Fe-stalactites were observed by LM (Fig. 1a).

2.4. Solution analysis

Fe(II) concentrations of initial water samples were measured by colorimetry using an *o*-phenanthroline method (Akai et al., 1999) in the field. The concentrations of NH₄⁺, some anions such as PO₄³⁻ and NO₃⁻ and dissolved oxygen (DO) were also analyzed by colorimetry in the field where Eh, pH and temperature in the water samples were measured. For the measurements of cation concentrations in the laboratory, 50 ml of each initial water sample was filtered at 0.22 μm, and then 1 ml of 50% HNO₃ was added to the water sample in the field to lower the pH and preserve the metal ions for analysis. They were analyzed for Si, Al, Fe (total), Mn, Mg, Ca, Na and K by ICP-AES. The chemistries of the initial water are given in Table 2.

2.5. Measurements of Fe precipitation rates in the field

As water runs down the side of or through an Fe-stalactite, it precipitates Fe-oxides along the way.

Table 2
Chemistries of initial water (ppm)

Si ^a	47.3	PO ₄ ³⁻ ^b	< 0.1
Al ³⁺ ^a	n.d.	NO ₂ ⁻ ^b	< 0.02
Fe(II) ^b	14.1	NO ₃ ⁻ ^b	< 0.1
	(13.8–14.2) ^c		
Fe(total) ^a	14.3	SO ₄ ²⁻ ^b	51.8
	(14.1–15.5) ^d		
Mn ²⁺ ^a	6.3	Cl ⁻ ^b	19.8
Mg ²⁺ ^a	20.4	DO ^b	1.7 (1.4–2.1) ^d
Ca ²⁺ ^a	88.7		
Na ⁺ ^a	173	Temp. (°C)	20.8 (19–21) ^d
K ⁺ ^a	15.1	pH	6.0 (6.0–6.1) ^d
NH ₄ ⁺ ^b	2.4	Eh (mV)	236 (229–242) ^d

Values without parentheses were averages of three samples in November 2000.

n.d. = not detected.

^aMeasured by ICP-AES.

^bMeasured by colorimetry.

^cThe parentheses indicated the range of concentrations measured in September 1999.

^dThe parentheses indicated the range of concentrations measured in September 1999 and November 2000.

Therefore, the Fe precipitation rates in the field were estimated by decreases in Fe concentrations in water samples before and after cutting Fe-stalactites (Fig. 1b). A water sample was collected at the tip of an Fe-stalactite. The volume of this sample, divided by the time required to collect it, yielded a rate of flow for the water. Another water sample was collected at the tip of the Fe-stalactite for the measurement of Fe concentration. Then, 15–25 cm of the Fe-stalactite was cut from the tip and another water sample was collected at the new tip (Fig. 1b). Water samples, 50 ml each, was filtered at 0.22 μm and then 1 ml of 50% HNO₃ was added to the water sample in the field. The Fe concentrations in the water samples were analyzed by ICP-AES. The cut Fe-stalactites were used to measure the mass of water contained in them. Cut Fe-stalactites were weighed then heated to 110°C for 12 h and weighed again. The mass loss on drying corresponds to the mass of water in the original cut stalactite. We carefully collected cut Fe-stalactites in plastic bags so as not to spill water from them. Estimates of Fe precipitation rates obtained through this method are given in Table 3.

We measured the length of an Fe-stalactite in the cave in August and October, 1996 and April, June, August and October, 1997 to compare the growth of

the Fe-stalactite with the Fe precipitation rates obtained by the above method.

2.6. On-site Fe precipitation experiments with sterilized and unsterilized Fe-stalactites and without Fe-stalactites

To examine the effect of microorganisms on the Fe precipitation rate, we carried out two on-site Fe precipitation experiments with sterilized and unsterilized Fe-stalactites. We prepared 265 ml of initial water sample and 15 g of gently crushed Fe-stalactite for each trial (Fig. 1c). One of the two Fe-stalactites was used for the live experiment and the other was used after heating at 120°C for 1 h for sterilization. Sterilization was confirmed by attempting to cultivate microbes from heated stalactite samples; a sterilized Fe-stalactite was used to inoculate solid media (Kucera and Wolfe, 1957; Hallbeck and Pedersen, 1990) containing FeS at pH 5.6. No colony growth was observed in media inoculated with the sterilized stalactite. In contrast, we found several kinds of colonies in solid media inoculated with an unsterile Fe-stalactite under the same conditions.

Cave water samples were filtered at 0.22 μm before on-site experiments (Fig. 1c). Filtered water samples were poured into bottles containing sterilized and unsterilized Fe-stalactites (Fig. 1c) and gently stirred at intervals of 20 min. Solutions of 10 ml were taken from the bottles at intervals of 20–200 min, filtered at 0.22 μm , acidified with 0.2 ml of 50% HNO_3 , then were taken back to the laboratory for Fe concentration measurements by ICP-AES. We measured water temperature, pH, Eh and DO concentrations at each time interval. Part of the filtered

initial water sample before the on-site experiments was treated in the same way and measured for initial Fe concentrations.

During on-site experiments, the temperature was $18 \pm 2^\circ\text{C}$ and DO concentrations were constant at ~ 2 ppm. We heated an Fe-stalactite at 120°C for 1 h, examined it by XRD, IR, TEM and confirmed no change in the Fe-stalactite after heating.

We carried out a separate on-site Fe precipitation experiment in which the filtered initial water sample was placed into a reaction bottle with no Fe-stalactite for 13 h (Fig. 1c). The supernatant of this trial was filtered and acidified with 0.2 ml of 50% HNO_3 and the Fe concentration in this solution was measured by ICP-AES.

3. Results

3.1. Mineralogy and chemistry of the Fe-stalactites

The XRD pattern of the untreated Fe-stalactite had two broad bands, indicating that the Fe-stalactite was X-ray amorphous or poorly crystalline (Fig. 2). After removing silica from the Fe-stalactite by NaOH treatment, there appeared two weak bands at about 0.26 and 0.15 nm (Fig. 2), consistent with two-line ferrihydrite or protoferrihydrite (Chukhrov et al., 1973; Vempati and Loeppert, 1986). The presence of two-line ferrihydrite was confirmed by TEM and we also found a small amount of higher crystalline ferrihydrite (Eggleton and Fitzpatrick, 1988; Drits et al., 1993; Janney et al., 2000) by TEM.

The IR spectrum of the untreated Fe-stalactite indicated the presence of amorphous silica and cal-

Table 3
Data for the estimation of Fe precipitation rates in the field

Sample	Water ^a (ml)	Fe-stalactite ^b (g)	Velocity ^c (ml/min)	Residence time (min)	Fe conc. before cutting (ppm)	Fe conc. after cutting (ppm)	Fe precipitation rate (mol/l/s)
S5	99	32	39.8	2.5	11.7	15.0	4.0×10^{-7}
S6	147	29	11.9	12.4	8.4	11.2	6.8×10^{-8}
S7	256	111	18.5	13.9	9.0	14.3	1.1×10^{-7}
S8	88	48	2.3	38.2	1.9	15.0	1.0×10^{-7}

^aAmount of water contained in an Fe-stalactite, considering 1 g of water as 1 ml of water.

^bMass after heating at 110°C.

^cVelocity of the flowing water.

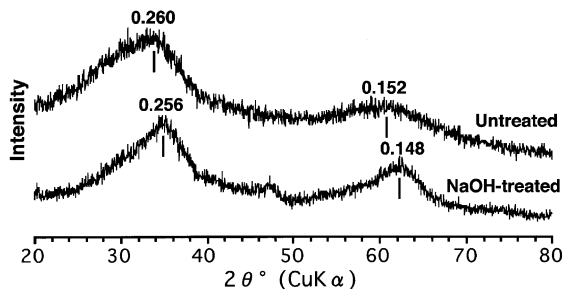


Fig. 2. XRD patterns of the untreated and NaOH-treated Fe-stalactites.

cite in addition to ferrihydrite (Fig. 3). TEM revealed that ferrihydrite coexisted with amorphous silica and calcite in the submicron scale. The NaOH treated Fe-stalactite had a band at 938 cm^{-1} (Fig. 3). The band at 938 cm^{-1} was related to Fe–O–Si bonding on the surface of ferrihydrite and the wave number of 938 cm^{-1} indicated that ferrihydrite contained 4–5 wt.% Si (Si/Fe = 0.14–0.17 in atomic ratio) (Schwertmann and Thalmann, 1976; Carlson and Schwertmann, 1981). Chemical analyses showed that the Fe-stalactites consisted of about 68 wt.% Fe_2O_3 and 17 wt.% SiO_2 (Table 1).

3.2. Microorganisms in the Fe-stalactites

The fluorescence image of an Fe-stalactite by LSM using ethidium bromide corresponded well to the

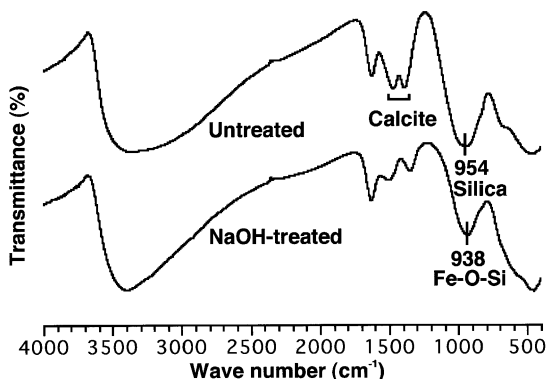


Fig. 3. IR spectra of the untreated and NaOH-treated Fe-stalactites.

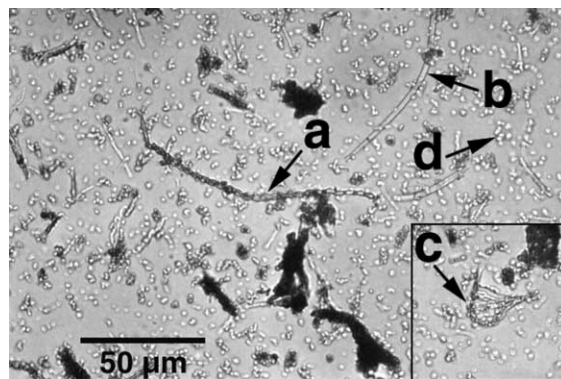


Fig. 4. Light micrograph of microorganisms in an Fe-stalactite. The major microorganisms are (a) stalk-like, (b) sheath-like, (c) fan-shaped, and (d) spherical. These microorganisms are not identified.

transmission one by LM, indicating that microorganisms were abundant in the Fe-stalactites. The major microorganisms present in the Fe-stalactites had stalk-like, sheath-like, fan-shaped and spherical structures (Fig. 4).

3.3. Relationships between ferrihydrite and microorganisms

The internal structure of an Fe-stalactite consisted of plate-like and filament-like materials (PI and Fi in Fig. 5) forming a framework. The plate- and fila-

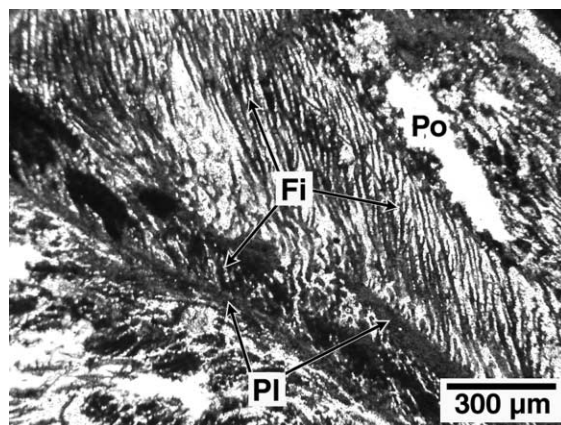


Fig. 5. Light micrograph of the interior of an Fe-stalactite. Fi: filament-like material, PI: plate-like material, and Po: pore.

ment-like materials were probably microorganisms on which ferrihydrite precipitated. The density of 0.52 g/cm^3 showed that the Fe-stalactites have a significantly porous structure (Po in Fig. 5).

Microorganisms were mostly covered with ferrihydrite that coexisted with amorphous silica and calcite (e.g. Fer in Fig. 6). TEM and EDS revealed that the atomic ratios of Si/Fe were similar to one another in the precipitates that occurred on the surfaces of microorganisms and distant from the surfaces. The average atomic ratio of Si/Fe of the precipitates, obtained by TEM-EDS, was 0.33. This value included the contribution of Si of amorphous silica as well as that of Si bound to ferrihydrite by Fe–O–Si bonding and thus, ferrihydrite and amorphous silica were distributed homogeneously in the precipitates. The crystallinity of ferrihydrite was not different between the positions of the precipitates, as was the atomic ratio of Si/Fe.

3.4. Chemistry of initial water

The initial water was slightly supersaturated with respect to ferrihydrite (Vempati and Loeppert, 1989). Comparison of Fe concentrations in the initial water between the field (*o*-phenanthroline method) and laboratory (ICP-AES) revealed that all the Fe in the initial water occurred as Fe(II) (Fe(II) and Fe(total) in Table 2).

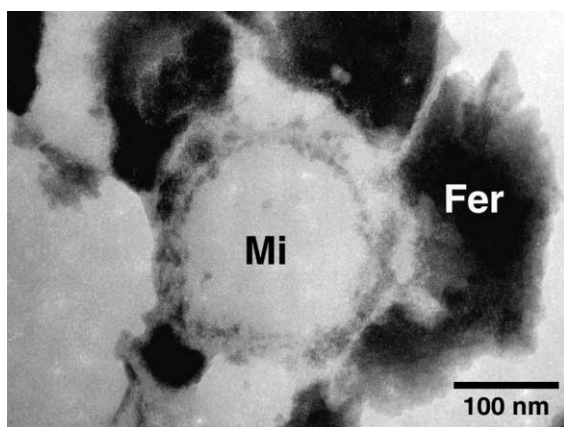


Fig. 6. TEM image of a microorganism (Mi) encrusted with ferrihydrite (Fer) that coexists with amorphous silica and calcite.

3.5. Iron precipitation rate in the field

We did find differences in Fe (substantially, Fe(II)) concentrations in the water samples before and after cutting the Fe-stalactites (Table 3). The differences in Fe concentrations were due to the formation of ferrihydrite in the Fe-stalactites. That is, the difference in Fe concentration was equivalent to the amount of Fe lost to the precipitation during transport. In the present study, all the mass of Fe calculated from the difference in Fe concentration before and after cutting an Fe-stalactite was assumed to be used to form ferrihydrite from dissolved Fe(II).

The Fe precipitation rates in the field (Table 3) were calculated by the equation

$$R = ([\text{Fe}_{\text{conc, after}}] - [\text{Fe}_{\text{conc, before}}]) / t \quad (1)$$

where R stands for the Fe precipitation rate (mol/l/s), $[\text{Fe}_{\text{conc, after}}]$ is the concentration of Fe (mol/l) after cutting an Fe-stalactite, $[\text{Fe}_{\text{conc, before}}]$ the concentration of Fe (mol/l) before cutting the Fe-stalactite, and t represents the residence time (s) of water contained in the cut Fe-stalactite ml/water velocity (ml/s). The residence time is the average time that water remains in an Fe-stalactite. The estimated Fe precipitation rate ranged from 6.8×10^{-8} to 4.0×10^{-7} mol/l/s (Table 3).

The growth rate of the Fe-stalactite for which we measured the length in the cave throughout more than a year was different between summer and winter, but it was constant in each season; 1.3 cm/month from April to October and 0.13 cm/month from October to April the following year. The growth rate of Fe-stalactites was very low in winter.

3.6. Iron precipitation with sterilized and unsterilized Fe-stalactites

The Fe concentrations in water samples decreased with time for the on-site Fe precipitation experiments with sterilized and unsterilized Fe-stalactites. The decrease in Fe concentration at each sampling interval from the initial Fe concentration (14.1 ppm) was converted to a precipitated Fe amount required to form ferrihydrite (Fig. 7), assuming all the decrease in Fe concentration was attributed to the formation of ferrihydrite. Iron loss by precipitation for the

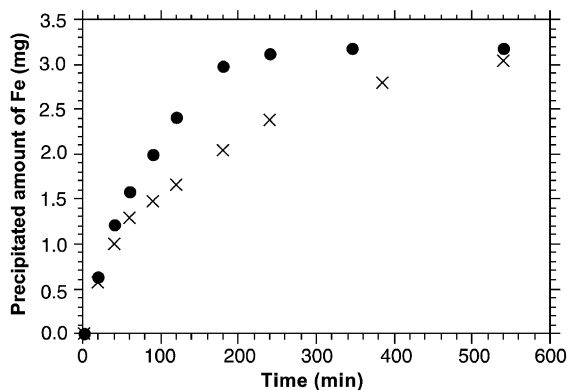


Fig. 7. Variations of precipitated Fe amounts with sterilized and unsterilized Fe-stalactites. Circles are with the unsterilized Fe-stalactite and crosses with the sterilized Fe-stalactite.

on-site experiment with an unsterilized Fe-stalactite was faster than with a sterilized Fe-stalactite. The Fe concentrations in the reactant solutions dropped to 10% from the initial value in 340 and 180 min for the on-site experiments with sterilized and unsterilized Fe-stalactites, respectively. During on-site experiments, the pH increased gradually and the Eh slightly decreased with time (Figs. 8 and 9, respectively). There was no significant difference in pH and Eh between experiments with sterilized and unsterilized Fe-stalactites.

The decrease in Fe concentration was extremely small for the reference experiment lacking an Fe-stalactite; the precipitated Fe amount was 0.042 mg for 13 h, which should be compared to about 3.2 mg

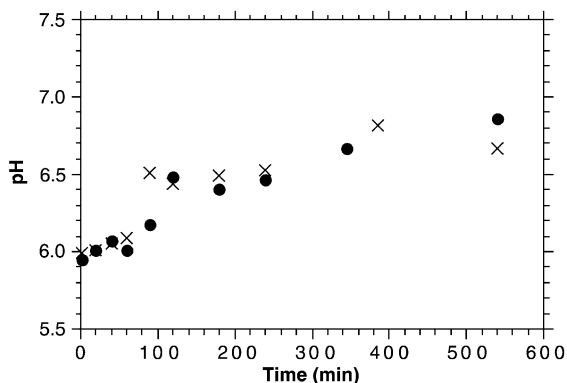


Fig. 8. Variations of pH in the solutions with sterilized and unsterilized Fe-stalactites. Circles are with the unsterilized Fe-stalactite and crosses with the sterilized Fe-stalactite.

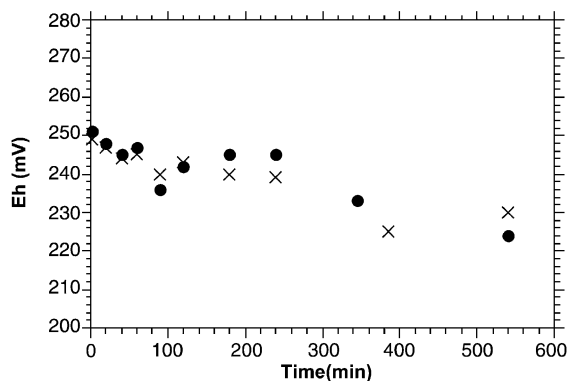


Fig. 9. Variations of Eh in the solutions with sterilized and unsterilized Fe-stalactites. Circles are with the unsterilized Fe-stalactite and crosses with the sterilized Fe-stalactite.

for 9 h in the experiment with the unsterilized Fe-stalactite (Fig. 7).

4. Discussion

4.1. Characteristics of ferrihydrite

Ferrihydrite surrounds the individual surfaces of the microorganisms (Fig. 6) and is a major constituent of the Fe-stalactites. The ferrihydrite shows poor crystallinity with a two-ring pattern, the chemical composition is rich in Si and there is no essential difference in crystallinity and composition of ferrihydrite on the surfaces of microorganisms and apart from cell surfaces. The presence of Fe–O–Si bonding on the surface of ferrihydrite (Fig. 3) prevents it from developing more ordered crystalline structures such as hematite and goethite (Henmi et al., 1980; Carlson and Schwertmann, 1981; Vempati and Loepert, 1989; Mayer and Jarrell, 1996). Therefore, ferrihydrite in the Fe-stalactites is stable and does not readily recrystallize to more ordered Fe phases.

4.2. Factors controlling Fe precipitation rates in the field

Estimated field Fe precipitation rates ranged from 6.8×10^{-8} to 4.0×10^{-7} mol/l/s (Table 3) and are dependent on Fe concentration in an Fe-stalactite during water transport, volume of water in the Fe-

stalactite and water through-flow rate. We observed independently that an Fe-stalactite grows in length by ~ 1.3 cm in a month, which is consistent with the mass of ferrihydrite estimated from our measured precipitation rates to grow in that time.

We calculated an Fe precipitation rate based on the equation by Stumm and Lee (1961) and compared it with our measured field Fe-precipitation rates. The assumed conditions for the calculation were a pH of 6, a DO concentration of 2 ppm, an Fe(II) concentration of 14 ppm and a temperature of 20°C (Table 2). The calculated, inorganic Fe precipitation rate is 9.4×10^{-12} mol/l/s, which is slower by about four orders of magnitude than the observed rates (Table 3).

The Fe(II) oxidation or Fe precipitation rate is affected by such factors as ferric hydroxide (Tamura et al., 1976; Sung and Morgan, 1980; Barry et al., 1994), organic acids (Theis and Singer, 1974; Miles and Brezonik, 1981), Cl^- and SO_4^{2-} (Sung and Morgan, 1980), Cu^{2+} , Mn^{2+} and Co^{2+} (Stumm and Lee, 1961), and the presence of microorganisms (Singer and Stumm, 1970; Pesic et al., 1989; Emerson and Revsbech, 1994b; Kirby et al., 1999). The precipitated Fe amount, 0.042 mg for 13 h for the reference on-site lacking an Fe-stalactite, yields an Fe precipitation rate of 8.0×10^{-11} mol/l/s, which is one order of magnitude larger than 9.4×10^{-12} mol/l/s, the calculated, inorganic Fe precipitation rate. The stalactite-free value, 8.0×10^{-11} mol/l/s, includes the effects of factors such as aqueous organic acids, anions and cations. Ferric hydroxide affects the rate when the pH is 7 and higher (Sung and Morgan, 1980). Therefore, factors other than microorganisms accelerate inorganic Fe precipitation rates only by one order of magnitude and cannot explain the observed Fe precipitation rates.

4.3. Effect of microorganisms on the Fe precipitation rate

An inorganic Fe precipitation rate can be related to Fe concentration at a given temperature if pH and DO concentration are known (Stumm and Lee, 1961). The temperature and DO concentration are almost constant during the on-site Fe precipitation experiments, and the pH difference between the sterilized

and unsterilized Fe-stalactite experiments is negligible (Fig. 8). In addition, factors other than microorganisms do not accelerate inorganic Fe precipitation rates significantly. Therefore, the Fe precipitation rate as a function of Fe concentration obtained from the on-site Fe precipitation experiments with sterilized and unsterilized Fe-stalactites can provide a quantitative estimate of the effect of microorganisms on the Fe precipitation rate if compared with an inorganic rate.

To estimate the effect of microorganisms on the Fe precipitation rate, we used the data in Fig. 7 and obtained a relationship between Fe precipitation rates and Fe concentrations in the solutions (Fig. 10). Each Fe precipitation rate in Fig. 10 was obtained from the slope of two neighboring data points in Fig. 7, and Fe concentration from an average of the two neighboring data points (see caption of Fig. 10). We also give calculated, inorganic Fe precipitation rates as a function of Fe concentration based on the equation by Stumm and Lee (1961) in Fig. 10,

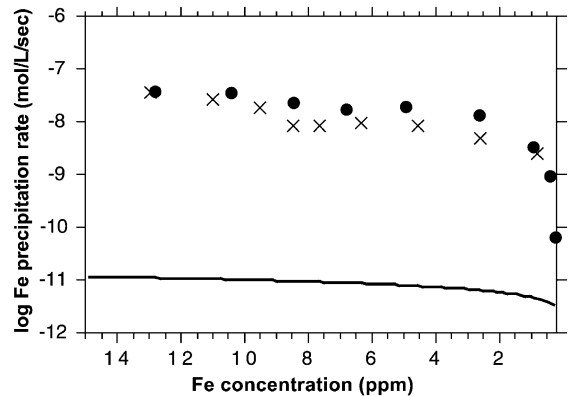


Fig. 10. Variations of Fe precipitation rates as a function of Fe concentration in solution deduced from the data points in Fig. 7. Circles are with the unsterilized Fe-stalactite and crosses with the sterilized Fe-stalactite. The curve is a calculated, inorganic Fe precipitation rate based on the equation by Stumm and Lee (1961). The data deduction from Fig. 7 to Fig. 10 is: the horizontal axis = $(\text{Fe}_{\text{conc},a} + \text{Fe}_{\text{conc},b})/2$ (ppm) and the vertical axis = $\log [(P_b - P_a)/(T_b - T_a)/(W_a + W_b)/2]$ (mol/l/s) where T_a (s) is the sampling time, T_b (s) the sampling time next to T_a , $\text{Fe}_{\text{conc},a}$ (ppm) the Fe concentration in the solution in the reaction bottle at T_a , $\text{Fe}_{\text{conc},b}$ (ppm) the Fe concentration in the solution in the reaction bottle at T_b , P_a (mol) the precipitated Fe amount at T_a , P_b (mol) the precipitated Fe amount at T_b , W_a (ml) the volume of the solution in the reaction bottle at T_a , and W_b (ml) the volume of the solution in the reaction bottle at T_b .

assuming a constant DO concentration, 2 ppm (see Section 2.6), and considering pH change with time (Fig. 8). The Fe precipitation rates with sterilized and unsterilized Fe-stalactites at an Fe concentration of 14 ppm are approximately within the range of those in the field (Fig. 10 and Table 3).

There are three possibilities for the effect of microorganisms on the Fe precipitation rate: (1) the metabolism of microorganism (e.g. Ehrlich, 1996); (2) the surface sites of microorganisms that might have $-\text{COOH}$, $-\text{OH}$, and $-\text{NH}_2$ groups (e.g. Stone, 1997), and exopolysaccharides (e.g. Clarke et al., 1997); and (3) a combination of the above two. The Fe precipitation rates with the sterilized Fe-stalactite (crosses in Fig. 10) correspond to those affected only by the surface sites of microorganisms and exopolysaccharides and those with the unsterilized Fe-stalactite (circles in Fig. 10) by a combination of the surface sites and exopolysaccharides, and the metabolism of microorganisms. Therefore, the difference between the crosses and circles in Fig. 10 is attributed to the effect of the metabolism of microorganisms.

There is a difference by three or four orders of magnitude between the calculated, inorganic Fe precipitation rate and those with sterilized and unsterilized Fe-stalactites, and the difference in Fe precipitation rate between sterilized and unsterilized Fe-stalactites is a factor of two to three (Fig. 10). Because the Fe precipitation rate without Fe-stalactite, 8.0×10^{-11} mol/l/s, is faster by at most one order of magnitude than the calculated, inorganic Fe precipitation rate, about 1×10^{-11} mol/l/s at an Fe concentration of 14 ppm, the above differences strongly indicate that the major factor to control the observed Fe precipitation rates in the field is exopolysaccharides and the surfaces of microorganisms. Although the activity of living microorganisms is lost by sterilization, dead microorganisms can supply exopolysaccharides and surface sites for catalysis of Fe precipitation. Thus, microorganisms control the Fe precipitation rate even at neutral pH and for water supersaturated with respect to ferrihydrite.

Emerson and Revsbech (1994b) concluded that 50–80% of Fe precipitation is attributed to the metabolism of microorganisms in laboratory experiment with the presence of *Gallionella*, *Leptothrix*

and unicellular bacteria and 3–6 ppm of Fe concentration at pH of 6.9–7.2. Similarly, our result shows a small difference in Fe precipitation rate between sterilization and unsterilization, which suggests the metabolism of microorganisms slightly accelerate the Fe precipitation rate in the field. However, because we carried out the on-site Fe precipitation experiments once and the difference in Fe precipitation rate between sterilization and unsterilization is small, the effect of the metabolism of microorganisms is not yet certain.

5. Conclusions

Iron-stalactites in which microorganisms were covered with ferrihydrite were found on the ceiling of a cave near the source of a hot spring in Niigata, Japan. Fe precipitation rates were measured in the cave and the effects of microorganisms on Fe precipitation rates at neutral pH was examined. Initial water was slightly supersaturated with respect to ferrihydrite, had a pH of 6 and an Fe concentration of approximately 14 ppm. The major conclusions drawn from the present study are as follows.

(1) Precipitated ferrihydrite is homogeneous structurally and chemically in the Fe-stalactites even on the surfaces of the microorganisms.

(2) The Fe precipitation rates in the field are estimated from decreases in Fe concentrations in water flowing through the Fe-stalactites, volumes of water contained in the Fe-stalactites, and velocities of water flowing through the Fe-stalactites. The Fe precipitation rate ranges from 6.8×10^{-8} to 4.0×10^{-7} mol/l/s. Fe-stalactite growth rates were approximately 1.3 cm/month (in summer season), based on the measured length of an Fe-stalactite measured in the cave over approximately 1 year.

(3) The estimated Fe precipitation rates in the field are faster by about four orders of magnitude than those calculated assuming inorganic Fe precipitation alone.

(4) Factors such as ferric hydroxide, organic acids, anions such as Cl^- and SO_4^{2-} and cations such as Cu^{2+} , Mn^{2+} and Co^{2+} do not affect significantly Fe precipitation rates in the field. This is confirmed by the on-site Fe precipitation experiment without an Fe-stalactite.

(5) The on-site Fe precipitation experiments with sterilized and unsterilized Fe-stalactites and without Fe-stalactite reveal that microorganisms accelerate the Fe precipitation rate by about four orders of magnitude compared with inorganic processes even with water supersaturated with respect to ferrihydrite and at neutral pH.

(6) The on-site Fe precipitation experiments suggest that the accelerated Fe precipitation rate is more related to exopolysaccharides and the surfaces of microorganisms than their metabolism.

Acknowledgements

We are grateful to J.F. Banfield at the University of Wisconsin-Madison and T. Watanabe and T. Oba at Joetsu University of Education for valuable suggestions and encouragement. We thank N. Kohyama and T. Sakai of National Institute of Industrial Health, for their technical assistance in preparing biological samples for TEM, and Y. Yoneyama, Joetsu University of Education, for the introduction of the ideal field for our purpose. This manuscript was greatly improved by the comments of J.R. Haas and three anonymous reviewers. The electron microscopy was performed at the Electron Microbeam Analysis Facility for Mineralogy in the Department of Earth and Planetary Science, the University of Tokyo. The present study was supported by a Science Grant of the Ministry of Education, Science and Culture.

References

- Akai, J., Akai, K., Ito, M., Nakano, S., Maki, Y., Sasagawa, I., 1999. Biologically induced iron ore at Gunma iron mine, Japan. *Am. Mineral.* 84, 171–182.
- Barry, R.C., Schnoor, J.L., Schlberger, B., Sigg, L., Stumm, W., 1994. Iron oxidation kinetics in an acidic alpine lake. *Water Res.* 28, 323–333.
- Brown, D.A., Kamineni, D.C., Sawicki, J.A., Beveridge, T.J., 1994. Minerals associated with biofilms occurring on exposed rock in a granitic Underground Research Laboratory. *Appl. Environ. Microbiol.* 60, 3182–3191.
- Brown, D.A., Sawicki, J.A., Sherriff, B.L., 1998. Alteration of microbially precipitated iron oxides and hydroxides. *Am. Mineral.* 83, 1419–1425.
- Brown, D.A., Sherriff, B.L., Sawicki, J.A., Sparling, R., 1999. Precipitation of iron minerals by a natural microbial consortium. *Geochim. Cosmochim. Acta* 63, 2163–2169.
- Carlson, L., Schwertmann, U., 1981. Natural ferrihydrites in surface deposits from Finland and their association with silica. *Geochim. Cosmochim. Acta* 45, 421–429.
- Casanova, J., Bodenau, F., Negrel, P., Azaroual, M., 1999. Microbial control on the precipitation of modern ferrihydrite and carbonate deposits from the Cezallier hydrothermal springs (Massif Central, France). *Sediment. Geol.* 126, 125–145.
- Chukhrov, F.V., Zvyagin, B.B., Ernilova, L.P., Gorshkov, A.I., 1973. New data on iron oxides in the weathering zone. *Proc. Int. Clay Conf.*, 333–341, Madrid.
- Clarke, W.A., Konhauser, K.O., Thomas, J.C., Bottrell, S.H., 1997. Ferric hydroxide and ferric hydroxysulfate precipitation by bacteria in an acid mine drainage lagoon. *FEMS Microbiol. Rev.* 20, 351–361.
- Crerar, D.A., Knox, G.W., Means, J.L., 1979. Biogeochemistry of bog iron in the New Jersey pine barrens. *Chem. Geol.* 24, 111–135.
- Drits, V.A., Sakharov, B.A., Salyn, A.L., Manceau, A., 1993. Structural model for ferrihydrite. *Clays Clay Miner.* 28, 185–207.
- Eggleton, R.A., Fitzpatrick, R.W., 1988. New data and a revised structural model for ferrihydrite. *Clays Clay Miner.* 36, 111–124.
- Ehrlich, H.L., 1996. Geomicrobiology of iron. In: Ehrlich, H.L. (Ed.), *Geomicrobiology*. Marcel Dekker, New York, pp. 312–388.
- Emerson, D., Revsbech, N.P., 1994a. Investigation of an iron-oxidizing microbial mat community located near Aarhus, Denmark: field studies. *Appl. Environ. Microbiol.* 60, 4022–4031.
- Emerson, D., Revsbech, N.P., 1994b. Investigation of an iron-oxidizing microbial mat community located near Aarhus, Denmark: laboratory studies. *Appl. Environ. Microbiol.* 60, 4032–4038.
- Emerson, D., Moyer, C., 1997. Isolation and characterization of novel iron-oxidizing bacteria that grow at circumneutral pH. *Appl. Environ. Microbiol.* 63, 4784–4792.
- Ferris, F.G., Fyfe, W.S., Beveridge, T.J., 1988. Metallic ion binding by *Bacillus subtilis*: implicating for the fossilization of microorganisms. *Geology* 16, 149–152.
- Fisar, Z., Hysek, J., Binek, B., 1990. Quantification of airborne microorganism and investigation of their interactions with non-living particles. *Int. J. Biometeorol.* 34, 189–193.
- Ghiorse, W.C., Ehrlich, H.L., 1992. Microbial biomineralization of iron and manganese. In: Skinner, H.C.W., Fitzpatrick, R.W. (Eds.), *Biomineralization: Processes of Iron and Manganese*. Catena Verlag, Cremlingen, Germany, pp. 75–99.
- Hallbeck, L., Pedersen, K., 1990. Culture parameters regulating stalk formation and growth rate of *Gallionella ferruginea*. *J. Gen. Microbiol.* 136, 1675–1680.
- Hallbeck, L., Pedersen, K., 1991. Autotrophic and mixotrophic growth of *Gallionella ferruginea*. *J. Gen. Microbiol.* 137, 2657–2661.
- Hallbeck, L., Stahl, F., Pedersen, K., 1993. Phylogeny and phenotypic characterization of the stalk-forming and iron-oxidizing bacterium *Gallionella ferruginea*. *J. Gen. Microbiol.* 139, 1531–1535.
- Henmi, T., Wells, N., Childs, C.W., Parfitt, R.L., 1980. Poorly

- ordered iron-rich precipitates from springs and streams on andesitic volcanoes. *Geochim. Cosmochim. Acta* 44, 365–372.
- Janney, D.E., Cowley, J.M., Buseck, P.R., 2000. Transmission electron microscopy of synthetic 2- and 6-line ferrihydrite. *Clays Clay Miner.* 48, 111–119.
- Kirby, C.S., Elder Brady, J.A., 1998. Field determination of Fe²⁺ oxidation rates in acid mine drainage using a continuously stirred tank reactor. *Appl. Geochem.* 13, 509–520.
- Kirby, C.S., Thomas, H.M., Southam, G., Donald, R., 1999. Relative contributions of abiotic and biological factors in Fe(II) oxidation in mine drainage. *Appl. Geochem.* 14, 511–530.
- Kucera, S., Wolfe, R.S., 1957. A selective enrichment method for *Gallionella ferruginea*. *J. Bacteriol.* 74, 344–349.
- Lutters-Czekalla, S., 1990. Lithoautotrophic growth of the iron bacterium *Gallionella ferruginea* with thiosulfate or sulfide as energy source. *Arch. Microbiol.* 154, 417–421.
- Mann, H., Tazaki, K., Fyfe, W.S., Kerrich, R., 1992. Microbial accumulation of iron and manganese in different aquatic environments: an electron optical study. In: Skinner, H.C.W., Fitzpatrick, R.W. (Eds.), *Bio-mineralization: Processes of Iron and Manganese*. Catena Verlag, Cremlingen, Germany, pp. 115–132.
- Mayer, T.D., Jarrell, W.M., 1996. Formation and stability of iron(II) oxidation products under natural concentrations of dissolved silica. *Water Res.* 30, 1208–1214.
- Miles, C.J., Brezonik, P.L., 1981. Oxygen consumption in humic-colored waters by a photochemical ferrous–ferric catalytic cycle. *Environ. Sci. Technol.* 15, 1089–1095.
- Noike, T., Nakamura, K., Matsumoto, J., 1983. Oxidation of ferrous iron by acidophilic iron-oxidizing bacteria from a stream receiving acid mine drainage. *Water Res.* 17, 21–27.
- Pesic, B., Oliver, D.J., Wichlacz, P., 1989. An electrochemical method of measuring the oxidation rate of ferrous to ferric iron with oxygen in the presence of *Thiobacillus ferrooxidans*. *Biotechnol. Bioeng.* 33, 428–439.
- Schrenk, M.O., Edwards, K.J., Goodman, R.M., Hamers, R.J., Banfield, J.F., 1998. Distribution of *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*: implications for generation of acid mine drainage. *Science* 279, 1519–1522.
- Schwertmann, U., Thalmann, H., 1976. The influence of (Fe(II)), (Si) and pH on the formation of lepidocrocite and ferrihydrite during oxidation of aqueous FeCl₂ solutions. *Clay Miner.* 11, 189–200.
- Singer, P.C., Stumm, W., 1970. Acidic mine drainage: the rate-determining step. *Science* 167, 1121–1123.
- Stone, A.T., 1997. Reaction of extracellular organic ligands with dissolved metal ions and mineral surfaces. In: Banfield, J.F., Nealson, K.H. (Eds.), *Geomicrobiology: Interactions between Microbes and Minerals*. Mineralogical Society of America, Washington, DC, pp. 309–344.
- Stumm, W., Lee, G.F., 1961. Oxygenation of ferrous iron. *Ind. Eng. Chem.* 53, 143–146.
- Sung, W., Morgan, J.J., 1980. Kinetics and product of ferrous iron oxygenation in aqueous systems. *Environ. Sci. Technol.* 14, 561–568.
- Tamura, H., Goto, K., Nagayama, M., 1976. The effect of ferric hydroxide in the oxygenation of ferrous ions in neutral solutions. *Corros. Sci.* 16, 197–207.
- Theis, T.L., Singer, P.C., 1974. Complexation of iron(II) by organic matter and its effect on iron(II) oxygenation. *Environ. Sci. Technol.* 8, 569–573.
- Vempati, R.K., Loepfert, R.H., 1986. Synthetic ferrihydrite as a potential Fe amendment in calcareous soils. *J. Plant Nutr.* 9, 1039–1052.
- Vempati, R.K., Loepfert, R.H., 1989. Influence of structural and adsorbed Si on the transformation of synthetic ferrihydrite. *Clays Clay Miner.* 37, 273–279.
- Williamson, M.A., Kirby, C.S., Rimstidt, J.D., 1992. The kinetics of iron oxidation in acid mine drainage. *Abst. Third V.M. Goldschmidt Conference*, Reston, Virginia.
- Yoneyama, Y., 1998. Personal communication.