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## X-ray photoelectron evidence for bacteria-enhanced dissolution of hornblende

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**Abstract**—An *Arthrobacter* species capable of extracting Fe from hornblende was isolated from a soil from the Adirondacks, NY (USA). This bacteria isolate, used in batch experiments with hornblende, accelerated the release of Fe from hornblende without measurably affecting Al release. The isolate produces both low molecular weight organic acids (LMWOA) and a catecholate siderophore. Polished hornblende (glass and crystal) discs were analyzed with X-ray photoelectron spectroscopy (XPS) before and after incubation with growing *Arthrobacter* sp. to investigate whether the bacteria caused a distinguishable chemical signature on the upper 100 Å of mineral surface. After removal of the arthrobacter grown on hornblende crystal or glass substrates using lysozyme, XPS revealed surface depletion of Fe for samples grown for several days in buffered (crystal) and unbuffered (crystal and glass) media. Fe/Si ratios of hornblende surfaces dissolved under biotic conditions are significantly lower than Fe/Si ratios on surfaces dissolved under abiotic conditions for similar amounts of time. Enhanced Fe release and the formation of Fe-depleted surfaces is inferred to be caused by catechol complexation at the mineral surface.

Because natural siderophore was not isolated in sufficient quantities to run bacteria-free leaching experiments, parallel investigations were run with a commercially available siderophore (desferrioxamine B). Desferrioxamine B was observed to enhance release of Fe, Si, and Al from hornblende both with and without added bacteria. Formation of desferrioxamine-Fe surface complexes were probed by studying the multiple splitting and shift in intensities of the N 1s line analyzed by XPS on siderophore ± Fe on gold surfaces and siderophore + hornblende crystal surfaces. Based upon the observed formation of an hydroxamate (desferrioxamine) surface complex on hornblende, we infer that catecholate siderophores, such as those produced by the arthrobacter, also complex on the hornblende surface. Surface complexation is favored because of the extremely high association constants for siderophore + Fe(III). X-ray photoelectron spectroscopic data is therefore consistent with a model wherein enhanced Fe release by these bacteria or desferrioxamine B is caused by Fe-siderophore complexation at the silicate surface. Such complexation presumably weakens bonds between the Fe and the oxide lattice, causing enhanced Fe leaching and an Fe-depleted surface. Some leaching may also be due to LMWOA, although this is interpreted to be of secondary importance. Copyright © 2000 Elsevier Science Ltd

### 1. INTRODUCTION

Many researchers are interested in identifying distinctive surface patterns on soil minerals related to etching by microbiota. Such bacteria-mineral interactions have been investigated for many years (e.g. Duff et al., 1963; Daragan et al., 1971; Kutuzova, 1973; Lyalikova and Petushkova, 1991; Sand and Bock, 1991; Grantham and Dove, 1996; Ullman et al., 1996; Forsythe et al., 1998; Barker et al., 1998). Bacteria, lichens, and fungi in soils produce organic acids such as lactic, succinic, oxalic, citric, acetic and  $\alpha$ -keto acids (e.g., Rozycki and Strzelczyk, 1986; Vandevivere et al., 1994; Stone, 1997). These dissolved acids and other organic exudates can affect pH in weathering solutions and thereby promote or inhibit etching. The dissolved organic molecules can also form surface complexes that affect weathered mineral surface characteristics by ligand-promoted dissolution or through inhibition of reactivity. Alternatively, organic ligands can complex cations in solution, inhibiting precipitation or lowering the saturation index in solution and enhancing dissolution indirectly. Studies by Welch and Vandevivere (1994) have also shown that insoluble

extracellular polysaccharides can both increase and decrease dissolution of minerals under different conditions. However, most researchers have only analyzed solution chemistry and surface topography on minerals interacting with bacteria, and relatively few attempts have been made to use ultrasensitive techniques of surface chemical analysis such as x ray photoelectron spectroscopy (XPS) to document the effects of bacteria-mediated reactions on oxides (see, however, Seal et al., 1995; Seal et al., 1996a; Seal et al., 1996b; Barr et al., 1996). Investigation with such surface-sensitive tools may yield new techniques for documenting the effects of microbes on naturally weathered samples.

Multidentate ligands may form especially strong surface complexes by complexing multiple metal sites on the oxide surface. A few authors have suggested that dissolution may be affected by the presence of multidentate Fe(III)-specific ligands (siderophores) produced by bacteria and fungi (e.g., Stone, 1997). Such siderophores, by definition, are more Fe(III)-specific and show higher association constants than low molecular weight organic acids (LMWOA) such as oxalic acid. In a previous paper (Liermann et al., 2000), we showed that siderophores accelerate the release of Fe from hornblende in buffered and unbuffered solutions. We inferred the formation of siderophore-Fe surface complexes. Hornblende was chosen

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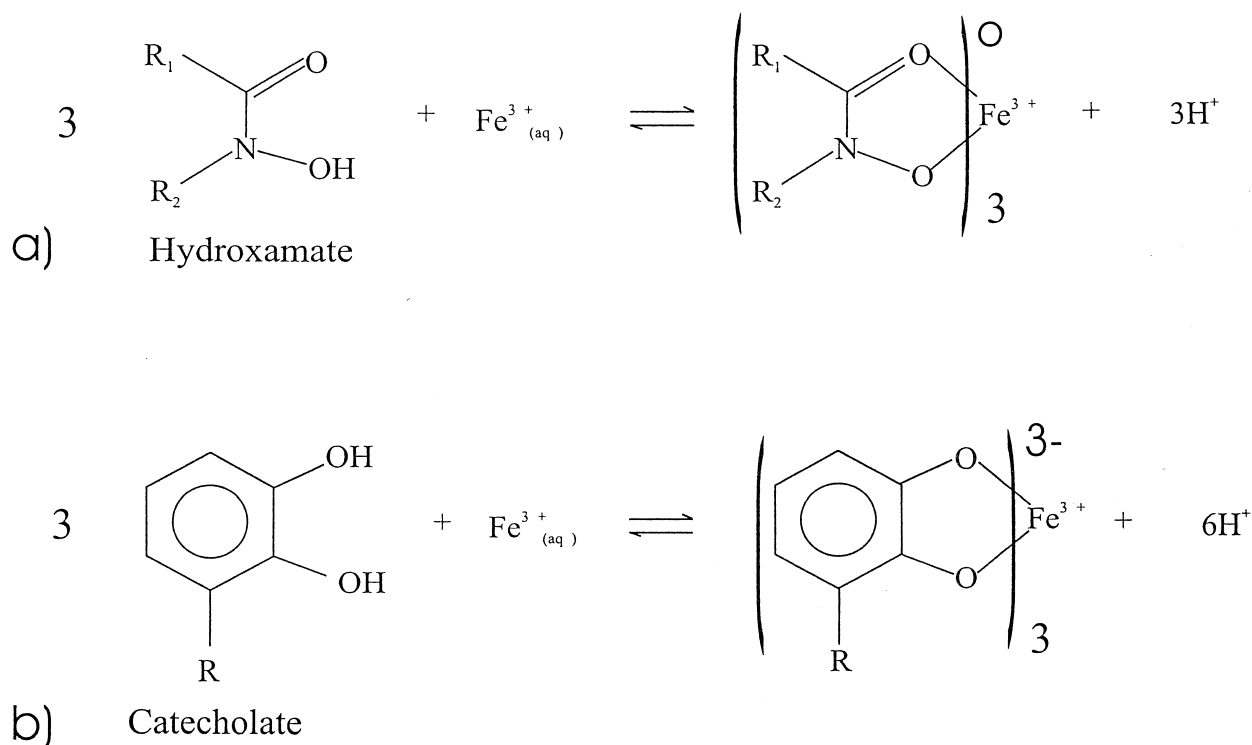


Fig. 1. Fe(III) complexation reactions of a) hydroxamate and b) catecholate siderophores.

for these studies because it often contains a relatively high content of minor or trace elements needed by soil microorganisms in granitic terrains (e.g., Fe, Mn, Cu, Zn, V, Mo, Ni and Co). Of these elements, Fe is of particular interest, because of its low concentration and lack of availability to cells in aerobic environments at neutral pH. Iron is needed by most organisms for enzyme function and as an important constituent in the respiratory pathway. Siderophores are therefore used by many organisms to provide this essential element.

The most common groups of siderophores are the hydroxamates and the catecholates (Winkelman, 1991), but novel groups of siderophores are still being discovered in nature (Telford and Raymond, 1997). Bacteria produce hydroxamate siderophores only in low-Fe surroundings and catecholates only in extremely low Fe surroundings (Bergeron and McManis, 1991). Hydroxamate siderophores form electrically neutral complexes when binding Fe(III), while catecholates form electrically charged complexes (Fig. 1). The presence of two adjacent hydroxyl hydrogens of high pKa ( $\text{pK}_{a1} = 9.2$  and  $\text{pK}_{a2} = 13$ ) in catechol generally explains why catecholates are predicted to be more powerful ligands for iron (III) than hydroxamates at non-acid pH values (association constants,  $K_{\text{ass}}$ , as high as  $10^{52}$ , Hider, 1984). However, at acidic pH, hydroxamates are more powerful chelators than catecholates (Winkelman, 1991).

Aqueous siderophore concentrations in nature may range from  $\sim 10 \mu\text{M}$  to 1–2 mM (Hersman et al., 1995). Typical concentrations for other common chelators in soils, including oxalic and ascorbic acids, are 2–3 mM respectively (Stevenson and Vance, 1989). Association constants for Fe(III) for siderophores are many orders of magnitude greater than the asso-

ciation constants for these latter LMWOA, however, so the effect of siderophores should be powerful (Powell et al., 1980).

Few studies have investigated siderophore-promoted dissolution, although several recent publications have investigated microbial interactions with iron-containing minerals or Fe(III)-(hydr)oxide coatings on mineral surfaces (Adams et al., 1992; Grantham and Dove, 1996; Hersman et al., 1996; Maurice et al., 1996; Grantham et al., 1997; Forsythe et al., 1998).

In a qualitative study of siderophore-promoted dissolution of minerals, Page and Huyer (1984) concluded that the soil bacterium *Azotobacter vinelandii* produced increasingly powerful siderophores in the presence of the following suites of minerals:

1. Pyrrhotite and marcasite;
2. Vivianite, olivine, and magnetite;
3. Hematite, siderite, pyrite, goethite;
4. Ilmenite, micaceous hematite, and illite.

Watteau and Berthelin (1994) reported dissolution of several soil minerals in the presence of the siderophore desferal (= desferrioxamine B) while Hersman et al. (1995) reported that a siderophore isolated from *Pseudomonas* sp. accelerated dissolution of hematite. Holmei and Casey (1996) investigated dissolution of goethite in the presence of acetohydroxamic acid, a ligand of smaller molecular weight but analogous to desferrioxamine B. The authors emphasize the relatively small enhancement of dissolution rates by the acetohydroxamate ligand as compared to rates in the presence of oxalate.

In the first paper of this series, Liermann et al. (2000) observed that increased concentrations of the mesylate salt of the hydroxamate siderophore desferrioxamine B, DFAM, in-

creased the Fe release rate,  $R$ , from hornblende non-linearly following the rate equation:

$$R = kC^n \quad (1)$$

where  $k$  is the rate constant =  $7.6 \times 10^{-13}$  mol Fe (mol DFAM) $^{-0.5}$  L $^{0.5}$  m $^{-2}$  s $^{-1}$ ,  $C$  is the concentration of siderophore, and  $n = 0.5$ . Comparison of average initial Fe release rates over a week long period showed that the presence of *Streptomyces* sp. caused an approximately 5-fold increase in the Fe release rate in buffered medium compared to cultures with hornblende only (no DFAM). However, when *Streptomyces* sp. was added along with DFAM, the presence of *Streptomyces* sp. resulted in a 2 to 3-fold rate increase over DFAM alone, regardless of DFAM concentration. We suggested that the *Streptomyces* sp. may use the DFAM and recycle it. In addition, the Fe release rate from hornblende in experiments with bacteria + elevated levels of DFAM was always higher than would have been predicted based upon the summation of release rates from bacteria-only and DFAM-only experiments. Although the streptomycete also produced LMWOA, the observation that *Streptomyces* sp. + DFAM always resulted in a doubled or tripled Fe release rate was cited as an argument that enhancement of Fe release in the bacteria experiments was due to siderophore-promoted dissolution as opposed to dissolution promoted strictly by complexation with LMWOA.

In the present study, X-ray photoelectron spectroscopy (XPS) is used to investigate surface chemical evidence of dissolution of hornblende as a result of bacterial growth. Instead of *Streptomyces* sp., however, the effect of an *Arthrobacter* sp. on hornblende reactivity is investigated for reasons described below.

## 2. MATERIALS AND METHODS

### 2.1. Bacteria and Growth Media

Bacterial isolates were obtained from hornblende-containing soil from Gore Mountain, New York in the Adirondacks (Liermann et al., 2000). Two isolates that exhibited robust growth in Fe-limited medium in the presence of hornblende were chosen for further study. Genomic DNA was extracted from these isolates and submitted for PCR amplification and 16S rRNA sequencing, as previously described (Liermann et al., 2000). One of these isolates, a streptomycete, was discussed in our previous papers (Liermann et al., 2000; Liermann et al., 2000); however, analysis of the silicate mineral surface under these bacteria after growth was difficult because the bacteria were impossible to remove without damaging the mineral surface, and so we focus on results from the second species, a putative arthrobacter, in this paper. A few Fe release experiments were conducted at room temperature with the arthrobacter.

Planchet leaching experiments were completed in both unbuffered and buffered media, following Liermann et al. (2000). Buffered medium was used to reduce effects due to changes in pH, whereas unbuffered medium was used to more closely simulate natural conditions. Dissolution experiments (with hornblende powder) were completed only in buffered medium.

All glassware used was soaked for >2 h in a Nochromix (GODAX Laboratories, Inc.) acid bath and rinsed 6 times with distilled H<sub>2</sub>O and 6 times with deionized H<sub>2</sub>O; plastic ware was soaked sequentially in HCl and HNO<sub>3</sub> acid baths (1:10 (v/v) dilution with deionized H<sub>2</sub>O), with a minimum of 12 rinsings, as above, between and after acid soaks. All equipment used in the experiments, including flasks, forceps, medium, mineral powder and planchets were autoclaved prior to the experiments.

### 2.2. Dissolution Experiments

Hornblende powder with a grain size of 250–429  $\mu\text{m}$  was ultrasonically cleaned several times in acetone until the acetone was transparent. The specific surface area was determined with BET on a Micromeritics ASAP 2010 Surface Area Analyzer to be 0.17 m<sup>2</sup> g<sup>-1</sup> using Kr as the adsorbent gas.

Dissolution experiments were completed with *Arthrobacter* sp. with and without the commercially available hydroxamate siderophore derivative, DFAM (Sigma Chemical Co.). This siderophore, although available commercially, is produced naturally by *Streptomyces pilosus* and *Arthrobacter simplex* (Winkelman, 1991). The experiments were completed using 7 g of hornblende powder, a starting volume of buffered medium of 500 ml, and 100  $\mu\text{l}$  inoculum from a late log to stationary phase culture of *Arthrobacter* sp. No attempt was made to analyze the number of bacteria added in the inoculum since our previous work had shown that dissolution experiments inoculated in this way were reproducible (Liermann et al., 2000). One set of duplicate flasks containing medium + hornblende was inoculated with both *Streptomyces* sp. and *Arthrobacter* sp. to see how interactions impact dissolution rates, since these bacteria coexist in natural soil.

Ten ml were sampled every second day from all flasks and another 10 ml were withdrawn at the end of the experiment for siderophore analysis. The solutions were analyzed for cations using a Leeman Laboratories PS 3000 UV inductively coupled plasma-atomic emission spectrometer (ICP-AES). Solution samples from dissolution experiments were tested for siderophores using the universal (Schwyn and Neilands, 1987), hydroxamate-specific (Arnold et al., 1983), and catecholamide-specific (Arnold, 1937; Neilands and Nakamura, 1991) assays. Filtered supernatant solutions from experiments with *Arthrobacter* sp. with and without hornblende were also analyzed with reversed phase high-performance liquid chromatography (HPLC) using ultraviolet (UV) detection.

### 2.3. Planchet Experiments

Mineral planchets with a diameter of 1 cm and polished to 0.25  $\mu\text{m}$  were cored from a slab of Gore Mountain hornblende. Eighteen hornblende crystal blanks, i.e., planchets that were polished but not exposed to medium, were prepared identically to planchets used in experiments. Because of second phase material in the hornblende (minor chlorite, biotite, feldspar, garnet), these samples were never homogeneous in composition. To investigate a similar substrate of homogeneous composition, a glass with major element composition similar to the hornblende crystal was synthesized as described in Liermann et al. (2000). Planchets of glass were polished identically as the hornblende planchets but rectangular prisms (2 mm  $\times$  8 mm  $\times$  8 mm) were used instead of discs.

Unbuffered planchet experiments were performed in 10–50 ml medium in culture tubes (see Liermann et al., 2000). Samples were inoculated with *Arthrobacter* sp. and incubated for 2, 4, or 21 days. All inocula were taken from late log to stationary phase *Arthrobacter* sp. cell culture. Experiments using the buffered medium were completed with *Arthrobacter* sp. for 5 or 21 days.

For all planchet experiments, the planchet was removed after incubation, immediately rinsed with deionized water, and ultrasonically cleaned either in ultrapure acetone or in lysozyme solution having a concentration of 0.1 mg ml<sup>-1</sup> for varying times. After lysozyme cleaning, the planchets were always rinsed in deionized water followed by ultrapure acetone and stored in a desiccator. For many samples, parallel non-inoculated experimental controls were also completed and treated identically.

For comparison, one planchet, prepared as described above, was also buried in an Adirondack podsol (at Gore Mountain) on 9/23/96 in the B horizon, and recovered 21 d later. This sample was saved for XPS analysis. The soil was the same soil where the bacteria isolates were collected.

### 2.4. X-ray Photoelectron Spectroscopy (XPS)

The elemental composition of the uppermost  $\sim 100$  Å of a roughly circular area (diameter  $\approx 3$  mm) of the crystal and glass planchets was

determined by XPS using a Kratos XSAM 800 pci electron spectrometer and Mg K $\alpha$  radiation. Where the same sample was measured twice, roughly the same spot was measured. The sensitivity factors for Na, Al, O and Si were determined using a vacuum fracture surface of Na<sub>2</sub>O · 0.8 Al<sub>2</sub>O<sub>3</sub> · 2.2 SiO<sub>2</sub> glass. The factor for C was calculated from a polyethylene terephthalate film, while the factor for Mg was determined from forsterite (Mg<sub>2</sub>SiO<sub>4</sub>) powder of known composition. The factors for Ca, K, Fe, S, N were obtained from the Handbook of X-ray Photoelectron Spectroscopy (Moulder et al., 1992). Before analysis, the blank, controls and samples were cleaned with ultraviolet ozone cleaning (UVOC, Vig, 1992; Zazzera and Evans, 1993), for 45–60 min.

To provide reference spectra to investigate siderophore surface complexation, desferrioxamine mesylate (DFAM) solutions with and without Fe<sup>3+</sup> were analyzed after evaporation onto gold-coated glass slides. Iron was added as Fe(III) nitrate (High Purity, ICP-standard), producing DFAM-Fe(III) solution at pH = 3.2. Desferrioxamine solution without added Fe<sup>3+</sup> had pH = 5.4.

Five individual hornblende planchets were also analyzed by XPS after evaporation of ~0.04 ml droplets of 15, 65, 150, 650, or 1500  $\mu$ M DFAM onto the surface. Three of these samples (65, 650, and 1500  $\mu$ M) were reanalyzed, except that the hornblende surface was rinsed ultrasonically with DI water after exposure to the DFAM solution. These samples with siderophore were not pre-treated by UVOC, since this would most likely have oxidized the organic siderophore. Spectra were deconvoluted using the software *Vision*, version 1.3.3. All peaks were Gaussian-Lorentzian mixed peaks.

## 2.5. Scanning Electron Microscopy (SEM)

To image intact bacteria, some samples were chemically fixed using 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 15 h at 4°C and washed 3 times for 5 min in 0.1 M phosphate buffer. Bacteria were dehydrated in 25, 50, 70, 85 and 95% ethanol for 5 min at each concentration and rinsed with 100% ethanol 3 times for 5 min. Critical point drying at 35°C and 1400 psi was completed using ethanol as transitional fluid and bone-dry liquid CO<sub>2</sub>. Exchange conditions were 16°C and a pressure of 1000 psi. The sample was coated with gold/palladium and imaged using a JEOL JSM 5400 Scanning Electron Microscope operated at 20 kV.

## 3. RESULTS

### 3.1. Isolates

Partial sequencing of the 16S rRNA gene (Nucleic Acid Facility, Life Sciences Consortium, The Pennsylvania State University) and analysis by the Ribosomal Database Project (RDP, Michigan State University) showed that the isolate used in these studies is almost as closely related to *Arthrobacter oxydans* as to *Micrococcus luteus* (similarity ranks of about 0.594 and 0.661 respectively). Scanning electron microscopy of the *Arthrobacter/Micrococcus* sp. showed that it has a rod-coccus growth cycle (Fig. 2), which is typical for arthrobacter but not for micrococcus (Keddie et al., 1989). This bacterium is therefore referred to as an arthrobacter in this paper.

### 3.2. Solution Chemistry

In the powder dissolution experiments, for all samples incubated with *Arthrobacter* sp. in buffered medium, pH decreased from 7.4 to about 6.8–6.9 after 7 days (Tables 1, 2). Fe release rates vs. time were constant in the first 7 d, and then decreased dramatically (see Liermann et al., 2000 for similar data for *Streptomyces* sp.). Initial release rates (over the first 7 days) of Fe in the experiments were calculated using a simplified version of the equation developed by Liermann et al. (2000):

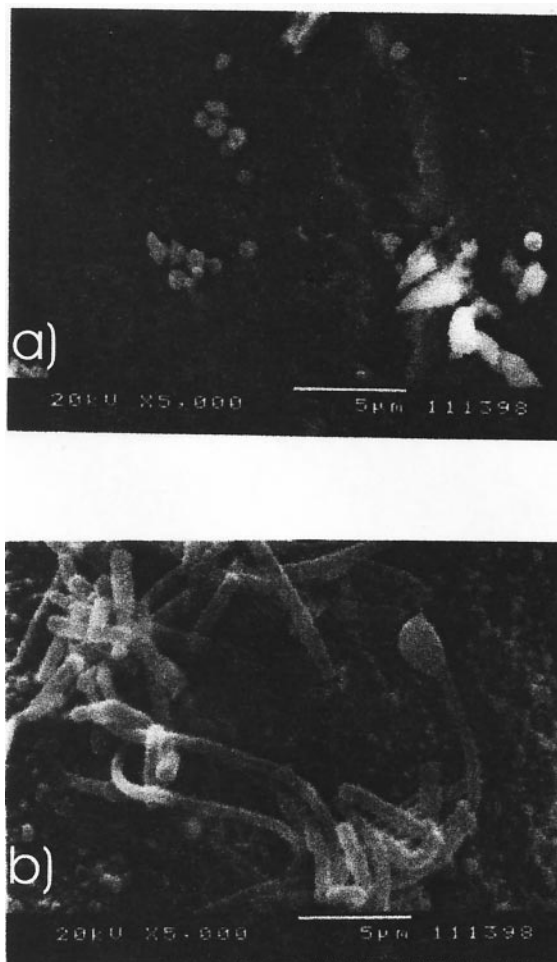


Fig. 2. SEM photomicrographs of chemically fixed, dehydrated and critical point-dried bacteria showing a rod-coccus growth cycle, typical for arthrobacter: a) shows the coccus phase b) shows the rod phase. Scale bars in both photos are 5 micron.

$$R_x = \left( \frac{d(C_x V)}{dt} \right) \frac{1}{Am} \quad (2)$$

where  $R_x$  is the release rate (mol  $x$  m<sup>-2</sup>s<sup>-1</sup>),  $C_x$  is the molar concentration of element  $x$  in solution sampled at time  $t$ ,  $V$  is the volume in the flask,  $A$  is specific surface area (m<sup>2</sup>g<sup>-1</sup>), and  $m$  is the mass of hornblende powder. These experiments were designed to use a large volume of medium so no correction was needed to account for changes in volume during sampling.

The initial release rate of Fe from hornblende crystal powder is faster with *Arthrobacter* sp. present compared to cultures with *Streptomyces* sp. (Liermann et al., 2000) and faster than abiotic experiments (Table 1). Like the streptomycete, *Arthrobacter* sp. does not measurably increase the release rate of Si or Al compared to abiotic experiments (Table 1). Addition of 24  $\mu$ M DFAM to the arthrobacter experiments accelerates the release of Fe (by a factor of ~2) and Al (by a factor of  $\geq 5$ ). Addition of *Streptomyces* sp. to the flasks with *Arthrobacter* sp. does not measurably increase the rate of Fe released to solution above those observed in the presence of the arthrobacter alone.

Table 1. Initial release rates ( $\times 10^{-13}$  mol m $^{-2}$  s $^{-1}$ ) calculated for buffered powder experiments.

Sample name	Fe		Si		Al	
	Replic. 1	Replic. 2	Replic. 1	Replic. 2	Replic. 1	Replic. 2
HB only	16 $\pm$ 6	n.d.	110 $\pm$ 20	n.d.	<0.5	n.d.
HB + Arth	58 $\pm$ 7	52 $\pm$ 4	140 $\pm$ 50	100 $\pm$ 30	<1	<3
HB + 24 $\mu$ M DFAM + Arth	120 $\pm$ 10	90 $\pm$ 10	110 $\pm$ 30	130 $\pm$ 50	14 $\pm$ 2	16 $\pm$ 2
HB + Stm + Arth	60 $\pm$ 10	80 $\pm$ 10	90 $\pm$ 70	120 $\pm$ 40	<0.5	<0.6

HB-Gore Mountain Hornblende, Arth-*Arthrobacter* sp., Stm-*Streptomyces* sp., DFAM-desferrioxamine mesylate, n.d.-no data. All error estimates represent 1 standard error in the slope. All experiments were run in replicate as indicated.

Because of the high content of Ca, Mg, K and Na in the culture medium, release rates of these elements were not investigated.

Higher Fe/Si release rate ratios ( $=R_{Fe}/R_{Si}$ ) were observed in the samples when bacteria were present compared to the non-inoculated samples (Table 2). The Fe/Si release rate ratio from hornblende for the arthrobacter-inoculated experiments exceed the Fe/Si ratio in the starting material ( $0.21 \pm 0.06$ ) as measured by electron microprobe. Fe/Si release rate ratios for abiotic experiments were observed in the range 0.07–0.14 (Liermann et al., 1999, Table 2), below the blank ratio. The Al/Si release rate ratios (<0.09) are below the blank ratio ( $0.40 \pm 0.07$ ) for all experiments without DFAM. Addition of DFAM increases the Al/Si ratio to 0.12, a value still below the blank ratio.

A catecholate-specific assay demonstrated that *Arthrobacter* sp. produced a catecholate siderophore (Arnou, 1937; Neilands and Nakamura, 1991). Typical absorption maxima for catechol were detected at 214, 254 and 280 nm (Fessenden and Fessenden, 1986) for the siderophore excreted from the *Arthrobacter* sp. with HPLC using UV detection.

### 3.3. X-ray Photoelectron Spectroscopy (XPS)

Only Fe, Al, and Si elemental surface compositions are reported here because of the high content of Ca, Mg, K and Na in the culture medium. For eighteen crystalline hornblende (unreacted) blanks analyzed with XPS (Table 3), the Fe/Si ratio averaged  $0.21 \pm 0.02$  ( $1\sigma$ , range from 0.17 to 0.25), and the Al/Si ratio averaged  $0.45 \pm 0.06$  ( $1\sigma$ , range from 0.26–0.53). One crystalline and two glass blanks were also treated with lysozyme for different lengths of time (Table 3). The Fe/Si ratio of the crystalline blank remained constant within the estimated error ( $\pm 3\%$ ) even after 1.5 h in lysozyme. The 3% error is an estimate of the error of the analysis including instrument error and error due to heterogeneity of the hornblende and the

impossibility of measuring identical spots on the mineral surface. In contrast, the initial Al/Si value of 0.43 decreased 9% after 1.5 h lysozyme treatment. Both these Fe/Si and Al/Si ratios measured on the blank after 1.5 h lysozyme treatment were well within the range of measured values for crystalline hornblende blanks (Table 3). The Fe/Si and Al/Si ratios of the glass blank were also relatively unchanged (<5%) after treatment for 0.3 h. Longer treatments of the glass caused measurable Fe depletion.

*Arthrobacter* sp. grown with a hornblende planchet in buffered medium for 3 weeks was not completely removed from the hornblende crystal surface after 0.3 h in lysozyme as determined by SEM. However, planchets that had been incubated with arthrobacter for 9 days or less (Liermann et al., 2000) and treated with lysozyme for 0.3 h show no remaining bacteria on the surface under SEM or differential interference microscopy. Lysozyme treatments for 0.3 h were therefore used for cleaning purposes for both crystal and glass. Note that a few control crystal samples are reported in Table 3 without lysozyme treatment since this treatment was strictly only needed for samples incubated with bacteria, and because the treatment did not cause a measurable Fe leaching effect on the hornblende crystal surface chemistry.

After lysozyme treatment, XPS analysis of crystalline samples that had been exposed to *Arthrobacter* sp. in unbuffered medium for 2 and 4 days (i.e., samples successfully cleaned of bacteria) showed Fe/Si ratios (0.14, 0.13 respectively) lower than the range of Fe/Si observed in blanks (Table 3), indicating depletion of Fe (Fig. 3). Fe/Si ratios of crystalline hornblende surfaces exposed to the arthrobacter in buffered medium for 5 days also showed relatively low ratios (0.14–0.17, Table 3). No depletion was observed for Al for buffered or unbuffered experiments with *Arthrobacter* sp., nor for control experiments.

Fe/Si ratios of hornblende glass blanks were lower (0.08–

Table 2. Elemental release rate ratios for buffered powder experiments calculated from solution chemistry.

Sample	pH		Fe/Si		Al/Si	
	0 d	7 d	Ratio <sub>1</sub>	Ratio <sub>2</sub>	Ratio <sub>1</sub>	Ratio <sub>2</sub>
HB blank <sup>a</sup>			0.21 $\pm$ 0.06		0.40 $\pm$ 0.07	
HB only	7.3	7.1	0.14 $\pm$ 0.06	n.d.	<0.004	n.d.
HB + Arth	7.3	6.9	0.4 $\pm$ 0.2	0.5 $\pm$ 0.06	<0.007	<0.03
HB + 24 $\mu$ MDFAM + Arth	7.3	6.8	1.1 $\pm$ 0.3	0.7 $\pm$ 0.2	0.12 $\pm$ 0.06	0.12 $\pm$ 0.05
HB + Stm + Arth	7.3	6.9	0.7 $\pm$ 0.5	0.7 $\pm$ 0.2	<0.006	<0.005

HB-Gore Mountain Hornblende, Arth-*Arthrobacter* sp., Stm-*Streptomyces* sp., DFAM-desferrioxamine mesylate, n.d.-no data.

<sup>a</sup> Ratios of element composition measured using electron microprobe on multiple spots.

Table 3. Elemental ratios (as atom%) as measured by XPS on planchets.

Medium and substrate <sup>a</sup>	Sample <sup>b</sup>	Duration (days) <sup>c</sup>	pH <sup>d</sup>	Fe/Si after lysozyme treatment (h) <sup>e</sup>				Al/Si after lysozyme treatment (h) <sup>e</sup>				
				0 <sup>f</sup>	0.3	1.5	4	0 <sup>f</sup>	0.3	1.5	4	
Blank crystal (N = 18)		0		00.17–0.25 <sup>e</sup>				0.26–0.53 <sup>e</sup>				
Blank crystal		0		0.19	0.18	0.19		0.43	0.40	0.39		
Unbuffered crystal	Control	4			0.22				0.51			
	Control	21	6.4	0.17				0.48				
	Control	21		0.21								
	Control	56	7.4	0.24				0.54				
	Control	56		0.53								
	Arth	2				0.14				0.41		
Buffered crystal	Arth	4	5.2		0.13				0.43			
	Control	5	7.5		0.20				0.50			
	Arth	5	6.6		0.14				0.46			
	Arth	5	6.6		0.17				0.49			
	Blank glass (N = 2)		0		0.080–0.10 <sup>h</sup>	0.096		0.048	0.51–0.56	0.58		0.51
	Unbuffered glass	Control	2	6.8		0.066				0.49		
Control		2	6.8		0.067				0.49			
Control		4	6.8		0.067				0.42			
Control		4	6.8		0.065				0.47			
Control		21	6.8		0.039				0.38			
Control		21	6.8		0.041				0.41			
Arth		4	5.1		0.023				0.33			
Arth		4	5.1		0.025				0.28			
Arth		21	7.6		0.022				0.33			
Arth		21	7.6		0.007				0.33			
Crystal buried in soil			21	~5	0.65				0.67			

<sup>a</sup> Information indicates whether unbuffered or buffered medium and glass or crystal of hornblende composition was used. Blanks are planchets that were polished but not incubated in media. N = number of blanks analyzed. One crystalline sample was buried in the B horizon of soil at Gore Mountain, and retrieved 21 d later.

<sup>b</sup> Control samples were incubated in medium for the number of days indicated in the duration column, and samples marked Arth were incubated in medium with the arthrobacter.

<sup>c</sup> Duration in days that controls were incubated in medium or that samples were incubated in medium inoculated with arthrobacter. For the one sample buried in a soil, the duration indicates the duration the sample was buried.

<sup>d</sup> The measured pH at the end of the duration of the incubation period. For the sample buried in the soil, the pH indicates the value of the pH measured on a few samples of soil water from lysimeters at the site.

<sup>e</sup> The Fe/Si or Al/Si ratio on planchets before any treatment other than polishing. Range as measured on 18 blanks.

<sup>f</sup> The Fe/Si or Al/Si ratio on planchets after reaction in medium for # of days indicated with bacteria (Arth samples) or without bacteria (controls). These analyses were completed on samples before lysozyme treatment.

<sup>g</sup> The Fe/Si or Al/Si ratio on planchets after treatment with lysozyme for the indicated period of time.

<sup>h</sup> Fe/Si ratios of the glass blanks were significantly lower than the bulk composition of the glass (0.22), presumably due to leaching of Fe during polishing.

0.10) than the bulk composition of the glass (0.22 = bulk composition of the glass). Hornblende glass controls incubated in unbuffered medium showed ~30% reduction in Fe/Si ratio after 4 d in medium, and ~60% reduction after 21 d. In comparison, glass incubated with *Arthrobacter* sp. for only 4 d exhibited an 80% reduction in Fe/Si ratio. Fe/Si ratios incubated with the arthrobacter for 21 d were not reproducible but were at least 80% lower than starting material. Al/Si ratios measured on the hornblende glass after incubation with bacteria were also lower than similar ratios measured on control samples.

Measured pH changes in the unbuffered experiments with bacteria and hornblende planchets were generally larger than in the buffered medium. For all cases (Table 3) of experiments run for less than a week, pH decreased from the initial near-neutral pH. Increases in pH after this decrease were only observed for the 21–56 d experiments.

The Fe/Si and Al/Si ratios measured by XPS on the horn-

blende planchet buried in a Gore Mountain soil increased above the range of values observed in 18 polished blanks (Table 3).

### 3.4. Detection of Fe-Chelates by XPS

Significant differences between the high-resolution spectra for DFAM with and without Fe evaporated on gold-coated slides were observed in the relative intensities of the N 1s peak (Fig. 4). For the Fe-free siderophore, the high resolution nitrogen spectra showed two peaks indicating two bonding configurations for nitrogen. These peaks, with maxima around 400.9 and 399.2 eV (Fig. 4a), are assigned to N-OH and N-H bonds, respectively (Odriozola et al. 1991). In the spectrum for siderophore with Fe (Fig. 4b), the relative intensities of these 2 peaks reverse and the N-OH peak has lower intensity. Consistent with the presence of Fe in the solution, an extra peak was fitted in this spectrum and assigned to N-O-Fe.

The XPS spectrum of a hornblende planchet after evaporat-

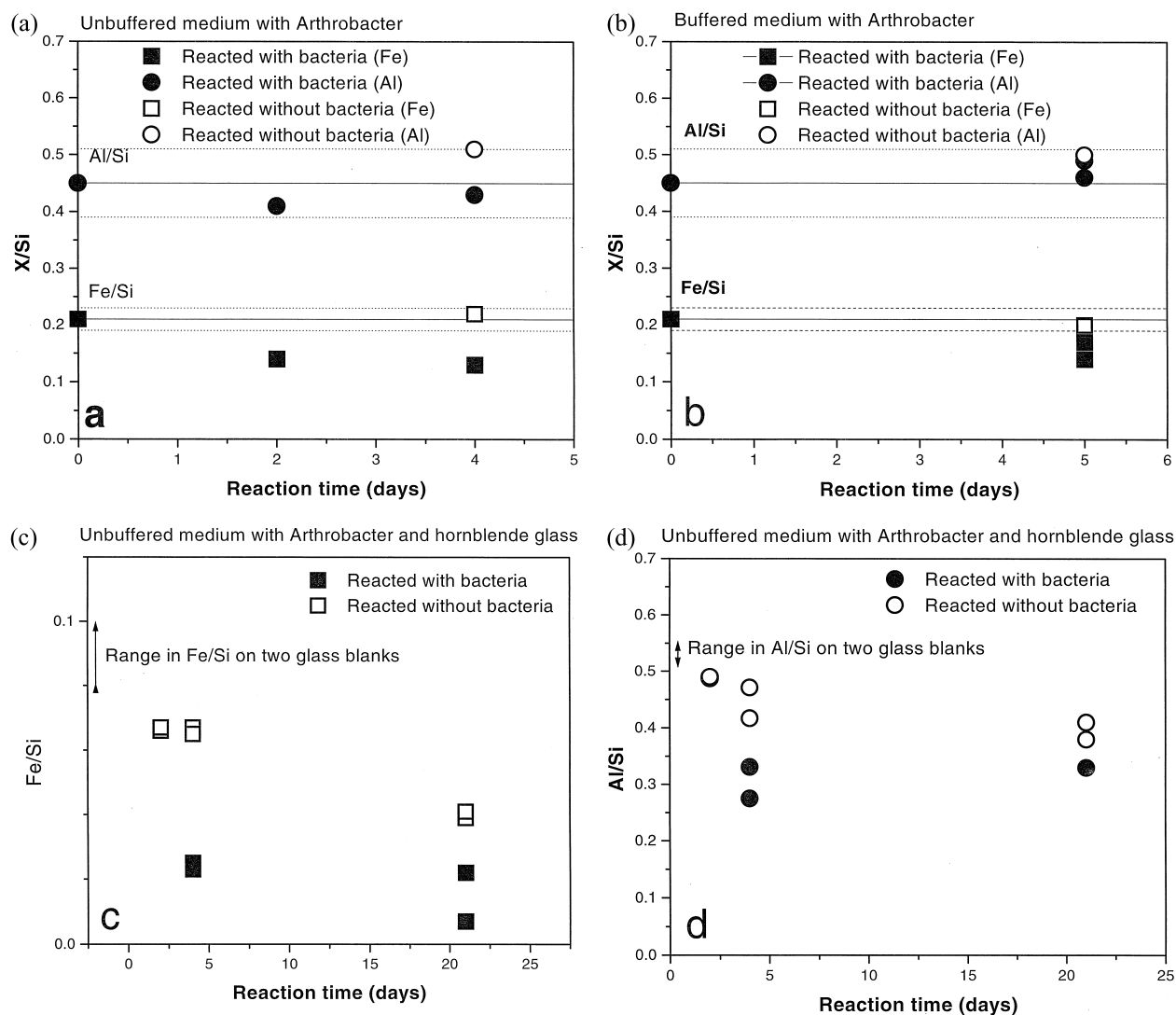


Fig. 3. Elemental ratios of the mineral surface as measured by XPS versus reaction time for: a) hornblende crystals in unbuffered medium; b) hornblende crystals in buffered medium; c) hornblende glass in unbuffered medium. Solid symbols indicate samples inoculated with *Arthrobacter* sp.; d) Controls (open symbols) are samples incubated in media w/o bacteria. For crystalline samples, the time zero data point is the average value for 18 measured blanks (polished unreacted planchets). The solid line indicates this average value for crystal samples, bracketed by  $\pm 1\sigma$  for the crystalline samples (delineated by dotted lines: note that these lines extending across the figure are for convenience, and are not meant to indicate that the blanks were submitted to reaction). Range in elemental ratios for two glass blanks is also noted in c) and d). All samples except blanks were cleaned with 0.3 h lysozyme treatment as discussed in text prior to analysis.

ing a drop of 15  $\mu\text{M}$  DFAM solution yielded a spectrum in which the N-O-Fe peak was undetectable. However, the spectrum for the sample treated with 1500  $\mu\text{M}$  DFAM solution revealed three peaks in the 400 eV binding energy region; again, the middle-energy (lowest intensity) peak was assigned to the N-O-Fe environment (Fig. 5a). Analysis of the same planchet after excess siderophore had been rinsed away by ultrasonication with deionized water showed that the intensity of the N-O-Fe peak increased extensively relative to both the N-OH and N-H peaks (Fig. 5b). The hornblende surfaces analyzed after exposure to droplets of 65 and 650  $\mu\text{M}$  DFAM followed by rinsing yielded XPS spectra comparable to that of the rinsed 1500  $\mu\text{M}$  DFAM sample (Fig. 5b). Relative areas under each of the three N peaks were calculated as a %ratio

with respect to the total area under all three peaks using the *Vision* software. The atomic %N present in the N-O-Fe environment for rinsed samples varied between 44 and 65% for droplets with concentrations of 65  $\mu\text{M}$  DFAM or higher.

#### 4. DISCUSSION

##### 4.1. Adsorption of Bacteria to Mineral Surfaces

Lysozyme hydrolyzes the  $\beta(1-4)$  glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid in the polysaccharide backbone of peptidoglycan. It is effective in lysing bacteria by hydrolyzing the peptidoglycan, which is present in bacterial cell walls (Madigan et al. 1995). Lysozyme, when

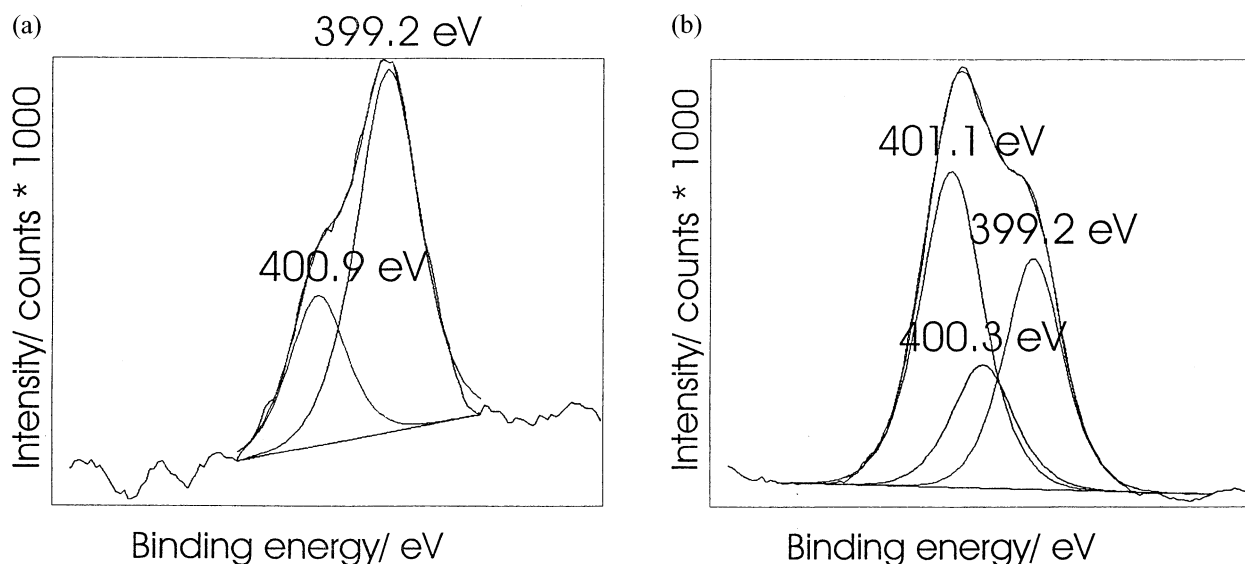


Fig. 4. Spectra showing intensity of photoelectrons emitted (counts per second) versus binding energy (eV) for A) nonchelated; B) Fe-chelated DFAM evaporated on gold-coated slides. Peaks are assigned to N-OH (400.9 and 401.1 eV respectively), N-H (399.2 eV) plus N-O-Fe (400.3 eV) for the Fe-chelated siderophore.

used immediately after termination of experiments conducted for  $< \sim 9$  d, removes *Arthrobacter* sp. completely or almost completely. Lysozyme was the most effective treatment as compared to ultrasonication, acetone immersion, and UVOC.

Planchets grown with *Arthrobacter* sp. for  $\geq 3$  weeks could not be completely cleaned with lysozyme. This may be in accordance with the hypothesis (Marshall et al., 1971) that bacteria initially sorb to a solid surface reversibly and later sorb irreversibly. This latter irreversible adhesion can be associated with the production of extracellular fibrils that attach the cell to the surface. Our results are consistent with a model whereby bacterial attachment becomes stronger with aging, perhaps

related to both physical (e.g., fibrils) and chemical factors (e.g., C-O-Si bonds such as those discussed by Seal et al., 1995).

#### 4.2. Release Rates of Fe, Al and Si

Leaching of Fe from hornblende increased significantly in the presence of an arthrobacter as compared to abiotic controls, while leaching of other elements such as Si and Al was unaffected (Table 1). The leaching rate of Fe was more enhanced in the presence of *Arthrobacter* sp. than *Streptomyces* sp. (see Liermann et al., 2000). While *Arthrobacter* sp. did not enhance the release of Al and Si, addition of DFAM to flasks inoculated

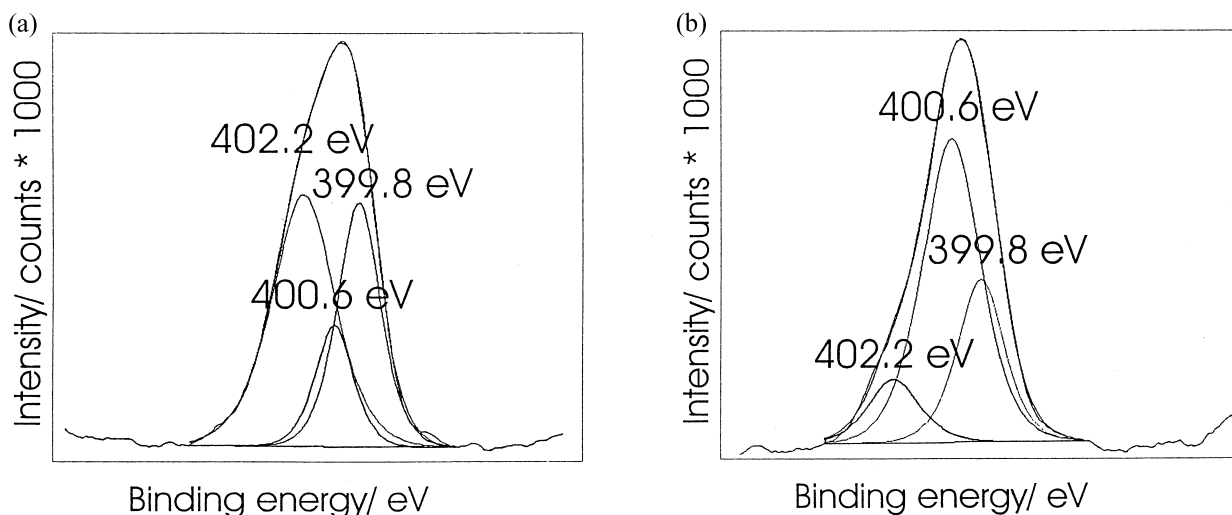


Fig. 5. A) XPS spectra measured for a droplet of 1500  $\mu$ M DFAM evaporated on a hornblende surface; B) same as (a) except that excess, nonadsorbed siderophore has been rinsed away with deionized water from the hornblende surface. Peaks are assigned to N-OH (402.2 eV), N-H (399.8 eV) and N-O-Fe (400.6 eV)

with *Arthrobacter* sp. increased the release rates of Fe and Al in comparison to abiotic experiments or experiments exposed to the arthrobacter alone (Table 1). This observation is consistent with our prior observation that DFAM alone increases release of Al, Si, and Fe from hornblende (Liermann et al., 2000). This enhanced leaching of Al by DFAM as compared to the bacteria-only experiments may be explained by the difference between catecholate (produced by *Streptomyces* sp. and *Arthrobacter* sp.) and hydroxamate siderophores such as DFAM.

Finally, addition of *Streptomyces* sp. to flasks with *Arthrobacter* sp. did not affect the release of Fe, Si and Al compared to experiments with only *Arthrobacter* sp. present. The arthrobacter extracts Fe at a faster rate than the streptomycete, and presumably dominates the chemistry in these experiments. Also, *Arthrobacter* sp. grows faster than *Streptomyces* sp.

### 4.3. XPS of Hornblende Surfaces

For crystalline hornblende experiments, the only planchets exhibiting Fe/Si ratios lower than the initial range of blank values were planchets incubated with *Arthrobacter* sp. XPS measurements document the formation of an Fe-depleted surface layer on these samples (Table 3), but no measurable change in Al relative to Si. Fe/Si release rate ratios calculated for all dissolution experiments with bacteria or siderophores present are also consistent with formation of Fe-depleted surfaces (Table 2). XPS results also document that the arthrobacter can enhance Fe release from the hornblende glass. For the glass, Fe/Si ratios were significantly lower for experiments with arthrobacter in comparison to those experiments without the bacteria. In general, however, leaching of Fe from the glass was enhanced relative to leaching of Fe from the crystal in experiments with and without bacteria.

These laboratory results (Tables 2, 3) document that dissolution under some biotic conditions creates an Fe-depleted surface layer on hornblende crystal while dissolution under near-neutral abiotic conditions does not. If these observations can be extrapolated to natural weathering environments, then hornblende surfaces weathered in the presence of soil microbiota should also show Fe depletion. However, XPS measurements on a planchet of hornblende buried in a Gore Mountain soil showed Fe- and Al-enrichment in the surface (Table 3). This Fe,Al-rich layer is probably an amorphous iron + aluminum oxyhydroxide precipitate.

Other workers reached contradictory conclusions with respect to leaching or deposition of Fe on naturally weathered hornblende surfaces. Berner and Schott (1982) observed no surface change in the Fe/Si ratio measured by XPS, while Mogk and Locke (1988) observed a decrease in Fe/Si ratio measured by Auger electron spectroscopy on weathered hornblende grains from different soils. It is possible that discrepancies between Fe-depletion vs. deposition on naturally weathered samples may result from differences in ultrasonication protocol. For example, an Fe-rich layer was reported by Schott and Berner (1983) to form on the surfaces of two other iron silicate minerals (bronzite and fayalite) weathered in the presence of oxygen in the laboratory. However, the layer was shown to be easily removed by ultrasonication. Further re-

search is needed to determine whether Fe depletion or deposition occurs in natural systems in the presence of microbiota.

### 4.4. Siderophores

Both the *Streptomyces* sp. used in Liermann et al. (2000) and the *Arthrobacter* sp. investigated here were found to produce catecholate siderophores. This latter observation is surprising given that published studies have shown that arthrobacter typically uses hydroxamate siderophores (Winkelmann, 1991). One possibility is that the bacterium is not an arthrobacter. However, very little is known about siderophores produced by soil bacteria, and, under iron starvation conditions, the bacteria may be stimulated to produce the most Fe-efficient chelator. In general, catecholate siderophores have higher Fe-complex formation constants than hydroxamate siderophores and may work as a "back-up" system for low Fe conditions (Bergeron and McManis, 1991). Note also that Liermann et al. (2000) concluded from experiments with *Streptomyces* sp. + DFAM that this bacterium produces a catecholate siderophore but that it may use both catecholate and hydroxamate siderophores. This latter conclusion was based upon the observation that addition of DFAM at different concentrations to hornblende + bacteria experiments always roughly doubled Fe release rates. Addition of DFAM to arthrobacter experiments also caused a doubling of rate as measured over 6–7 days, suggesting the arthrobacter also can use an hydroxamate (DFAM) as well as a catecholate siderophore (Table 1).

### 4.5. Mechanism of Leaching

In addition to the siderophores, Liermann et al. (2000) reported ion chromatographic evidence for production of formic, acetic, oxalic, and citric acids by the *Arthrobacter* sp. used in this study. Malonic and phthalic acids were not present at detectable levels but other acids that were not investigated may have been present. For example, it is possible that the *Arthrobacter* sp. produces organic acids such as pyruvic,  $\alpha$ -ketoglutaric (Ahmed et al., 1984; Rózycki and Strzelczyk, 1986), lactic, or succinic acids (Kutzner, 1981). Furthermore, Urzi et al. (1991) detected gluconic, lactic and minor pyruvic and succinic acids from *Micrococcus halobius*, and bacteria commonly produce these acids (Vandevivere et al., 1994). Decreases in pH of solutions in growth experiments reported here may therefore be presumed related to a mixture of these LMWOA, as well as siderophore molecules.

Enhanced leaching of Fe from hornblende during growth with bacteria is probably related to enhanced reactivity due to higher activities of the hydronium ion in solution; complexation effects of LMWOA; and/or chelate effects of siderophores. Although Fe depletion may be slightly greater in unbuffered medium (Table 3), leaching of Fe from hornblende crystal is not solely due to lower pH because Fe depletion of the hornblende surface also occurs in buffered medium. In addition, if lower pH were the dominant reason for the observed Fe leaching, we would also expect to see significant Al leaching from the hornblende surface, since both of these cations are commonly leached under acid conditions from silicate minerals. Furthermore, the effect of pH on silicate weathering at near-neutral pH is minimal (White and Brantley, 1995).

Distinguishing between the effects of complexation by LMWOA and siderophores is more difficult. Three lines of evidence suggest, however, that chelation by siderophores is the dominant mechanism of Fe leaching. First, Zhang and Bloom (1999) have reported experiments investigating the dissolution of hornblende in the presence of LMWOA. They have concluded that Al and Fe are preferentially released with respect to Si. In contrast, in experiments reported here with bacteria, only Fe release was enhanced. Second, release of Fe is accelerated in the presence of the siderophore DFAM without bacteria. This provides evidence that siderophores can promote Fe release from hornblende. Third, release of Fe is accelerated when *Arthrobacter* sp. cultures are injected with DFAM. For example, we have observed in a few flask experiments using the small-volume batch reactors of Liermann et al. (2000) that the rate of Fe release measured with 240  $\mu\text{M}$  DFAM, when added to the rate expected for the arthrobacter alone, is significantly less than the rate observed for *Arthrobacter* sp. + 240  $\mu\text{M}$  DFAM, suggesting the bacteria may be using and recycling DFAM when it is present, as mentioned previously. A similar observation was made for experiments with DFAM and *Streptomyces* sp. (Liermann et al., 2000). These observations are indirect evidence that DFAM and the catecholates produced by the streptomycete and the arthrobacter are used by the bacteria to harvest Fe, and that the siderophores are responsible for much of the Fe release. Therefore, although the effects of LMWOA cannot be ruled out entirely, we conclude that chelation by siderophores is the dominant mechanism causing enhanced Fe release.

Effects of complexation by organic ligands (either siderophores or LMWOA) could be explained through either complexation of Fe in solution and the effect of this complexation on the chemical affinity of the system, or by complexation of the siderophore at the solid-water interface. Such surface complexation could weaken bonds and accelerate hydrolysis. As described below, the XPS evidence is consistent with surface complexation.

#### 4.6. XPS Spectra of Surface Complexes

The geometry of the non-chelated DFAM molecule in solution differs significantly from the chelated molecule which forms a hexadentate cage complex with Fe (III) in the center (Fig. 6). The XPS spectrum of non-chelated DFAM on gold shows at least two different N environments in the siderophore, since a splitting of the N 1s line occurs. It is likely that these environments correspond to N-H and N-OH bonds since the siderophore is built up of a number of amino acids with carbonyl and amino groups (Fig. 6). The intensities of these two peaks are reversed when Fe is added to the siderophore solution (Fig. 4) and a third peak can be used to fit the data. Since chelation would involve formation of N-O-Fe and C-O-Fe bonds (Fig 6), it is likely that this new environment for nitrogen is due to replacement of the hydroxyl H for Fe on the hydroxamate siderophore. Correspondingly, the peak assigned to the N-OH environment decreases in intensity, since these proposed N-O-Fe sites are formed at the expense of N-OH.

An N-OH peak is still observed in the spectrum for the siderophore + Fe (on gold) even though chelation with Fe should occur in a 1:1 molar ratio (Jalal and van der Helm, 1991;

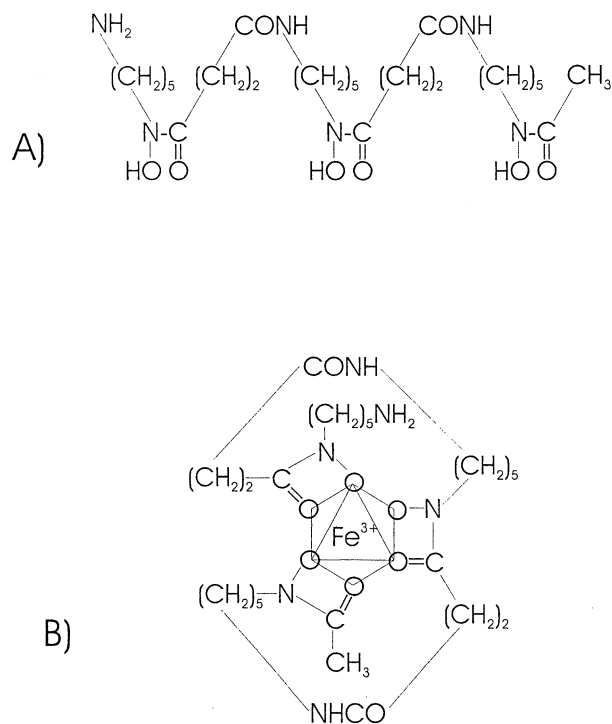


Fig. 6. The structure of the A) non-chelating and B) Fe(III)-chelating hydroxamate siderophore desferrioxamine (DFA). Drawing modified after Cramer et al. (1984) and Konetschny-Rapp et al. (1988).

Matzanke, 1991). Presumably, the decrease in pH from 5.4 to 3.2 upon addition of the acid Fe nitrate solution to the siderophore causes both addition of an NO environment and competition between Fe<sup>3+</sup> and H<sup>+</sup> for the chelating sites (Fig. 7). The protonation constants of desferrioxamine B are relatively high (pK<sub>a1</sub> = 8.50, pK<sub>a2</sub> = 9.24 and pK<sub>a3</sub> = 9.69, Dictionary of Organic Compounds, 1996). DFAM does not stop chelating Fe as pH is decreased below the pK<sub>a1</sub> (Winkelmann, 1991), however, because this protonation occurs at the nonchelating amino group (Fig. 7). For pH values lower than neutral (Fig. 7), however, hydroxamate complexation with Fe<sup>3+</sup> is a strong function of pH: for example, at pH < 2 the monohydroxamate species dominates. Between pH 2 and 4 the bishydroxamate species dominates and at pH > 4 the trishydroxamate is formed (Holmén et al., 1997).

One planchet of crystalline hornblende with an evaporated drop of 15  $\mu\text{M}$  DFAM showed insignificant evidence for surface N in the N-O-Fe environment, suggesting complexation was unmeasurable for this concentration of DFAM. In contrast, evaporation of 1500  $\mu\text{M}$  solution of DFAM onto a hornblende planchet resulted in a spectrum that appeared intermediate between the siderophore + Fe and siderophore - Fe spectra observed on gold. This spectrum was deconvoluted using three peaks, since the siderophore was assumed to form surface complexes with Fe at the mineral surface, thus creating N-O-Fe environments. The peak assigned to N-O-Fe was significantly less intense than the other two peaks due to the limited availability of Fe at the hornblende surface or due to excess physisorbed siderophore. (Note that the XPS signal is averaged over roughly the upper 100 Å of the surface.) The same

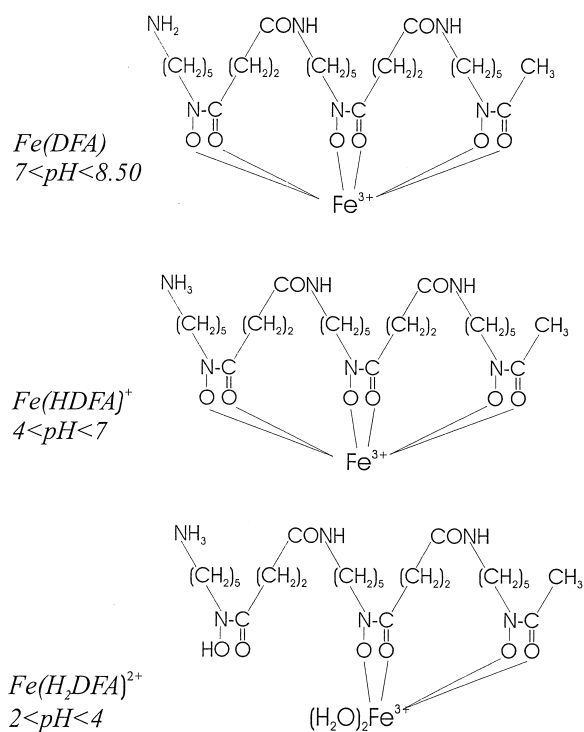


Fig. 7. The DFA siderophore complexed to Fe(III) as a function of pH. For  $pH > 7$ , the neutral trishydroxamate complex forms; for  $4 < pH < 7$ , the protonated trishydroxamate complex forms, and for  $pH < 4$  the doubly protonated bishydroxy complex forms. All complexes shown are mononuclear. Drawing modified after Crumbliss (1991).

planchet was therefore rinsed and ultrasonicated for 8 minutes with deionized water to remove excess siderophore. After rinsing, the intensity of the peak assigned to N-O-Fe increased significantly whereas the intensity of the N-OH peak decreased (Fig. 5). In the unrinsed specimen, most of the detected siderophore was probably present as a physisorbed or precipitated species, whereas in the rinsed spectrum the siderophore complexed to the hornblende surface was exposed. A planchet exposed to  $65 \mu M$  DFAM also showed significant atom %N in the N-O-Fe environment after rinsing. All samples with evaporated DFAM solution  $> 15 \mu M$  yielded values between 44 and 65% for %N in the N-O-Fe environment after rinsing.

At the nominal pH of solutions evaporated onto the planchets ( $\sim pH 5$ ), 3 out of 6 nitrogen atoms could be present in the N-O-Fe environment (trishydroxamate). Similarity between the ratio 3/6 and the value of atomic %N-O-Fe observed after rinsing (44–65%) may therefore indicate that this strong chelate ligand forms a hexadentate complex even at the mineral surface where it is sterically unable to form the full cage complex expected in solution (Fig. 6). Such a hexadentate complex, if it is forming, is most likely binuclear rather than mononuclear. Holmén and Casey (1996) previously suggested that it may be possible for two or three of the hydroxamate groups of desferrioxamine to bind multiple surface Fe atoms on goethite (binuclear complexes), since the siderophore cannot completely encapsulate a single structural iron atom when complexed at a surface.

## 5. CONCLUSIONS

1. A hornblende-containing soil yielded an arthrobacter isolate capable of extracting Fe from the mineral hornblende under laboratory conditions. The arthrobacter increases the Fe release rate of hornblende over abiotic conditions. This is the second bacterial isolate cultured from an Adirondack (NY, USA) soil that can enhance Fe release from hornblende. Both isolates excrete catechololate siderophores under low Fe conditions. Neither isolate enhances the release of Al from hornblende. When both isolates are incubated with hornblende, the Fe and Al release rates are comparable to those observed with the arthrobacter alone.
2. In the presence of a commercially available hydroxamate siderophore (DFAM), Fe release from hornblende is doubled when the arthrobacter is present. In the presence of DFAM, the Al release rate is also enhanced over abiotic experiments.
3. The arthrobacter adheres strongly to the hornblende surface during growth. Adherent arthrobacter, grown for  $< \sim 9$  d in the presence of the hornblende, can be removed with a 0.3 h lysozyme treatment, without significantly changing the hornblende surface chemistry. Adherent arthrobacter grown for several weeks cannot be removed with a 0.3 h lysozyme treatment.
4. After the arthrobacter (grown for up to 5 d in both unbuffered and buffered media) was removed from the hornblende crystal, XPS analysis documented decreased Fe/Si ratios of the upper  $100 \text{ \AA}$  of the mineral surfaces, consistent with solution chemistry observations that Fe was removed from the mineral faster than Si during bacteria growth. Al/Si ratios of the surfaces were unchanged relative to the starting material. Under abiotic conditions, Fe/Si and Al/Si ratios of the outermost surface of hornblende crystal as measured by XPS were relatively unchanged.
5. For experiments with hornblende glass incubated in unbuffered medium, Fe/Si ratios were significantly more depleted on the glass surface for biotic as compared to abiotic experiments.
6. A surface complex between Fe and DFAM was detected at the hornblende surface. Enhanced release of Fe from hornblende in the presence of the arthrobacter is inferred to result from similar siderophore surface complexes.
7. Enhancement of Fe release is inferred to be predominantly due to siderophore surface complexation and rate enhancement; however, complexation by LMWOA may also contribute to Fe release.

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