

Etch pit formation on iron silicate surfaces during siderophore-promoted dissolution

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Abstract

Understanding the effects of microbiota on mineral alteration requires the ability to recognize evidence of bacteria-promoted dissolution on mineral surfaces. Although siderophores are known to promote mineral dissolution, their effects on mineral surfaces are not well known. We have utilized atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), and Mirau vertical scanning interferometry (VSI) to investigate surfaces after incubation with the siderophore desferrioxamine-B mesylate (DFAM) and under colonies of bacteria. Iron-silicate glass planchets chemically similar to hornblende were incubated in buffered growth medium with siderophore-producing bacteria (*Bacillus* sp.) for 46 days with parallel abiotic experiments conducted with and without 240 μM DFAM, with and without 0.01 g l^{-1} of microbially produced extracellular polysaccharides (EPS, alginate or xanthan gum). Some glass planchets were protected by dialysis tubing from direct contact with the EPS. Weekly sampling and analysis of all filtered sample solutions showed negligible Fe and Al release in the control experiments and significant release of Fe and Al in the presence of DFAM, with negligible changes in pH. Concentration of Fe in the filtered solutions after incubation with bacteria was below detection, consistent with uptake of Fe by cells. Release of Fe, Al, and Si in control, xanthan-only, and alginate-only experiments was negligible. Release of these elements was enhanced in all experiments containing DFAM, and greatest in alginate + DFAM experiments.

AFM and VSI analyses reveal widespread, small etch pits and greater root mean squared roughness on siderophore-exposed surfaces and fewer, localized, larger etch pits on bacteria-exposed surfaces. This is the first documented case of etch pit development during siderophore-promoted dissolution. Roughness was not affected by the growth medium, alginate, or xanthan gum alone. The roughness trends among samples correlate with trends in Fe depletion documented by XPS. Enhanced dissolution and roughness cannot be attributed to direct contact with EPS because no significant chemical or physical differences were observed between surfaces directly exposed to EPS and those protected by dialysis tubing. Acetate released from the EPS may have enhanced the siderophore-promoted dissolution. Siderophores produced by *Bacillus* sp. may be responsible for some of the 'biopits.' The difference in size and distribution of the biopits may be related to colonization.

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1. Introduction

1.1. Surface colonization

Microbial colonization of mineral surfaces is rapid and extensive in aqueous and soil environments because organic macromolecules adsorb to surfaces and form a layer that encourages attachment of microorganisms (Baier, 1980; Characklis, 1989; Brisou, 1995; Little et al., 1997). As a result, free microorganisms represent only 0.1 to 1.0% of total microorganisms in an aquatic ecosystem, with the remainder of the microorganisms attached to surfaces (Brisou, 1995; Madigan et al., 2000).

To colonize a surface, microorganisms form large aggregates of cells, proteins, lectins, and polysaccharides, collectively termed “biofilms” (e.g., Brisou, 1995; Little et al., 1997; e.g., Wilderer and Characklis, 1989). A number of researchers have documented the attachment of microorganisms to mineral surfaces via the formation of a biofilm (e.g., Barker et al., 1998; e.g., Thorseth et al., 1995). The nutrient content of mineral surfaces drives the attachment (Brisou, 1995; Bennett et al., 1996a; Madigan et al., 2000). In fact, in environments depleted in one or more nutrients, microorganisms preferentially colonize mineral surfaces containing essential macro- or micro-nutrients (Bennett et al., 1996a; Grantham and Dove, 1996; Rogers et al., 1998; Sawyer and Hermanowicz, 1998; Kalinowski et al., 2000).

The effects of colonization on mineral surfaces remain, for the most part, un-quantified. Effects such as the formation of etch pits by microorganisms on mineral surfaces are of interest as potential biosignatures. Several researchers have documented etch pits on colonized mineral surfaces using scanning electron, transmission electron, vertical scanning interferometry or atomic force microscopies (SEM, TEM, VSI, AFM, respectively). Barker et al. (1998) and Rogers et al. (1998) saw etch pits on feldspars near attached microbial colonies. Fisk et al. (1998) observed remnants of cells within etched channels on basaltic glass collected from the sea floor and found the etchings to be consistent with microbial weathering. Similarly, Furnes et al. (2004) found tubular and segmented etchings that were likely microbial in origin on formerly glassy rims of Archean pillow basalts. Irregular etchings on hematite particles (Maurice et al., 1996) and muscovite surfaces (Maurice et al., 2001) were observed after incubation with bacteria in laboratory and field experiments, respectively. Others have documented etch pits on surfaces from which colonies have been removed (Thorseth et al., 1995; Bennett et al., 1996a). Whether the etch pits were formed by way of direct cellular

attachment or chemical interactions with one or more microbial exudates is unknown. Conversely, Lüttge and Conrad (2004) found bacteria to inhibit etch pit formation on calcite surfaces.

Microorganisms produce and secrete a variety of substances that may influence mineral dissolution by lowering pH, by complexing with surface or solution ions, or by catalyzing redox reactions. Some of these substances include enzymes, alcohols, low molecular weight organic acids (LMWOA), high molecular weight extracellular polymeric substances (EPS), and highly Fe (III)-specific ligands called siderophores. Some high affinity ligands may also be released to extract other metals (e.g., Liermann et al., 2005).

The EPS that bacteria secrete are primarily composed of glycocalyx, which are primarily polysaccharides and serve to anchor and give structure to the biofilm and to concentrate and store enzymes, ions, other bioessential molecules, and heavy metals (Morel and Palenik, 1989; Brisou, 1995; Roane and Kellogg, 1996; Madigan et al., 2000; Templeton et al., 2003).

1.2. Siderophores

Most microorganisms need $\sim \mu\text{M}$ concentrations of Fe to thrive (Neilands, 1995). Fe(III)-oxides (including oxides, oxyhydroxides, and hydrated oxides), specifically goethite ($\alpha\text{-FeOOH}$), are the dominant forms of Fe in most aerobic soils (Hersman, 2000). Fe in these secondary minerals ultimately derives from the common rock-forming Fe-silicates: olivines, pyroxenes, amphiboles (notably hornblende), and biotite (Allen and Hajek, 1989; Huang, 1989). In soils containing these primary Fe minerals, the actions of microorganisms may affect silicate weathering rates (e.g., Bennett et al., 1996b; Liermann et al., 2000b; Buss et al., 2005) with implications for the global regulation of CO_2 over geologic timescales (e.g., Berner, 1995). However, the low solubility products of most Fe minerals and especially Fe(III)-oxides, limit the aqueous Fe concentration at equilibrium and near-neutral pH to as low as 10^{-17} M in inorganic solutions (Schwartzman and Volk, 1991). Many microorganisms have evolved the ability to produce siderophores in order to overcome the ~ 10 orders of magnitude difference between available Fe and Fe needed for metabolism (Hersman, 2000).

Typical aqueous siderophore concentrations in nature are estimated to range from approximately equal to as much as three orders of magnitude less than concentrations of other chelators in soils, such as LMWOA (Hersman et al., 1995; Hersman, 2000; Kalinowski et al., 2000). LMWOA can increase

weathering via proton- or ligand-promoted dissolution. However, siderophores have greater affinity for Fe than LMWOA and previous studies have shown that siderophores are more effective than LMWOA for inducing release of Fe(III) from minerals at near-neutral pH (Holmen and Casey, 1996; Kalinowski et al., 2000; Brantley et al., 2001).

Here we investigate the effects on Fe-silicate surfaces of siderophores, EPS, and microorganisms that only use Fe as a micronutrient (i.e., that do not respire Fe). We performed batch dissolution experiments in which flasks containing a polished Fe-silicate substrate and an Fe-free, buffered, pH-neutral growth medium were each supplemented with desferrioxamine-B mesylate (DFAM, the salt of a commercially available siderophore) or a strain of *Bacillus* sp., an obligately aerobic soil bacterium that produces an acidic glycocalyx (Brantley et al., 2001) and a catecholate siderophore (Kalinowski et al., 2000). To more specifically investigate the influence of EPS on siderophore-promoted dissolution, we also incubated hornblende glass planchets in batch experiments with alginate or xanthan gum (Sigma), two commercially available extracellular polysaccharides, with and without DFAM.

Surfaces were analyzed with AFM, Mirau vertical scanning interferometry (VSI), and XPS to document changes in microtopography and chemistry of the surfaces. Solution analyses were performed to document glucose consumption, pH changes, and Fe, Al, and Si release.

2. Materials and methods

2.1. Experimental setup

In order to isolate biogenic features from mineralogical features (e.g., heterogeneities among crystals, preferential dissolution of inclusions or along crystal grain boundaries), glass planchets were synthesized with a composition similar to the Fe-silicate mineral hornblende (Liermann et al., 2000a) and polished to provide a smooth, chemically analogous substrate. The composition of this “hornblende glass” (in wt.%: 48.2% SiO₂, 14.5% Al₂O₃, 11.0% Fe₂O₃, 8.33% CaO, 10.9% MgO, 2.31% Na₂O, 0.51% K₂O, where Fe₂O₃ includes Fe(II) and Fe(III)) was determined using the lithium metaborate fusion technique (Suhr and Ingamells, 1966; Medlin et al., 1969) and inductively coupled plasma-atomic emission spectrometry (ICP-AES). The glass was cut into planchets approximately 1 × 1 × 0.5 cm with a diamond blade and polished with diamond slurries to 0.25 μm. Polished samples were ultrasonicated in acetone for 10 min, air-dried, and stored in a dessicator.

The soil bacterium selected for this study was previously identified as an *Arthrobacter* but was subsequently found to be a *Bacillus* sp. (Buss et al., 2003). This bacterium was isolated from a hornblende-containing soil from Gore Mountain, New York, and has been shown to grow vigorously and produce siderophores in Fe-deficient growth medium in the presence of hornblende or hornblende glass (Kalinowski et al., 2000; Liermann et al., 2000a; Brantley et al., 2001). Two polished hornblende glass planchets were placed in each 500-ml glass culture flask and sterilized by autoclaving at 250 °C for 20 min. After cooling, 150 ml of sterilized, modified iron-free MM9 medium (Schwyn and Neilands, 1987; Liermann et al., 2000a) was added aseptically. The medium composition was: 6.0 g l⁻¹ Na₂HPO₄, 0.3 g l⁻¹ KH₂PO₄, 0.5 g l⁻¹ NaCl, 1.0 g l⁻¹ NH₄Cl, and 6.06 g l⁻¹ (50 mM) TRIS buffer, prepared from ultrapure chemicals and deionized water and buffered at pH 7.4. The medium was supplemented with 2% (v/v) Chelex-100-treated 10% (w/v) casamino acids (Bio-Rad Laboratories, Difraco Laboratories, respectively), 0.2% (v/v) 1 M MgSO₄, 1% (v/v) filter-sterilized 20% (w/v) glucose, and 0.01% (v/v) 1 M CaCl₂, each prepared and sterilized separately. Some experiments were supplemented with filter-sterilized 240 μM DFAM, or sterilized 0.1 g ml⁻¹ alginate or xanthan gum. Alginates are polysaccharides produced by several species of bacteria and algae. These polymers contain monomers of D-mannosyluronic and L-gulosyluronic acids (Budavari, 1996) or β-D-mannuronic and α-L-guluronic acids (Larsen and Haug, 1971). Xanthan gum is produced by *Xanthomonas campestris* and is composed of monomers of D-glucose, D-mannose, and D-glucuronic acid (Sloneker and Jeanes, 1962). The mannuronic acid and guluronic acid monomers of alginate have pK_a's of 3.38 and 3.65, respectively and the glucose, mannose, and glucuronic acid monomers of xanthan gum have pK_a's of 12.28, 12.08, and ~2.9, respectively (Wang et al., 1991; Rohrer and Olechno, 1992). The pK_a's of these polymers' acid groups are below the experimental pH of 7.4 indicating that they remain deprotonated during the experiments. Although the protonation constants for DFAM are relatively high (pK_a's of 8.50, 9.24, and 9.69), protonation occurs at non-chelating amino groups and thus does not interfere with Fe(III) chelation at pH values below the pK_a's (Winkelman, 1991).

Duplicate flasks for each of six conditions were set up. Abiotic conditions included 1) controls (medium+planchets), 2) DFAM-only (medium+planchets+DFAM), 3) xanthan-only (medium+planchets+xanthan gum), 4) alginate-only (medium+planchets+alginate), 5) xanthan+DFAM (medium+planchets+xanthan gum+DFAM), and 6) alginate+DFAM (medium+planchets+

Table 1
Si, Fe, and Al released from hornblende glass

Experiment ^a	Days incubation	[Si] μM	[Fe] μM	[Al] μM
Controls	39	24.6 \pm 1.7	0.82 \pm 0.00	bd
DFAM	39	159.2 \pm 2.7	3.1 \pm 0.3	9.5 \pm 0.9
DFAM	46	191.5 \pm 2.9	4.4 \pm 0.4	12.3 \pm 0.9
Bacteria	46	24.0 \pm 1.4	bd	0.58 \pm 0.33
Bacteria	46	47.5 \pm 1.4	bd	0.84 \pm 0.36
Alginate	47	7.4 \pm 14.1	0.28 \pm 0.08	0.67 \pm 0.39
Alginate	47	12.0 \pm 20.0	0.30 \pm 0.06	0.50 \pm 0.32
Xanthan gum	47	6.2 \pm 9.9	0.36 \pm 0.06	0.55 \pm 0.29
Xanthan gum	47	7.5 \pm 10.5	0.27 \pm 0.04	0.62 \pm 0.30
Alginate+DFAM	47	507.9 \pm 39.7	8.6 \pm 0.3	40.6 \pm 0.39
Alginate+DFAM	47	496.3 \pm 26.1	8.3 \pm 0.5	37.9 \pm 0.32
Xanthan+DFAM	47	328.3 \pm 16.6	2.6 \pm 0.1	17.4 \pm 1.4
Xanthan+DFAM	47	337.7 \pm 23.4	2.7 \pm 0.1	19.0 \pm 0.3

^a Control experiments contained hornblende glass planchets incubated in Fe-free MM9 medium only. DFAM experiments contained hornblende glass planchets incubated in Fe-free MM9 medium with 240 μM desferrioxamine-B mesylate. Bacteria experiments contained hornblende glass planchets incubated in Fe-free MM9 medium with *Bacillus* sp. Alginate and xanthan gum experiments contained hornblende glass planchets incubated in MM9 medium with 0.1 g l⁻¹ alginate or xanthan gum.

alginate+DFAM). To determine whether direct attachment to mineral surfaces is required for EPS to affect dissolution, in the flasks with EPS (alginate or xanthan gum) one of the two glass planchets was enclosed in 12–14,000 Da dialysis tubing. The molecular weights of alginate and xanthan gum are about 240,000 and >10⁶ Da, respectively (Budavari, 1996). Thus, the dialysis tubing prevented the polymers from contacting the surfaces while permitting free flow of siderophores and other small molecules and ions. A sixth set of 2 flasks contained live bacteria (medium+planchets+2.0 ml of an inoculum of stationary stage cultures of *Bacillus* sp.). Inocula contained 2.5 \times 10⁷ cells ml⁻¹, as counted on streak plates. All experiments were incubated at room temperature for 46 days on a shaker table continuously agitated at 120 rpm.

To monitor chemical changes over time and to replenish nutrients to sustain microbial growth, solutions were aseptically sampled from each flask and replaced with equivalent amounts of fresh medium \pm DFAM \pm EPS approximately once a week. Sampled solutions were syringe-filtered through 0.2 μm Nuclepore polycarbonate membranes and aliquots were measured for pH immediately. Of the remaining filtered supernatant, 2 ml was frozen for glucose analysis, and the remainder acidified to 1% with nitric acid for elemental analysis of Fe, Al, and Si by inductively coupled plasma-atomic emission spectrometry (ICP-AES) and ICP-mass spectrometry (ICP-MS).

After 47 days, the EPS experiments were terminated and the planchets were gently rinsed with fresh MM9

medium followed by distilled and deionized water. Planchets were then imaged using an FEI Quanta 2000 Environmental SEM operated at 5 $^{\circ}\text{C}$ and 4.5–5 Torr.

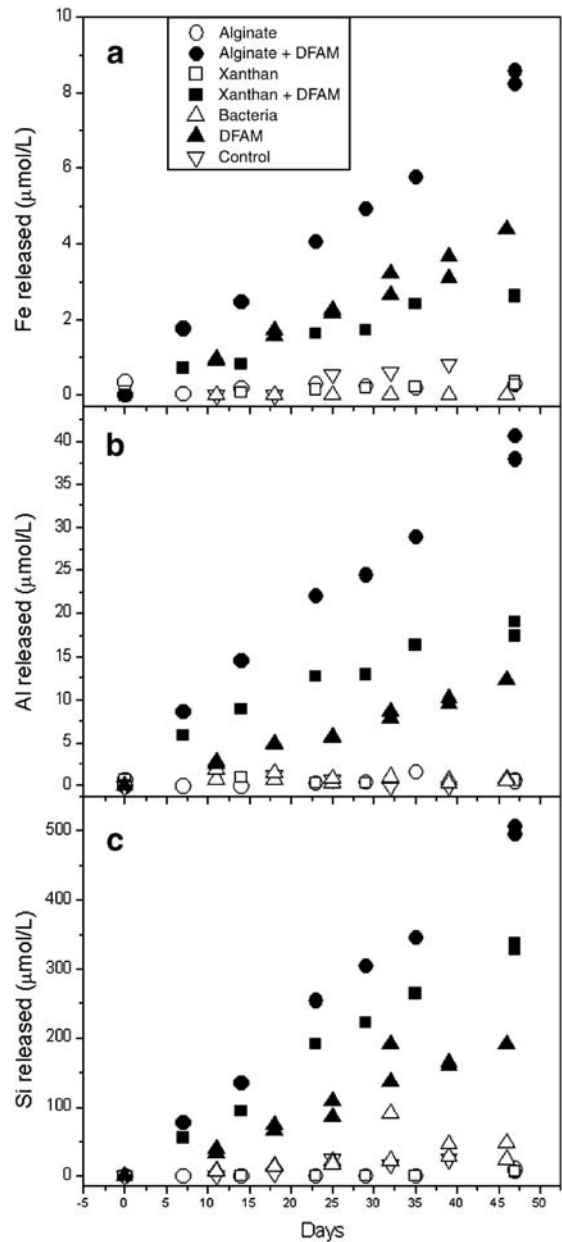


Fig. 1. Release of Fe, Al, or Si (μM) from Fe-silicate glass surfaces to MM9 medium. (a) The [Fe] in the bacteria-containing experiments is below the lower detection limits of the ICP-AES, consistent with uptake of Fe by the bacteria. Fe release was comparable in all solutions without DFAM, whereas the DFAM-containing solutions enhanced Fe release. Alginate+DFAM solutions showed the greatest release. Similar trends are seen for (b) Al release and (c) Si release, except these elements were not completely removed from solution by the bacteria.

Following ESEM analysis, all plachets were ultrasonicated for 45 min in a 2% solution of sodium dodecyl sulfate (SDS), then rinsed in distilled and deionized water and ultrasonicated for 30 min in spectroscopic grade acetone before air-drying. SDS has been shown to effectively remove biomatter from hornblende glass without chemically or physically altering the surfaces (Buss et al., 2003).

At the end of the bacteria-containing experiments, the bacteria in the solutions were pelleted by centrifugation, dried overnight at 65 °C, and weighed.

2.2. XPS

X-ray photoelectron spectroscopy (XPS) has been used to study a variety of chemical changes at mineral surfaces such as the bioleaching of metals, leached-layer formation, or adsorption of organics (e.g., Balaz et al., 1996; Blight et al., 2000; Hamilton et al., 2000; Kalinowski et al., 2000; Maurice et al., 2001; Buss et al., 2003).

Elemental concentrations of the upper ~100 Å of 3 oval areas (1 × 0.7 mm each) of each of the hornblende glass plachets were analyzed by XPS using a Kratos Analytical Axis ULTRA XPS with a 1486.6 eV Al monochromatic X-ray source at 280 W at a takeoff angle of 90° with respect to the sample plane. The three measurements for each individual plachet were averaged. Prior to XPS analysis, samples were cleaned with spectroscopic grade acetone followed by 15 min of ultraviolet

ozone cleaning (UVOC) to remove organic contamination (Fig. 1992; Zazzera and Evans, 1993; Kalinowski et al., 2000). Such contamination can distort elemental ratios such as Fe/Si as measured by XPS (Buss et al., 2003).

2.3. AFM

All hornblende glass plachets were imaged in air with a Digital Instruments Dimension™ 3100 Atomic Force Microscope in Tapping-Mode® (TM-AFM) using a tapping-mode etched silicon probe tip (TESP-70) at a scan-rate of 0.75–1.00 Hz.

Three types of images were collected of each scan, including height images, showing features both above and below the average surface level; amplitude images, showing only the positive features; and phase-contrast images, revealing variations in surface adhesive properties (Digital Instruments, 1997). Third-order plane fitting was performed on each image to eliminate tilt and S-shaped bow distortions caused by curvature of the piezoelectric stylus, thermal drift, or lateral forces (Ruppe and Duparee, 1996). Fifteen to 26 randomly chosen 100 μm² areas of each surface were scanned in addition to 4–10 μm² areas, which were scanned to examine surface features in detail.

The images were analyzed using Digital Instruments Nanoscope IIIa Controller software to measure the dimensions of surface features and to calculate the root mean square (RMS) roughness – the standard deviation of

Table 2
Surface data

Experiment ^a	Days incubation	Fe/Si (at.%) ^b	Fe/Si (at.%) In dialysis bag ^c	AFM ^d RMS (nm)	AFM RMS (nm) In dialysis bag	VSI ^e RMS (nm)
Blanks	0	0.102–0.110	na ^f	2.83–5.40	na	nd ^f
Controls	39	0.104–0.117	na	3.12–5.20	na	5.35–26.2
DFAM	39	0.072±0.016	na	3.80–8.60	na	4.14–29.8
<i>Bacillus</i>	46	0.072±0.016	na	3.22–7.33	na	5.19–21.5
Alginate	47	0.154±0.016	0.084±0.016	3.57–4.71	3.32–4.85	nd
Xanthan gum	47	0.102±0.016	0.092±0.016	3.00–4.72	2.98–5.34	nd
Alginate + DFAM	47	0.107±0.016	0.091±0.016	4.32–6.39	4.16–6.06	nd
Xanthan + DFAM	47	0.038±0.016	0.018±0.016	3.98–11.5	4.28–12.9	nd

^a Blanks are the starting material: polished hornblende glass plachets that were not incubated. Control experiments contained hornblende glass plachets incubated in Fe-free MM9 medium only. DFAM experiments contained hornblende glass plachets incubated in Fe-free MM9 medium with 240 μM desferrioxamine-B mesylate. Bacteria experiments contained hornblende glass plachets incubated in Fe-free MM9 medium with *Bacillus* sp. Alginate and xanthan gum experiments contained hornblende glass plachets incubated in MM9 medium with 0.1 g l⁻¹ alginate or xanthan gum.

^b Surface Fe relative to Si measured by XPS. Each XPS measurement is an average of three measurements on a single sample. Ranges are given for replicate blanks and controls. Error in these values is based on measurement error of 5% for Si and 10% for Fe. This error is shown in Fig. 2. Measurements on other replicate samples are reported as averages with error given as the sample variability based on the range and precision of measurement on the blanks.

^c “In dialysis bag” indicates that the surfaces were protected from EPS by dialysis tubing.

^d Range of AFM root mean square roughness values measured on 10 × 10 μm AFM images using Digital Instruments Nanoscope IIIa Controller software.

^e Range of VSI root mean square roughness values calculated from raw numerical VSI data collected from 124 × 163 μm areas.

^f na = not applicable, nd = not determined.

the height measurements relative to the basal plane – for each image. Only 100 μm^2 scans at the same resolution (39 nm lateral resolution) were compared because RMS roughness varies with scan size and resolution (e.g., Mellott et al., 2002). Fifteen RMS roughness measurements were made of the untreated, polished glass surfaces (blanks) to obtain a range of RMS roughness values for the initial variations of the glass surfaces.

2.4. VSI

Mirau vertical scanning interferometry (VSI) is a light-optical technique that provides an additional source of microtopographic data, providing approximately 1 nm vertical resolution in white light mode. In green light mode (i.e., a narrow band of green light centered at 550 nm), VSI has a vertical resolution of 0.7 Å. Although VSI is commonly used in industry for quality assurance applications, its use as a research tool is still relatively novel (Lüttge et al., 1999; Lasaga and Lüttge, 2001). Scanning surfaces with light prevents many of the analytical artifacts that result from the physical probing involved in AFM. Additionally, VSI can scan up to a 760 × 840 μm area with a vertical scan range of up to 100 μm , while AFM scans are limited to a maximum of 90 × 90 μm with a vertical scan range of only ~6 μm . Although AFM suffers from pixelization, which limits resolution to the pixel size (Digital Instruments, 1997), the maximum lateral resolution of AFM (1–5 nm) is far superior to VSI (0.5–1.2 μm) making both techniques indispensable and complementary tools for analyzing surfaces in detail. A thorough description of the VSI technique is found in Lüttge et al. (1999).

A vertical scanning phase shift interferometer (MP8 8, ADE Phase Shift, Tuscon) was used to image the hornblende glass samples. For each sample, an overall scan of 800 × 600 μm was made with a 10× objective followed by 25 scans of adjacent ~124 × 163 μm areas to form a 5 × 5 grid pattern using a 50× objective. The raw VSI data were analyzed using software we developed to format the data and measure RMS roughness. Topographic height images, 3-D plots, and cross-sectional traces were produced from the digitized interferograms of some scans using MAPVUE software (ADE Phase Shift, Tuscon, Arizona).

3. Results

3.1. Solution chemistry

Concentrations of Fe in the filtered solutions, [Fe], increased with time for all conditions except those

incubated with bacteria (Table 1, Fig. 1a), for which [Fe] was below the lower limit of detection (0.36 μM for [Fe] by ICP-AES). Release of Fe, Al, and Si in control, xanthan-only, and alginate-only experiments was negligible (Fig. 1). Release of these elements was enhanced in all experiments containing DFAM, with alginate+DFAM experiments showing the greatest release. By day 11, xanthan+DFAM experiments showed higher [Al] and [Si], but approximately equal [Fe] compared to the DFAM-only experiments.

Throughout the experiments, changes in pH were negligible, with pH ranging from 7.2 to 7.6, as compared to a starting pH of 7.4. Glucose levels in abiotic experiments remained relatively constant (>1500 mg l^{-1}). Only in the experiments with bacteria were fluctuations in glucose levels observed, increasing to 600–680 mg l^{-1} immediately after replenishment with fresh medium at each sampling time point and then dropping to 4.3–6.8 mg l^{-1} by the following week. The drastic reductions in glucose levels between sampling days in the biotic solutions confirmed that the bacteria remained viable for the duration of the experiments.

3.2. Surface chemistry

Ratios of Fe/Si on polished hornblende glass surfaces are identical within error for untreated blanks and abiotic

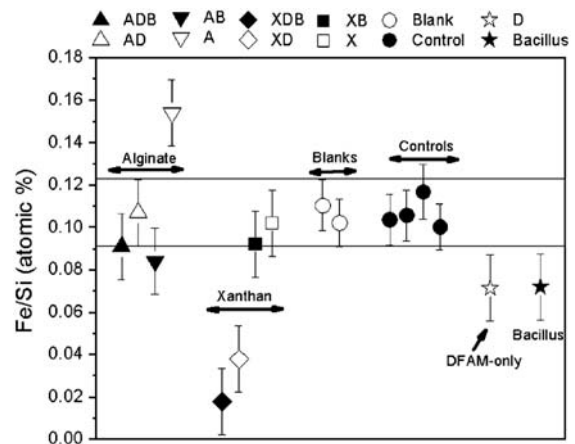


Fig. 2. Fe/Si ratios of the upper ~100 Å of the hornblende glass surfaces measured by XPS. Error in the XPS measurements is estimated to be about 5% for Si and 10% for Fe. Measurements on replicate samples are shown as averages except for the blanks and controls, for which all replicates are shown. Sample variability is greater than the XPS measurement error for blanks: the highest and lowest Fe/Si ratios are bracketed by lines for the two blanks. This variability is used as the error on the samples. Standard deviations of the averaged values are within this variability range. The x-axis is arbitrary. Sample codes: A = alginate, X = xanthan gum, D = DFAM, B = inside dialysis bag, control = growth media only.

controls incubated in medium for 39 days (Table 2, Fig. 2). For our analysis, ratios are considered “unchanged” when they fall within the range of the ratios measured on the

untreated blanks ($\text{Fe/Si}=0.102\text{--}0.110$, ± 0.012). This range of values includes measurement errors of 5% for Si and 10% for Fe. This “blank range” (± 0.016) indicates sample variability and is used as an estimate of error in the XPS analyses. DFAM-only experimental surfaces have lower Fe/Si ratios (0.072 ± 0.016) than the blank range. Fe/Si ratios are significantly lower on surfaces exposed to xanthan+DFAM, whether inside or outside the dialysis bags (0.018 ± 0.016 and 0.038 ± 0.016 , respectively). In contrast, Fe/Si ratios on xanthan-only experimental surfaces are unchanged. The surfaces exposed to alginate+DFAM have unchanged Fe/Si ratios regardless of the dialysis bags. The alginate-only sample incubated inside the bag also has an unchanged Fe/Si ratio but the sample incubated outside the bag has an elevated Fe/Si ratio (0.154 ± 0.016).

3.3. Etch pits

No evidence for widespread adsorption of EPS on surfaces was detected by ESEM. The planchet exposed to alginate-only (and not protected by dialysis tubing) did rarely contain a few strands of hydrated material within some of the larger polishing scratches; such strands were not observed on any other samples.

In AFM images, surfaces from all experiments were observed to contain polishing scratches, 7–30 nm deep and <600 nm wide as seen previously (Buss et al., 2003). All surfaces also contain very small pits <20 nm deep. However, these pits were rare (0–3 pits per $100\ \mu\text{m}^2$ scan) except on surfaces exposed to DFAM. Regardless of the sample treatment and pit size, etch pits were roughly circular to oval-shaped. Thus, different treatments do not produce differently shaped etch features as has been demonstrated for anisotropic materials such as crystals (e.g., Honess, 1929).

The control and EPS-only surfaces appear identical to the blanks as observed by AFM. Specifically, these surfaces exhibit no distinguishable changes in the shapes or dimensions of the etched polishing scratches or the bulk

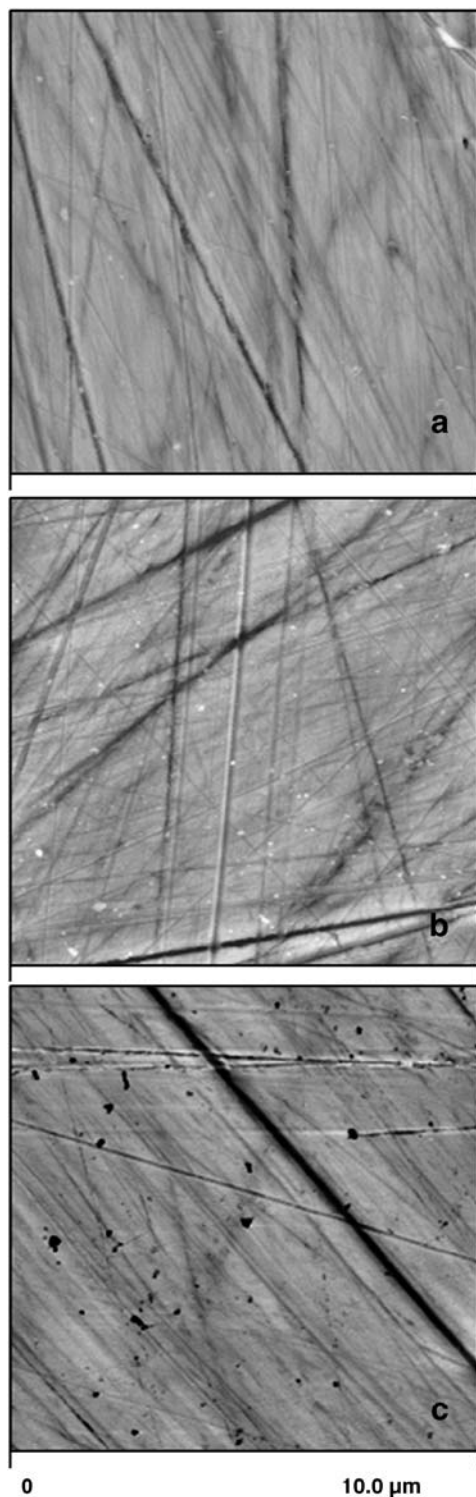


Fig. 3. AFM height images of $10\times 10\ \mu\text{m}$ areas of hornblende glass planchets. The vertical scale is $\pm 200\ \text{nm}$ from the average plane (gray). Lighter and darker areas represent positive and negative topography, respectively, relative to the average plane of the surface. (a) Starting surfaces, or “blanks,” were polished but not incubated in solution. AFM images of blanks reveal polishing scratches visible on all sample surfaces. (b) Control surfaces were polished and incubated in MM9 growth medium for 46 days and appear unchanged relative to blanks. (c) Surfaces exposed $240\ \mu\text{M}$ DFAM in MM9 medium for 46 days reveal numerous, widely distributed etch pits (<450 nm wide, <60 nm deep). Pits were seen on un-scratched areas of the surfaces as well as along polishing scratches.

surfaces relative to the blanks. In contrast, some polishing scratches appeared enlarged on the DFAM experiments as a result of etch pit formation along the scratches (Fig. 3); in addition, many etch pits were not associated with polishing scratches. The DFAM-exposed surfaces also contained numerous (4–50 per $100 \mu\text{m}^2$ area), scattered, small etch pits, measuring less than 450 nm wide and less than 60 nm deep. In fact, a $100 \mu\text{m}^2$ region on any DFAM-treated planchet could not be scanned with the AFM without observing from 4 to 20 or more pits.

Etch pits on surfaces exposed to alginate+DFAM or xanthan+DFAM are comparable in size and frequency to

etch pits on DFAM-only surfaces regardless of dialysis tubing (Figs. 3 and 4). The glass surfaces in the EPS experiments did not show any topographic variability according to presence or absence of dialysis tubing. In comparison to the DFAM-only surfaces, non-pitted regions of the surfaces exposed to alginate+DFAM or xanthan+DFAM appear more corroded in that the polishing features are more prominent (Figs. 4 and 5).

The etch pits on the bacteria-exposed surfaces tended to be fewer (0–5 pits per $100 \mu\text{m}^2$ image), larger (~ 300 – 1800 nm wide, <95 nm deep), and grouped together, unlike those observed on the DFAM

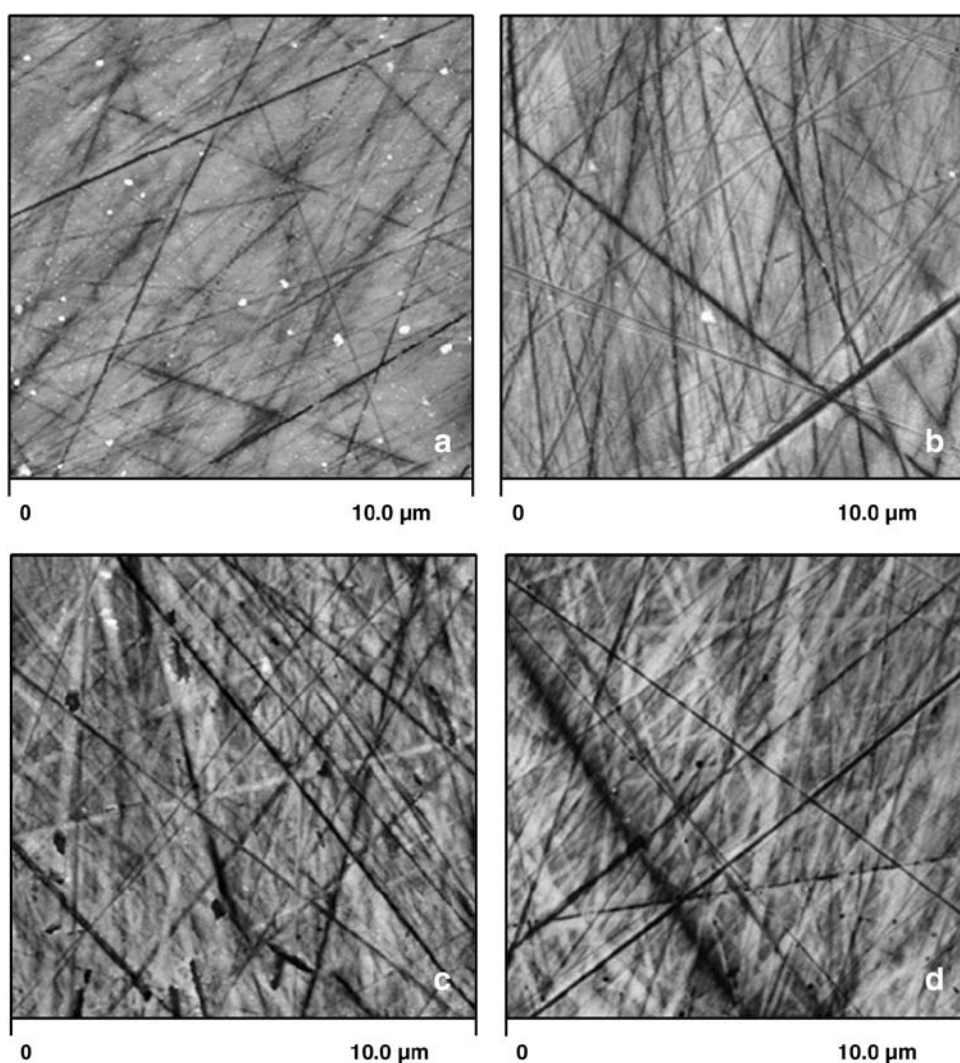


Fig. 4. AFM height images of $10 \times 10 \mu\text{m}$ areas of hornblende glass planchets. The vertical scale is ± 200 nm from the average plane. Lighter and darker areas represent positive and negative topography, respectively, relative to the average plane of the surface. (a) On surfaces incubated in MM9 medium with xanthan gum or (b) alginate, polishing scratches appear slightly more prominent than on control and blank surfaces (Fig. 3a–b). (c) When the siderophore DFAM was combined with xanthan gum or (d) with alginate, etch pits, enlarged polishing scratches and more prominently etched background texture were observed in AFM images.

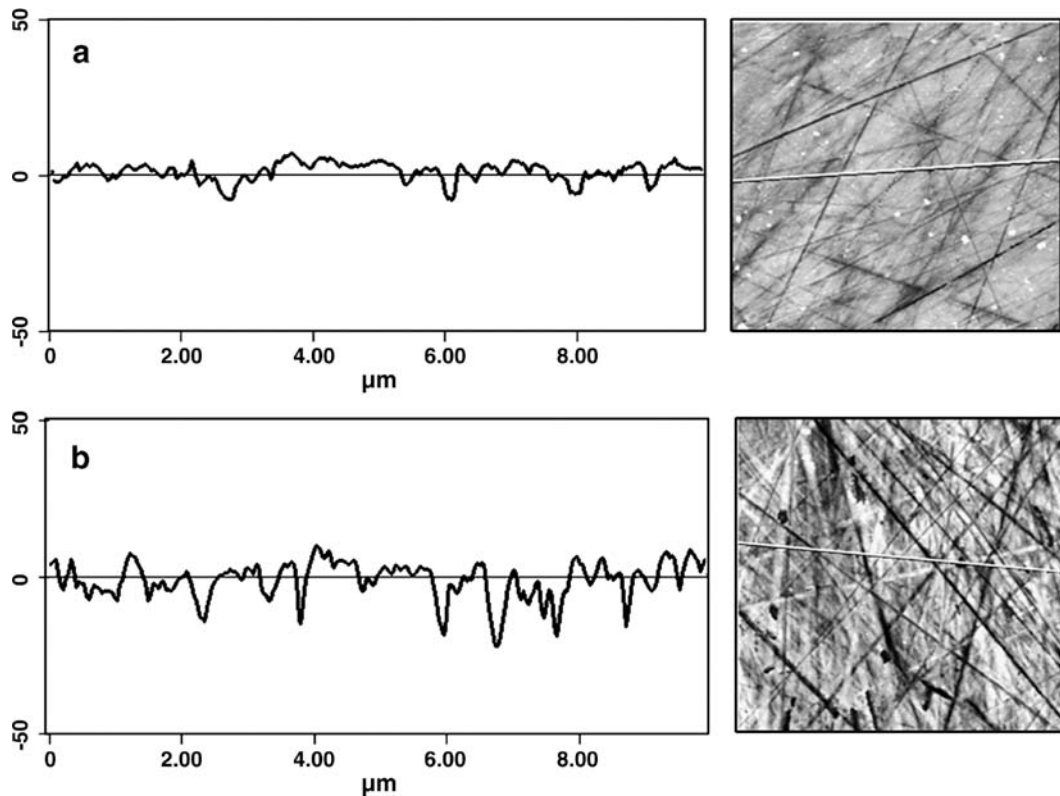


Fig. 5. Cross sections of AFM imaged surfaces exposed to (a) xanthan gum or (b) xanthan gum and the siderophore DFAM. The white lines on the AFM images at the right of the figure indicate the locations of the cross sections. The vertical scale is ± 50 nm.

experiments (Figs. 3 and 6). Although etch pits also formed along the polishing scratches of the surfaces that were exposed to *Bacillus* sp., these pits were too few to impact the overall shape of the polishing scratches.

3.4. RMS roughness

RMS roughness, is the root mean square average of height deviations from the average plane, calculated from the relative heights of each data point

$$\text{RMS} = \sqrt{\frac{(z_1^2 + z_2^2 + \dots + z_n^2)}{n}} \quad (1)$$

where z_i is the height difference relative to the mean plane for each point i and n is the total number of points measured. Fifteen AFM height images of the blanks (polished, untreated glass surfaces) were collected and analyzed for RMS roughness (Table 2, Fig. 7). The range of values for these blanks (2.83–5.36 nm) was used as a comparison to the glass surfaces exposed to experimental solutions. Unusually large polishing

scratches on all samples have larger RMS roughness, and were therefore avoided during imaging.

Roughness values (AFM) measured on the control, alginate-only, and xanthan-only experimental surfaces fall within the range of the blanks. All surfaces exposed to DFAM or bacteria have elevated RMS roughness as follows: (alginate+DFAM) \approx bacteria < DFAM-only < bacteria \approx DFAM-only \ll (xanthan+DFAM). No such correlation was observed between RMS roughness and Al-depletion.

For control, bacteria, and DFAM-only surfaces, 25 adjacent $164 \times 124 \mu\text{m}$ VSI scans were performed to obtain a representative analysis of the surfaces. The larger VSI scans show numerous etch pits on the DFAM-exposed surfaces (Fig. 8), but due to the lower lateral resolution of the VSI scans, the majority of the DFAM-pits visible in the AFM images (< 450 nm wide) are not visible at the VSI scale. Many of the pits visible in the VSI scans may represent etching at inherent glass defects (e.g., air bubbles; Buss et al., 2003) that were avoided during AFM imaging. Rather than rely on visual analyses of these pits and other surface features, we developed algorithms to calculate

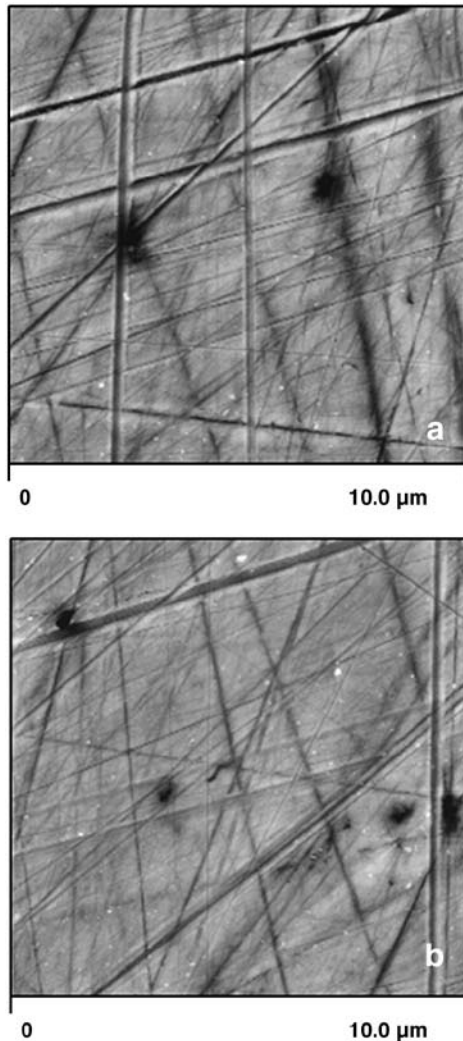


Fig. 6. (a) and (b) AFM height images of $10 \times 10 \mu\text{m}$ areas of hornblende glass surfaces after incubation with *Bacillus* sp. Vertical scale is $\pm 200 \text{ nm}$ from the average plane, which is represented by gray. Lighter and darker areas represent positive and negative topography, respectively, relative to the average plane of the surface. The etch pits caused by the bacteria tend to form in groups as shown here, leaving most of the surface un-pitted. Such “biopits” also tend to be larger than those formed in abiotic experiments (Figs. 3 and 4).

RMS roughness values from the raw numerical VSI data. RMS roughness values of the VSI images were more variable than RMS roughness values determined on AFM images, but followed the same general trend: controls < bacteria < DFAM, confirming AFM results (Table 2).

4. Discussion

Characteristic etching and non-stoichiometric chemical changes demonstrate the coupled physical–chemical

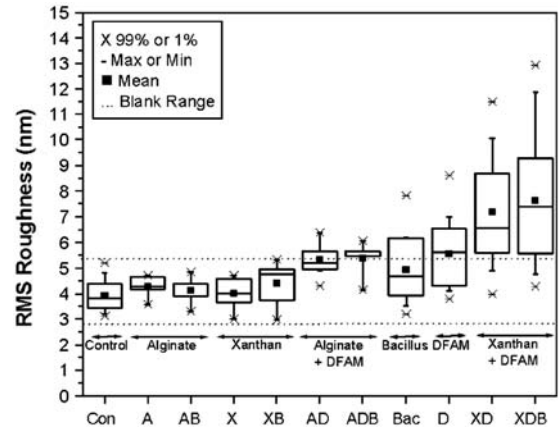


Fig. 7. Box and whisker statistical plot of root mean squared (RMS) roughness measured by AFM on polished Fe-silicate sample surfaces. Sample codes: A = alginate, X = xanthan gum, D = DFAM, B = inside dialysis bag, Bac = bacteria, Con = control (growth media only). Dotted lines indicate the RMS roughness range of the untreated starting surfaces (blanks) measured on 15 surfaces. Boxes represent 25–75% of the data, whiskers (vertical lines) indicate 5–95% of the data, × symbols bracket the range between 1 and 99% of the data and fall coincident with the dash (–) symbols, which indicate the maximum and minimum values. Solid squares (■) represent the mean.

nature of microbial dissolution. It has been established that siderophores promote mineral dissolution (Hersman et al., 1995; Kraemer et al., 1999; Liermann et al., 2000b; Cervini-Silva and Sposito, 2002; Coccozza et al., 2002; Cheah et al., 2003; Rosenberg and Maurice, 2003) and

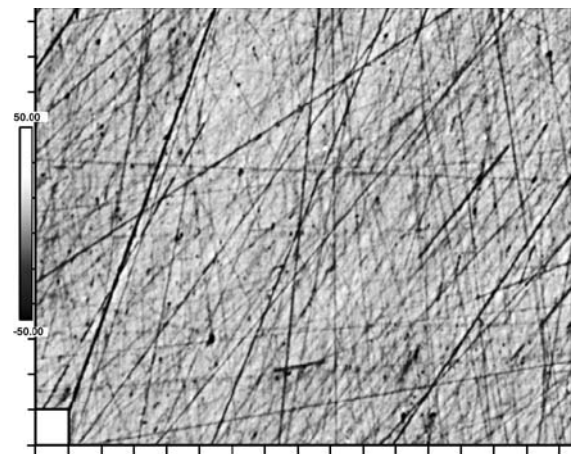


Fig. 8. VSI height image of a $164 \times 124 \mu\text{m}$ area of a hornblende glass planchet incubated in $240 \mu\text{M}$ DFAM in MM9 growth medium. Vertical scale is $\pm 50 \text{ nm}$ from the average plane. As with the AFM height images, the lighter and darker areas represent positive and negative topography, respectively, relative to the average plane of the surface, which is gray. The white square in the bottom left corner of the image indicates the average size of the AFM scans ($10 \times 10 \mu\text{m}$).

we have now shown that siderophores can alter the topography of mineral surfaces during dissolution. This is the first documented case in which the growth of etch pits in response to siderophores has been observed. Etch pits either were nucleated by siderophores or, if undetectable etch pits had previously nucleated, grew in the presence of siderophores. However, the presence of pre-existing etch pits is unlikely because the surfaces were polished prior to use in the experiments.

It has been well established that most microorganisms reside in biofilms, composed in large part by EPS, and exist attached to surfaces in natural environments (e.g., Brisou, 1995; Watnick and Kolter, 2000). Here we show that interaction of EPS with siderophores affects mineral dissolution in a manner distinct from either substance alone.

To determine the extent of dissolution, we can compare the solution chemistry, etch pits, surface roughness, and XPS data. For example, according to the solution chemistry data, the release of Fe was most enhanced by alginate+DFAM. This dissolution was close to stoichiometric because the Fe/Si ratio of the surface was not changed from the blanks or controls (Fig. 2). In contrast, although the solution chemistry would suggest that xanthan+DFAM experiment did not release the most Fe to solution, the surface roughness was significantly higher than for other experiments, and the Fe/Si ratio measured by XPS was significantly lower. These surface measurements indicate significant dissolution and preferential Fe release that was not evident from the solution data alone.

4.1. Surface effects of siderophores

Differences in size, distribution, and number of etch pits on the DFAM-exposed surfaces as compared to controls and other samples indicate that dissolution by siderophores promotes the widespread growth of etch pits on the Fe-silicate glass surfaces. Although etch pits were frequently seen along polishing scratches, the bulk surfaces (areas not marred by polishing scratches) also contained numerous pits of comparable size. Etch pits contribute to surface roughness and increased surface area, promoting further dissolution. The 240 μM concentration of DFAM used in these experiments lies within the range of siderophore concentrations estimated for soil solutions ($\sim 10 \mu\text{M}$ –mM, Hersman et al., 1995). The extensive pitting produced by DFAM under these relatively dilute conditions demonstrates the potential importance of siderophore-promoted pitting to mineral dissolution in the environment. Indeed, etch pits have been observed on hornblende crystals in the environment (e.g., Berner and Schott, 1982; Anand and Gilkes, 1984;

Hall and Martin, 1986; Velbel, 1989; Brantley et al., 1993; Hall and Horn, 1993).

Dissolution stoichiometries can be documented by changes in the ratio of Fe/Si on the surfaces of the glasses. XPS evidence documents Fe depletion in the layer of glasses exposed to bacteria or DFAM alone, consistent with previous experiments with *Bacillus* sp. and hornblende crystal (Kalinowski et al., 2000) or hornblende glass (Buss et al., 2003) where Fe depletion was also shown. Lateral resolution of XPS is poor: Kalinowski et al. used a spot size of 3 mm on polished hornblende crystal and Buss et al. used a spot size of 700 μm on polished hornblende glass. XPS yields mol% elemental composition averaged over some area and depth into the surface, $\sim 100 \text{ \AA}$ depth based upon the angle of measurement. Therefore, if the depletion observed on DFAM-only or bacteria-exposed experimental surfaces is localized to etch pits less than 2 μm wide and up to 1 μm deep, the actual depletion at the pits may be much greater than estimated based on XPS analyses. Such a possibility has been suggested for other phases: Berner et al. (1985) proposed that localized dissolution via etch pit formation may explain apparent discrepancies between XPS-based and solution chemistry-based estimations of feldspar leached-layer thicknesses (Schott et al., 1981).

4.2. Surface effects of EPS

Dissolution of hornblende glass (as measured by Si release, Fig. 1c) was not enhanced in the presence of EPS alone. This is consistent with observations by Welch and Vandevivere (1994), who also saw no enhanced dissolution when incubating either alginate or xanthan gum with feldspars. They did observe enhanced dissolution in the presence of fresh EPS extracted from bacterial cultures in their laboratory, and proposed that low molecular weight metabolites present in the fresh EPS may have contributed to dissolution. Likewise, Malinovskaya et al. (1990) also found that EPS produced by *Bacillus mucilaginosus* enhanced dissolution of silicate minerals only when incubated with minerals in combination with low molecular weight metabolites such as organic acids. Similarly, in our experiments, EPS did not enhance release of Fe to solution without the addition of a siderophore. Welch and Vandevivere (1994) concluded that bacterial EPS may aid dissolution by affecting the affinity of reaction through complexation of dissolved cations, thereby changing the saturation state of the cation with respect to the dissolving solid.

Siderophores increase solubility by complexing aqueous Fe(III). Simulations of our experiments run

using Geochemist's Workbench 4.0 (Bethke, 2002) using the thermodynamic database of Delany and Lundeen (1991) with added constants from NIST (1998) showed that our experiments were consistent with this effect. Concentrations of elements in solution for each experiment on each sampling day were entered into the simulations. The results demonstrated that if the solution were allowed to equilibrate, control solutions (without DFAM) would precipitate iron as a ferric mineral, whereas solutions with DFAM would retain all Fe(III) as aqueous species.

In our experiments, when DFAM was added to alginate or xanthan gum, Fe release to solution more than doubled over what was observed in the presence of the siderophore alone. A similar effect was seen by Cervini-Silva and Sposito (2002) and Cheah et al. (2003) on goethite dissolution when mixing the siderophore desferrioxamine-B with oxalate. In those studies, dissolution in the presence of both the siderophore and oxalate was greater than the sum of the dissolution effects of the two ligands alone. Siderophores have significantly higher affinity for Fe(III) than does oxalate but due to their large size may be sterically hindered from forming surface complexes as easily as oxalate. Cheah et al. (2003) concluded that the siderophores in solution complexed Fe(III) from aqueous oxalate-Fe(III) complexes, freeing the oxalate ions to complex additional surface-bound Fe(III). In this way, the siderophores effectively use oxalate as an Fe shuttle.

Similarly, the presence of acetate led to an increase in the dissolution rate of amorphous Cr(III) hydroxide by each of 6 different aminocarboxylate chelators (Carbonaro, 2005). In that study, the adsorption of chelators onto the mineral surface was reduced at the same time that the dissolution rate increased. Carbonaro (2005) proposed a mechanism by which a chelator adsorbed to the Cr(III) hydroxide surface may more effectively remove a metal atom when acetate is adsorbed to a neighboring metal atom because acetate may increase the rate of ligand exchange on the bridging oxygen atom. Although acetate may not be a strong enough ligand to measurably enhance the rate of dissolution alone, in this way it may aid dissolution by other, stronger, ligands such as siderophores. Carbonaro (2005) also proposed a second mechanism in which the adsorption of acetate alters the speciation of adsorbed chelators. In this scenario, acetate occupies neighboring coordination sites, which deters chelators from forming multinuclear surface complexes in favor of mononuclear complexes, which more effectively remove the metal atoms from the surface, increasing the dissolution rate.

In our study, we see no evidence for strong adhesion or direct physical effects from EPS-surface contact in ESEM

or AFM images, or in surface Fe/Si ratios. Therefore, enhanced dissolution and roughness cannot be attributed to direct contact with the polymer. Xanthan gum is a complex polysaccharide polymer that forms highly viscous, gel-like solutions. The primary monomers of the polysaccharide backbone are D-glucose, D-mannose, and D-glucuronic acid, but the polymer also contains 4.7% acetic acid and 3.0–3.5% pyruvic acid by weight (Sloneker and Jeanes, 1962). The acetic acid is present as an ester (Sloneker and Jeanes, 1962), which is susceptible to hydrolysis at room temperature; thus xanthan gum is likely to release acetate ions into solution. Both acetate and pyruvate are small enough (60.05 and 88.06 g mol⁻¹, respectively) to pass easily through the dialysis tubing (12,000–14,000 Da) and may have interacted with the surface and enhanced siderophore-promoted dissolution via one of the aforementioned mechanisms (Cheah et al., 2003; Carbonaro, 2005). Removal of Fe could de-stabilize the glass structure, enhancing overall dissolution in addition to discrete dissolution at etch pits.

Although monodentate ligands such as acetate are not as effective at enhancing mineral dissolution as multidentate ligands such as oxalate, small increases in dissolution in the presence of acetate have been recorded (e.g., Hamer et al., 2003; e.g., Miller et al., 1986). In similar experiments using the same MM9 medium and constant agitation (as used here), Brantley et al. (2004) documented increased iron release from hornblende crystal in the presence of acetate.

To investigate the possibility of acetate release from xanthan gum and alginate in our experiments, we filtered 1-week old solutions of 0.1 g l⁻¹ alginate, 0.1 g l⁻¹ xanthan gum, and 240 μM DFAM, each with and without 240 μM acetic acid and analyzed them with an ion chromatograph (Dionex 2010i) using a 0.005 M Na-borate eluent and compared to a 240 μM acetic acid standard. Results were consistent with the presence of a LMWOA in solutions containing alginate or xanthan gum (as well as in all solutions spiked with acetate for comparison). Resolution of the peaks was not sufficient to positively identify acetate versus other LMWOA's. Therefore, to confirm the presence of acetate in solutions containing alginate or xanthan gum, samples of these polymers at 0.1 g l⁻¹ were filtered and analyzed on a gas chromatograph mass spectrometer. Peaks were positively identified as acetate in the spectra for both polymer samples. The absence of other LMWOA's was not verified and thus other LMWOA's could have been present at much lower concentrations than acetate. However, enhanced dissolution in the presence of a siderophore and a bidentate LMWOA such as oxalate would likely be observed even at concentrations of a few micromolar oxalate (S. Kraemer, pers. comm.).

Exopolysaccharides from a wide variety of bacteria have been shown to contain acetyl groups, e.g., succinoglycan produced by the nitrogen-fixing soil bacterium *Sinorhizobium meliloti* (González et al., 1996), the EPS of thermophilic *Streptococcus thermophilus* (Nordmark et al., 2005), and the EPS of *Klebsiella aerogenes* (Atkins et al., 1987). Thus, siderophore-promoted dissolution enhanced by EPS-derived acetate or other small organic moieties could be an important component of biogeochemical iron cycling in a variety of bacterial biofilm systems.

Although general dissolution, as measured by Si release into solution, was enhanced by combining DFAM and xanthan gum, Fe release into solution was not enhanced over that observed in the presence of DFAM alone. In contrast, Fe release was more than doubled over DFAM alone when DFAM was combined with alginate. However, XPS results show the Fe/Si ratio to be extremely low on the xanthan+DFAM surfaces (Fig. 2), indicating either a non-stoichiometric, preferential loss of Fe or precipitation of Si onto the surface. Neither of these possibilities was reflected in the solution data. If some Fe were trapped within, or complexed to the polymer, it may have been filtered out of the solution before ICP-MS analysis. Considering the highly viscous nature of the xanthan gum in solution at 0.1 g l^{-1} , this is plausible. Furthermore, trivalent metal ions including Al^{3+} and Fe^{3+} can crosslink xanthan gum inducing gelation (Nolte et al., 1992), which would have increased viscosity and Fe sequestration within the polymer. In addition, removal of acetyl groups from bacterial EPS has been shown to increase the viscosity and crystallinity of the polymer (Atkins et al., 1987; Sutherland, 1997, 2001), which could also restrict movement of metal ions within the polymer. Elevated Fe/Si ratios on surfaces exposed directly to alginate (without DFAM) may indicate back precipitation of an Fe(III)-containing phase, demonstrating a lesser tendency to sequester metals compared to xanthan gum.

4.3. Surface effects of *Bacillus* sp.

In experiments containing *Bacillus* sp., concentrations of Fe in solution that are below detection are consistent with uptake of Fe by cells (Brantley et al., 2001). XPS data confirms preferential removal of Fe from the glass surfaces exposed to bacteria (Fig. 2).

Proton-promoted dissolution is an unlikely mechanism for etch pit formation in the presence of bacteria in these experiments because 1) etch pits were not seen on control surfaces, and 2) silicate dissolution should not be affected by the small pH changes observed within the range of this study (7.2–7.6, White and Brantley, 1995).

Therefore, the etch pits produced in the presence of bacteria were likely caused by ligand-promoted dissolution. Although lower pH values in micro-environments at the microbe–mineral interface can contribute to pitting (Barker and Banfield, 1998), Liermann et al. (2000a) detected a pH change of less than 0.04 across biofilms of *Bacillus* sp. grown in the same buffered medium used here in the presence of hornblende.

The production of glycocalyx by *Bacillus* sp. grown in the presence of hornblende was verified previously by Alcian Blue staining, which revealed a layer of acidic polysaccharides surrounding the cells when grown in an iron-depleted medium with hornblende crystals (Brantley et al., 2001). However, in the present experiments, *Bacillus* sp. did not produce enough polymeric material to harvest for experimentation. Iron release in the DFAM and EPS experiments cannot be directly compared to the *Bacillus* sp. experiments because these experiments contain different siderophores and EPS in different quantities. Semi-quantitative universal (Schwyn and Neilands, 1987) and catechol-specific (Arnou, 1937; Neilands and Nakamura, 1991; Liermann et al., 2000a) siderophore assays on this strain of *Bacillus* sp. growing in Fe-free MM9 medium for 3 weeks indicated approximately 180–200 μM catecholate siderophore in the culture solutions (B. Kalinowski, unpublished data). Our abiotic siderophore experiments contained 240 μM DFAM. The lower concentrations of EPS and siderophore in the *Bacillus* sp. experiments compared to the alginate+DFAM or xanthan+DFAM experiments are consistent with the trends observed in dissolution and roughness (Figs. 1c and 3).

The difference in size and distribution between the ‘biopits’ and the ‘DFAM-pits’ may be related to the EPS produced by the bacteria. In contrast to those produced abiotically by DFAM alone, the etch pits produced in the presence of *Bacillus* sp. are larger and more localized, often forming small groups of pits (Fig. 6). Additional components (besides polysaccharide) in the *Bacillus* sp. EPS may have adhered more strongly to the surfaces than did alginate or xanthan gum. Indeed, *Bacillus* sp. cells and cellular debris were observed by SEM and AFM, respectively, on hornblende glass plachets rinsed with distilled and deionized water in our previous work (Buss et al., 2003). Significant debris was not observed on plachets incubated with alginate or xanthan gum, suggesting that these substances either did not adhere to the surfaces or did not adhere strongly.

The initial step in the development of a biofilm is the adsorption of a conditioning film, which may contain polysaccharides, but is thought to be primarily glycoproteins (e.g., Baier, 1980; Characklis, 1989). This film

is dynamic, that is, constantly exchanging with solution molecules, and may be heterogeneously distributed over the surface (Characklis, 1989). *Bacillus* sp. likely colonized the hornblende glass surfaces by secreting a glycoprotein conditioning film for the biofilm to adhere to. This film would not have formed in the alginate and xanthan gum experiments.

Colonization-related pitting has been documented on silicate minerals before. For example, in a study of natural basaltic glass, irregular localized pitting was observed after incubation for 181 days in seawater while the majority of the surface appeared unaltered (Thorseth et al., 1995). This pitting was presumably caused by a consortia of bacteria attached to the glass surfaces that were observed using SEM and TEM. Similarly, when cyanobacteria were grown on polished glass, Staudigel et al. (1995) observed irregularly shaped, localized etch pits clustered along zones parallel to polishing scratches. And Bennett et al. (1996a) also observed significant etching in microbially colonized regions of microcline surfaces, but no etching on the uncolonized regions.

Enzymes, molecules, and ions become concentrated in EPS, which limits their diffusion into solution (Morel and Palenik, 1989; Roane and Kellogg, 1996; Madigan et al., 2000). It follows that a biofilm would contain a higher concentration of siderophores than the bulk solution. The viscosity of EPS may restrict movement of siderophores and other ligands; in a strongly adhering biofilm, this could explain the localization of the biopits. The large biopits could therefore represent assemblages of the same-sized pits as on the DFAM-exposed surfaces. Therefore, EPS may be instrumental in weathering minerals because they sorb to surfaces and create micro-environments with higher concentrations of ligands in close proximity to mineral surfaces. Our experiments with siderophores and EPS highlight additional mechanisms by which microbial communities can interact with surfaces, contributing to chemical weathering of silicate minerals.

5. Conclusions

This study is the first to document the growth of etch pits during siderophore-promoted dissolution. The widespread pitting and enhanced Fe release from siderophore-exposed surfaces is consistent with chelation of Fe by siderophores, which is likely responsible for the etch pits formed in the presence of *Bacillus* sp. at near-neutral pH. Small, approximately circular pits formed on hornblende glass dissolved with DFAM document that siderophore-promoted dissolution is localized to pits that are ubiquitous on the surface and contribute to an increase in surface roughness. EPS may

contribute to dissolution by providing a sink for released ions, enhancing apparent solubilities; by providing additional ligands that act as Fe-shuttles for siderophores; or by releasing additional ligands (such as acetate) that could alter the speciation of siderophore-surface complexes or accelerate ligand exchange on surface atoms. When sorbed strongly to surfaces via a conditioning film, microbial EPS may affect the size and distribution of ligand-produced etch pits. Widespread pitting on siderophore-exposed surfaces demonstrates the ability of siderophores to alter surface morphology. By comparison, the distribution and size of microbial etch pits suggest that the 'biopits' may be caused by siderophores concentrated in biofilms. Non-stoichiometric depletion of Fe on surfaces exposed to bacteria supports the interpretation of localized dissolution by metal-specific ligands. Mineral surface features such as etch pits provide non-exclusive evidence of microbial activity but may, when used in combination with other biomarkers, provide clues to the character of microbial communities.

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