Contrasting effects of Al substitution on microbial reduction of Fe(III) (hydr)oxides

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Abstract

Aluminum, one of the most abundant elements in soils and sediments, is commonly found co-precipitated with Fe in natural Fe(III) (hydr)oxides; yet, little is known about how Al substitution impacts bacterial Fe(III) reduction. Accordingly, we investigated the reduction of Al substituted (0–13 mol% Al) goethite, lepidocrocite, and ferrihydrite by the model dissimilatory Fe(III)-reducing bacterium (DIRB), Shewanella putrefaciens CN32. Here we reveal that the impact of Al on microbial reduction varies with Fe(III) (hydr)oxide type. No significant difference in Fe(III) reduction was observed for either goethite or lepidocrocite as a function of Al substitution. In contrast, Fe(III) reduction rates significantly decreased with increasing Al substitution of ferrihydrite, with reduction rates of 13% Al-ferrihydrite more than 50% lower than pure ferrihydrite. Although Al substitution changed the minerals’ surface area, particle size, structural disorder, and abiotic dissolution rates, we did not observe a direct correlation between any of these physiochemical properties and the trends in bacterial Fe(III) reduction. Based on projected Al-dependent Fe(III) reduction rates, reduction rates of ferrihydrite fall below those of lepidocrocite and goethite at substitution levels equal to or greater than 18 mol% Al. Given the prevalence of Al substitution in natural Fe(III) (hydr)oxides, our results bring into question the conventional assumptions about Fe (hydr)oxide bioavailability and suggest a more prominent role of natural lepidocrocite and goethite phases in impacting DIRB activity in soils and sediments.

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1. INTRODUCTION

Fe(III) oxide, hydroxide, and oxyhydroxide minerals (hence referred to as Fe (hydr)oxides) such as ferrihydrite (Fe₅(OH)₃·4H₂O), lepidocrocite (γ-FeOOH), goethite (α-Fe₂O₃), and hematite (α-Fe₂O₃) are ubiquitous in sediments and soils, contributing up to 50% of the bulk mass of soils (Schwertmann, 1991). As a result of their high surface areas and density of reactive surface sites, Fe(III) (hydr)oxides are important sorbents of nutrients (PO₄³⁻, HCO₃⁻), trace metals (Co²⁺, Cu²⁺, Mn²⁺, Ni²⁺, Zn²⁺), and pollutants (As), playing a critical role in controlling the aquatic chemistry of soils and sediments (Cornell and Schwertmann, 2003). After the discovery of bacteria that could conserve energy through the reduction of Fe(III) (hydr)oxides coupled with organic acid oxidation in the 1980s (Lovley et al., 1987; DiChristina et al., 1988), Fe(III) (hydr)oxides were recognized as an important terminal electron acceptor in anaerobic sediments. Subsequent research found dissimilatory Fe(III)-reducing bacteria (DIRB) to be important in the bioremediation of organic contaminants (Lovley et al., 1989; Lovley, 1997), the sequestration of toxic and radioactive metals (Lloyd, 2003; Wilkins et al., 2006), and the production of electricity in microbial fuel cells (Bretschger et al., 2007; Lovley, 2008).

Given the ecological and environmental importance of bacterial Fe(III) reduction, a significant amount of research has been conducted determining the rates and solid-phase products of Fe(III) reduction by DIRB (e.g., Roden and
2. EXPERIMENTAL METHODS AND PROCEDURES

2.1. Synthesis of Fe(III) (hydr)oxides

We synthesized and purified 2-line ferrihydrite (Fe₃O₄·4H₂O), lepidocrocite (γ-FeOOH), and goethite (α-FeO(OH)) containing between 0 and 13 mol% Al [mole Al/(mole Fe + mole Al)]. To minimize other co-precipitates in the Fe(III) (hydr)oxides, all mineral syntheses were conducted with acid-washed equipment, and all stocks were made in acid-washed, glass containers with double deionized water and ACS grade chemicals. Four ferrihydrite mineral phases containing 0, 3, 9, and 13 mol% Al were created by first combining 0.2 M FeCl₃ stock with a 0.2 M Al(NO₃)₃ stock in proportion to the final Al mol%, to a final volume of 500 mL. The solutions were then rapidly titrated with 1 M KOH to a pH of 7.5, followed by centrifugation and dialysis to remove any contaminants (Schwertmann and Cornell, 2000). The Fe(III) (hydr)oxides were dialyzed using Spectra/Por cellulose dialysis tubing (MWCO 12,000 to 14,000) until a steady conductivity value was obtained.

Al-substituted lepidocrocite (0, 3, 10, and 13 mol%) were produced by a controlled oxidation of ferrous chloride salt and aluminum nitrate in a NH₃/NH₄Cl buffer with CO₂-free air at pH 8 and a temperature of 13–15 °C (Schwertmann and Wolska, 1990). FeCl₂ and Al(NO₃)₃, added in proportion to the final mol% Al, were dissolved in 300 mL of 0.2 M NH₃/NH₄Cl buffer (50 mL 0.2 M NH₃OH and 950 mL 0.2 M NH₄Cl) in a 600 mL jacketed beaker placed on top of a stir plate and attached to a continuous-flow chiller. The pH, which was continuously monitored, was initially adjusted with 1 M NH₃ to a pH of 8.0, after which CO₂-free air was introduced in the solution through a fritted glass dispersion tube. During the ferrous chloride oxidation, the pH was held at 8.00 ± 0.05 with the addition of 1 M NH₃ and the temperature was held between 13 and 15 °C for approximately 1.5 h. Counter-ions were subsequently removed by dialysis.

Goethite substituted with 0, 2, 4, and 6 mol% aluminum were synthesized by incubating freshly made Al-ferrihydrite in an alkaline system at 70 °C for 4 days, followed by a 1 M KOH wash and dialysis (Schwertmann and Cornell, 2000). Residual ferrihydrite was removed from the goethite mineral slurries by 5 rinses with 0.25 M NH₂OH in 0.25 M HCl, incubated at 50 °C for 5 periods of 30 min, followed by centrifugation, and finally dialysis (Zachara et al., 2001). All mineral slurries were stored at 4 °C.

A subset of Al-doped ferrihydrite minerals were coated on quartz sand following the method of Brooks et al. (1996) and as conducted previously (Hansel et al., 2003, 2004). Pure quartz sand (Unimin Corporation) was mixed with ferrihydrite slurry (10 mg Al-ferrihydrite per g quartz sand), excess water was decanted, and the mixture was allowed to evaporate at room temperature under convection with periodic stirring. The coated sand was dried, washed with DI water to remove ferrihydrite not attached to the sand, and dried for 3 days. Ferrihydrite-coated sands were sterilized by gamma irradiation (25 kGy, by Food Technology Service Inc., Mulberry, FL).

2.2. Mineral characterization

The identity and purity of the 12 synthetic minerals were confirmed using X-ray diffraction (XRD) and extended X-ray absorption fine structure (EXAFS) spectroscopy. XRD was conducted on a Scintag XDS2000 with CuKα radiation.
radiation (Department of Chemistry and Chemical Biology at Harvard University). EXAFS spectra were acquired at the Stanford Synchrotron Radiation Laboratory (SSRL) on beamline 11–2. Spectra acquisition and analyses followed the procedures previously described in detail (Benner et al., 2002; Hansel et al., 2003, 2004). Purity was confirmed by comparing the synthesized phases to reference standards for ferrihydrite, lepidocrocite, and goethite. Linear combination k^2-weighted EXAFS (LC-EXAFS) spectral fitting was also conducted on lepidocrocite and goethite samples to define the fractional abundance of ferrihydrite within the samples, serving as a proxy for phase disorder (Hansel et al., 2004). LC-EXAFS was conducted using SiXpack (Webb, 2005).

The Fe and Al content of the minerals were determined by dissolving 1 mL of the mineral slurries in 2 mL concentrated HCl followed by dilution with double deionized water and Fe and Al analysis by ICP. Throughout the manuscript, the mol% Al of all minerals is based on their measured Al and Fe content. Mineral surface area was determined with a BET analyzer (Beckman Coulter SA 3100) after degassing for 24 h at 25 °C. Particle size and shape of the minerals were determined by transmission electron microscopy (TEM) analysis. Dried, powdered samples for TEM analysis were diluted in double deionized water, ultrasonicated for 15 min, and placed on carbon/formvar supported copper grids until the liquid evaporated. Samples were analyzed on a JEOL 2000-FX TEM (University of Oklahoma's Samuel Roberts Noble Electron Microscopy Laboratory).

Acid dissolution experiments with the mineral slurries were conducted to better understand the dissolution properties of the Al-containing minerals and to determine if Al co-precipitation occurred congruently or incongruently. For ferrihydrite minerals, 50 mg of the slurry was added to 100 mL of 0.1 M HCl. 50 mg of lepidocrocite minerals were added to 100 mL of 0.5 M HCl, and for goethite, 250 mg of the mineral slurry was added to 6 M HCl. All dissolution experiments were conducted at room temperature and shaken at 200 rpm, since previous research demonstrated that at this speed and above, dissolution is not diffusion controlled but instead depends solely on the surface reaction rate (Cornell et al., 1974). The dissolution experiments were sampled 10 times for dissolved metals (5 mL passed through a 0.2 µm filter) and total metals (2 mL digested in 6 M HCl). Diluted samples were analyzed for Fe and Al concentration by ICP.

2.3. Bacterial medium and preparation of cultures

Iron(III) reduction batch experiments were conducted in anaerobic medium with a pH of 7.0 modified from Fredrickson et al. (1998). The medium contained 18 mM sodium lactate, 4.7 mM NH₄Cl, 1.2 mM KCl, 0.61 mM CaCl₂, 1.1 mM MgSO₄, 1.5 mM NaCl, 4.5 mM PIPES, 0.4 mM NaH₂PO₄, A 1 L batch of the medium was amended with 1 mL of an acidified trace metal stock (excluding NTA) based on Widdel and Bak (1992) and 0.1 mL of a concentrated vitamin stock (Widdel and Bak, 1992). The trace metal stock, made in 0.05% HCl contained 0.5 mM H₂BO₃, 0.5 mM MnCl₂, 0.8 mM CoCl₂, 0.1 mM NiCl₂, 0.01 mM CuCl₂, 0.5 mM ZnSO₄, 0.15 mM Na₂MoO₄, and 0.02 mM Na₃SeO₃. The concentrated vitamin stock contained 100 mg 4-aminobenzoic acid, 100 mg D(+)-biotin, 100 mg nicotinic acid, 100 mg calcium D(+)-pantothenate, 100 mg pyridoxine HCl, 100 mg thiamine hydrochloride, 100 mg riboflavin, 100 mg folic acid, 100 mg niacinamide, 100 mg thiamine pyrophosphate, and 50 mg vitamin B₁₂ in 100 mL H₂O. The medium was boiled under a stream of O₂-free N₂ gas for 10 min to sparge out any oxygen and subsequently dispensed into anaerobic Balch tubes or serum bottles using the Hungate method (Widdel and Bak, 1992) and autoclaved.

Bacterial Fe(III) reduction experiments were conducted with S. putrefaciens strain CN32, a facultative, dissimilatory Fe(III)-reducing bacterium (DIRB), (Fredrickson et al., 1997, 1998) that couples the oxidation of lactate to acetate with Fe(III) reduction. Cultures were prepared for experiments according to the methods described in Hansel et al. (2004). Late log phase cells (~3 × 10⁷ cells/mL) grown in TSB were harvested by centrifugation (4500 rpm, 10 min, 10 °C), washed twice in 100 mM tris(hydroxymethyl)aminomethane buffer (130 mM NaCl, 7 mM Na₂HPO₄, 3 mM NaH₂PO₄, pH 7.2), and resuspended in 10 mL of anaerobic medium in an anaerobic chamber (Coy Laboratories Inc., Grass Lake, MI) under 90% N₂/10% H₂ gas.

2.4. Bacterial Fe reduction experimental design and analysis

Batch reduction experiments were run in duplicate in 25 mL balch tubes containing 15 mg Fe of the mineral with anaerobic medium added up to 15 mL (1 mg Fe/mL). Prior to experiments, the mineral slurries were added to sterile balch tubes, bubbled with O₂-free N₂ gas for 30 min, sealed, flushed with 0.2 µm filtered N₂ gas with sterile needles, and autoclaved for 15 min (Straub et al., 2005). Anaerobic medium was added steriley using the Hungate method. All treatments were inoculated with 0.1 mL of a concentrated cell culture (~3 × 10⁷ cells) within a time span of 15 min, resulting in a final cell concentration of ~2 × 10⁷ cells/mL within each vial. For the duration of the 20 day experiment, treatments were shaken on their side at 115 rpm at room temperature. Fe(II) and acetate production was measured over the length of the 20 day incubation. Monitoring the concentration of acetate, produced through the microbial consumption of 1 mol of lactate coupled with the reduction of 4 mol of Fe(III), helped confirm the trends observed with the Fe(III) reduction data. The experimental treatments were sampled 8 times by vigorously shaking the tube and immediately extracting the slurry using a sterile syringe in an anaerobic chamber. To decrease any potential oxidation of Fe(II), subsamples for metal analysis were immediately dispensed into glass scintillation vials containing 1 mL of concentrated HCl. The efficiency of HCl (1 N and higher) in completely dissolving secondary phases (e.g., green rust, sidereite, magnetite) following bioreduction of ferrihydrite has been previously confirmed (Fredrickson et al., 1998; Benner et al., 2002). The mineral extracts were shaken for a maximum of 24 h and subsequently analyzed for Fe(II). Samples for organic acid analysis were immediately frozen.
Bioreduction experiments with Al-ferrihydrite coated sand were designed and sampled with comparable methods as described for the mineral slurry bioreduction experiments. One gram of sterilized sand (coated with 10 mg of ferrihydrite) was added to each batch tube containing 10 mL of autoclaved anaerobic media in the anaerobic chamber using sterile techniques. To sample the bioreduction experiment for Fe reduction, duplicate tubes were sacrificed for analysis at each timepoint. In the anaerobic chamber, the liquid suspension was filtered with a 0.45 μm filter, and analyzed for dissolved Fe(II) (ferrozine assay; Stookey, 1970), organic acids (IC), and dissolved Fe and Al (ICP). The ferrihydrite coated sand was rinsed with sterile and anaerobic double deionized water and used for solid Fe(II) analysis.

Total Fe(II) in extracts was determined using the ferrozine assay (Stookey, 1970), adding 0.1 mL of the extract to 9.9 mL of ferrozine reagent in the anaerobic chamber. To determine the concentration of acetate produced by the S. putrefaciens during Fe reduction, subsamples were run on a Dionex IC (ICS-2000) with eluent generation on AG11-HC column following Dionex Application Note 123. Prior to analysis, samples were diluted 100-fold with sterile water due to high lactate levels and filtered with a 0.4 μm filter. Total Fe and Al concentrations were determined on diluted acid extracts on a Jobin Yvon ICP (D. Schrag Lab, Harvard University).

3. RESULTS AND DISCUSSION

3.1. Impact of Al substitution on mineral physiochemical properties

To determine the role of Al substitution in altering bacterial Fe(III) reduction, we synthesized ferrihydrite containing 0, 3, 9, and 13 mol% Al [mole Al/(mole Fe + mole Al)], lepidocrocite containing 0, 3, 10, and 13 mol% Al, and goethite containing 0, 2, 4, and 6 mol% Al. The identity of the synthesized phases was confirmed using X-ray diffraction (XRD) and extended X-ray absorption fine structure (EXAFS) spectroscopy. Peaks indicative of ferrihydrite, goethite, and lepidocrocite were identified in the XRD spectra (Fig. 1). Substantial differences between the XRD patterns were not evident for ferrihydride and goethite as a function of Al substitution. However, Al substitution in lepidocrocite leads to a subtle shift in diffraction peaks towards lower 2θ degrees as observed previously (Schwertmann and Wolska, 1990). Defining changes in the unit cell, however, is complicated due to the broadening of XRD lines with increasing Al content (Taylor and Schwertmann, 1980). Previously, Al substitution in lepidocrocite led to a contraction in the unit cell size, with a linear decrease in the unit cell edge lengths a, b, and c with increasing Al content (Schwertmann and Wolska, 1990). Here, we also see the (200), (511), and (521) reflections (0.627, 0.174, and 0.137 nm, respectively) disappear at the higher Al levels for lepidocrocite. For all of the Fe(III) (hydr)oxides, no other peaks indicative of separate aluminum phases were observed in the diffraction data.

Fig. 1. X-ray diffraction patterns for pure and substituted (A) goethite, (B) lepidocrocite, and (C) ferrihydrite.

Comparison of the pure and substituted (hydr)oxide k^3-weighted EXAFS spectra further confirms the identity of the substituted phases (Fig. 2). Similar to the observed increase in disorder for the lepidocrocite XRD spectra, a dampening of the lepidocrocite EXAFS spectra (Fig. 2; Supplementary information Fig. S1) is indicative of a decrease in the long-range order of the Al-substituted phases, which may be attributed to changes in the size or order of the precipitates as discussed further below. Although the pure and substituted goethites are similar, slight differences in the high k region of the EXAFS spectra are evident (Fig. 2; Supplementary information Fig. S2), suggesting that there are some structural differences between the phases warranting further investigation beyond the scope of this study. There are no observable differences in the EXAFS spectra for the pure and substituted ferrihydrites.
In acid dissolution experiments, all minerals dissolved congruently (Fig. 3), indicating that both Al and Fe dissolved at equal rates. Congruent dissolution is indicative of isomorphic substitution, in which Al is dispersed throughout the entire mineral phase instead of becoming concentrated on the surface or as discrete domains within the (hydr)oxide (Cornell and Schwertmann, 2003).

Aluminum substitution impacted the physicochemical properties of the Fe(III) (hydr)oxides, including acid dissolution, surface area, particle size, and morphology. In regards to the acid dissolution behavior of the (hydr)oxides, since dissolution curves were sigmoidal, a modified first order rate law (Kabai equation) was used to determine the rate constant of dissolution, $k$ (min$^{-1}$) (Table 1), as previously done for Al-substituted Fe(III) (hydr)oxides (Kabai, 1973; Schwertmann, 1991; Alvarez et al., 2007). The Kabai equation is expressed in its linear form as $\ln \ln \left[1/(1- c_{Fe})\right] = \ln k + a \ln t$, where $c_{Fe}$ is the fraction of Fe dissolved at time ($t$), $k$ is the dissolution rate constant, and $a$ is a fitting coefficient, which is characteristic of the structure of the solid-phase (Kabai, 1973; Schwertmann et al., 1985). Al substitution in the three Fe(III) (hydr)oxides affected acid dissolution in different ways. Al substitution in goethite resulted in minerals more resistant to acid dissolution since the dissolution rate constants sequentially decrease with increasing Al and show a 4.1 times decrease in the rate for the highest Al level (6%) compared to unsubstituted goethite. These results are consistent with previous findings for goethite containing up to 12 mol% Al (Alvarez et al., 2007; Schwertmann, 1984; Torrent et al., 1987). In contrast, increasing Al substitution in lepidocrocite resulted in faster acid dissolution, as shown by the higher dissolution rate constants at high Al concentrations, up to 5.8 times faster for 13% Al lepidocrocite compared to the pure phase. The difference in dissolution behavior between lepidocrocite and goethite has been previously attributed, in part, to the impact of Al on the strength of the hydrogen bond due to crystallinity changes. In brief, an increase in OH-stretch frequency and decrease in out-of-plane bending frequency was observed for lepidocrocite, which was opposite to those observed for Al substituted goethites (Schulze and Schwertmann, 1984, 1987; Schwertmann and Wolska, 1990). Acid dissolution rate constants varied little (maximum 1.4 times) with increasing Al in ferrihydrite, indicating that Al-substitution did not substantially change the dissolution properties of ferrihydrite.

Surface area did not change substantially for ferrihydrite and only slightly decreased for goethite with increasing Al substitution (Table 2). Previous research by Gonzalez et al. (2002) also found that the surface area of goethite did not change considerably with Al substitution. In contrast, however, Al substitution within lepidocrocite resulted in a substantial increase in surface area – increasing from...
144 to 255 m² g⁻¹ with the incorporation of 13 mol% Al (Table 2). The change in surface area is likely a consequence of a number of factors as discussed below, including particle size, morphology, crystallinity, and/or presence of ferrihydrite.

Mineral particle dimensions measured by TEM mirror the surface area trends, with increasing surface area correlating with decreasing particle size. Typical particle dimensions of 13% Al-lepidocrocite decreased over 50 times relative to 0% Al-lepidocrocite (Table 2, Fig. 4 E and F). Schwertmann and Wolska (1990) also observed a decrease in particle size with Al substitution (up to 10%) of lepidocrocite. In contrast, the typical particle dimensions measured for 6% Al-goethite was only 2 times smaller than 0% Al-goethite (Table 2). No observable changes to particle dimensions were found for Al-containing ferrihydrite (Table 2).

Aluminum substitution also modified particle morphology for lepidocrocite and goethite. Goethite particles without Al were multidomainic acicular crystals, while 6% Al-goethite had more monodomainic crystals (Fig. 4 C and D) with a decrease in the aspect ratio relative to 0% Al-goethite (Table 2). Similar trends have been previously observed for Al-substituted goethite (Gonzalez et al., 1987; Schulze and Schwertmann, 1987) and lepidocrocite (Schwertmann and Wolska, 1990). The most pronounced change due to Al substitution was observed in lepidocrocite. Without Al-doping, blocky laths volumetrically dominate sample morphology (Fig. 4 E). However, small blocky and <10 nm rounded particles are numerically abundant (Supplementary information Fig. S3). Morphology of the 13% Al lepidocrocite (Fig. 4 F) consist of extremely thin “crumpled sheets” (Kassim et al., 1982) with <10 nm rounded particles. Linear structures observed in the TEM (Supplementary information Fig. S4 also) may represent curved or folded edges of sheets or perhaps thin filaments elongated along the chains of Fe octahedra.

The results of the XRD (Fig. 1), EXAFS (Fig. 2), and acid dissolution (Fig. 3) analyses indicate that Al substitution may change the crystallinity of the Fe(III) (hydr)oxide, which is particularly evident for lepidocrocite. The impact of Al on the crystallinity of the synthetic lepidocrocite and goethite was determined by LC-EXAFS using ferrihydrite as a proxy for disorder within the phases (Hansel et al., 2004). The ferrihydrite component within lepidocrocite increased with Al substitution, with the fraction of ferrihydrite measured for 6% Al-goethite was only 2 times smaller than 0% Al-goethite (Table 2). No observable changes to particle dimensions were found for Al-containing ferrihydrite (Table 2).

### Table 1
Acid dissolution rate constants using the Kabai equation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rate constant k (min⁻¹)</th>
<th>α</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goethite (250 mg in 100 mL 6 M HCl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% Al goethite</td>
<td>0.00135</td>
<td>1.342</td>
<td>0.996</td>
</tr>
<tr>
<td>2% Al goethite</td>
<td>0.00101</td>
<td>1.264</td>
<td>0.998</td>
</tr>
<tr>
<td>4% Al goethite</td>
<td>0.00101</td>
<td>1.423</td>
<td>0.996</td>
</tr>
<tr>
<td>6% Al goethite</td>
<td>0.00033</td>
<td>1.325</td>
<td>0.999</td>
</tr>
<tr>
<td>Lepidocrocite (50 mg Fe in 100 mL 0.5 M HCl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% Al lepidocrocite</td>
<td>0.00357</td>
<td>0.680</td>
<td>0.995</td>
</tr>
<tr>
<td>3% Al lepidocrocite</td>
<td>0.00499</td>
<td>0.774</td>
<td>0.985</td>
</tr>
<tr>
<td>10% Al lepidocrocite</td>
<td>0.00567</td>
<td>0.643</td>
<td>0.999</td>
</tr>
<tr>
<td>13% Al lepidocrocite</td>
<td>0.02071</td>
<td>0.719</td>
<td>0.886</td>
</tr>
<tr>
<td>Ferrihydrite (50 mg Fe in 100 mL 0.1 M HCl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% Al ferrihydrite</td>
<td>0.10056</td>
<td>1.275</td>
<td>0.972</td>
</tr>
<tr>
<td>3% Al ferrihydrite</td>
<td>0.07296</td>
<td>1.190</td>
<td>0.982</td>
</tr>
<tr>
<td>9% Al ferrihydrite</td>
<td>0.07849</td>
<td>1.107</td>
<td>0.999</td>
</tr>
<tr>
<td>13% Al ferrihydrite</td>
<td>0.08909</td>
<td>0.773</td>
<td>0.979</td>
</tr>
</tbody>
</table>

### Table 2
Mineral surface area, particle size, and morphology.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Surface area (m² g⁻¹)</th>
<th>Particle dimensions (nm)ᵃᵇ</th>
<th>Particle morphologyᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% Al goethite</td>
<td>41</td>
<td>88 ± 29 × 551 ± 193</td>
<td>Multidomainic acicular crystals</td>
</tr>
<tr>
<td>2% Al goethite</td>
<td>45</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4% Al goethite</td>
<td>39</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6% Al goethite</td>
<td>36</td>
<td>66 ± 13 × 340 ± 68</td>
<td>Acicular crystals with a decrease in aspect ratio and multidomainic crystals</td>
</tr>
<tr>
<td>0% Al lepidocrocite</td>
<td>144</td>
<td>46 × 280</td>
<td>Blocky lath-shaped particles with trace ferrihydrite. Highly polydisperse</td>
</tr>
<tr>
<td>3% Al lepidocrocite</td>
<td>131</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10% Al lepidocrocite</td>
<td>216</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>13% Al lepidocrocite</td>
<td>255</td>
<td>3 × 10 to 5 × 50</td>
<td>Small laths with subrounded grains that mimic size &amp; morphology of ferrihydrite</td>
</tr>
<tr>
<td>0% Al ferrihydrite</td>
<td>314</td>
<td>&lt;5</td>
<td>Small aggregated spheres</td>
</tr>
<tr>
<td>3% Al ferrihydrite</td>
<td>329</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>9% Al ferrihydrite</td>
<td>318</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>13% Al ferrihydrite</td>
<td>328</td>
<td>&lt;5</td>
<td>Small aggregated spheres</td>
</tr>
</tbody>
</table>

ᵃ Particle dimensions and morphology determined by TEM on only mineral samples containing the least and most substituted Al for each mineral type.
ᵇ Number of particles used in particle dimension calculations were (top to bottom): 21, 63, and 7. Values for 13% lepidocrocite are highly approximate due to the poorly defined morphology.
required to reconstruct the EXAFS spectra approaching 65% for 13% Al-lepidocrocite (Supplementary information Table S1 and Fig. S5). Inclusion of ferrihydrite within the EXAFS fitting may be indicative of ferrihydrite impurities and/or regions of enhanced disorder within the lepidocrocite structure. In fact, trace amounts of small, sub-round spheres indicative of ferrihydrite were observed in the 0% Al-lepidocrocite sample (Supplementary information Fig. S3), while more grains indicative of a ferrihydrite-type phase were observed in 13% Al-lepidocrocite (Fig. 4, Supplementary information Fig. S4, Table 2); yet, lepidocrocite remained the dominant phase consistent with the preservation of XRD lines (Fig. 1B) and EXAFS oscillations (Fig. 2) representative of lepidocrocite. Thus, a decrease in particle size and crystallinity and increase in ferrihydrite impurities are all likely contributing to the observed differences in the XRD and EXAFS spectra and acid dissolution behavior of lepidocrocite in response to Al substitution.

Although the goethite minerals were washed with hydroxylamine prior to analysis (Zachara et al., 2001), still, a disordered fraction (measured as ferrihydrite component) was required to fit the spectra using LC-EXAFS (Supplementary information Table 1 and Fig. S6). In contrast to lepidocrocite, increasing Al-substitution in goethite resulted in a slight decrease in the degree (%) of disorder, with the percent ferrihydrite in the fit decreasing from 22% (0% Al) to 8% (6% Al). Maurice et al. (2000) also observed an increase in structural order with increasing Al substitution in goethite.

3.2. Microbial Fe(III) (hydr)oxide reduction

3.2.1. Pure Fe(III) (hydr)oxides

The synthesized Al-containing Fe(III) (hydr)oxides were used to explore the role that Al substitution has on the rates and extent of bacterial Fe(III) reduction by S. putrefaciens CN32. Iron(III) reduction rates (mM Fe(II) L\(^{-1}\) d\(^{-1}\)) were determined by calculating the rate of total Fe(II) production during the period of linear Fe(II) production coinciding with initial stages of the experiment. The period of linear Fe(II) production for ferrihydrite, lepidocrocite, and goethite was 13, 20, and 4 days, respectively. Rates were also calculated for acetate production over the same timeframe; yet, due to low respiration rates, acetate measurements were compromised by the high lactate background resulting in deviation from the expected 4:1 stoichiometry between Fe(II) and acetate generation. Furthermore, only soluble acetate was measured and therefore total acetate levels may be underestimated if acetate undergoes sorption to the Fe(III) (hydr)oxides.

As previously observed (Roden and Zachara, 1996), more crystalline Fe(III) (hydr)oxides are reduced at a slower rate (mM Fe(II) L\(^{-1}\) d\(^{-1}\)) than less crystalline phases such as ferrihydrite. Initial Fe(III) reduction rates of pure lepidocrocite and goethite were approximately half and one-third of pure ferrihydrite reduction rates, respectively (Fig. 5, Table 3). The lower rates of Fe(III) reduction for lepidocrocite and goethite resulted in a lower amount (%) of Fe(III) (hydr)oxide reduced. After 20 days,
S. putrefaciens reduced only 26.5% and 7.1% of the Fe(III) provided in lepidocrocite and goethite incubations, respectively, compared with 43.5% for ferrihydrite (Fig. 5, Table 3). We did not observe a significant difference in Fe(III) reduction (single-factor ANOVA, \( P = 0.69 \)) or acetate production rates (single-factor ANOVA, \( P = 0.77 \)) as a function of Al substitution within goethite. Although the measured Fe(III) reduction rates for 10% Al-lepidocrocite are slightly lower than other treatments, there does not appear to be a consistent trend in response to Al substitution, and there is no significant difference in acetate production rates for the Al substituted lepidocrocite incubations (single-factor ANOVA, \( P = 0.51 \)). In addition, the amount (%) of Fe(II) produced after 20 days did not vary considerably for either the lepidocrocite or goethite series as a function of Al substitution (Fig. 6). Although only a small fraction of the goethite has been reduced (Fig. 6), microbial reduction ceases for both the pure and substituted phases following ca. 5 days (Fig. 7A) as observed previously (Hansel et al., 2004). In contrast, lepidocrocite reduction is ongoing at 20 days for both the pure and substituted phases (Fig. 7A). The majority of the Fe(II) produced remains in the aqueous phase for both goethite (84%) and lepidocrocite (87%) and is not significantly different as a function of Al substitution (83% for 6% Al-goethite and 93% for 13% Al-lepidocrocite).

Kukkadapu et al. (2001) found that bacterial Fe(III) reduction of a natural Al-substituted goethite containing 13–17 mol% Al by S. putrefaciens CN32 did not occur at a significantly different rate relative to that of pure, synthetic goethite. In contrast, a 50% decrease in bacterial Fe(III) reduction was observed when an anaerobic fermenting bacterium, Clostridium butyricum, was grown with synthetic Al-goethite containing 5 mol% Al versus pure synthetic goethite (Bousserrhine et al., 1999). Furthermore, dissolution by C. butyricum was negatively correlated with the degree of Al substitution in goethite, ranging from 1.4 to 32 mol% (Dominik et al., 2002). Other substituted cations (e.g., Cr) were also found to decrease the bioreduction of goethite by C. butyricum (Bousserrhine et al., 1999). Yet, the cell population of the aerobic bacterium Pseudomonas mendocina growing on goethite increased with increasing Al substitution (Maurice et al., 2000). Although we performed our bioreduction experiments with Al-goethite minerals in the range of previous studies (0–6 mol% Al) (Bousserrhine et al., 1999; Dominik et al., 2002), we, like Kukkadapu et al. (2001) found no significant difference in Fe(III) reduction by S. putrefaciens CN32 for Al-containing goethite. Similarly, Ni and Co substitution does not significantly impact the reduction of goethite by S. putrefaciens CN32 (Zachara et al., 2001). Interestingly, the discrepancy of these findings may be due to the microorganism used in the incubations. In particular, Al and Cr impact the reduction of goethite by C. butyricum or P. mendocina, but Al, Co, and Ni do not impact reduction by S. putrefaciens. While Fe(III) reduction by S. putrefaciens is performed for cellular respiration, it is unclear whether fermenting organisms conserve energy through Fe(III) reduction (Dobbin et al., 1999) or if Fe(III) (hydr)oxides act only as a supplementary terminal electron acceptor (Lovley, 1987). Furthermore, Pseudomonas mendocina is not capable of using Fe(III) as a terminal electron acceptor and instead
the organism dissolves goethite to assimilate Fe (Maurice et al., 2000). Thus, the inhibitory impact of Al on Fe(III) reduction may be related to the mode of electron transfer or acquisition of Fe(III). To the best of our knowledge, the impact of Al substitution on lepidocrocite has not been previously investigated and thus this research is the first to illustrate that the bioreduction of lepidocrocite is not substantially impacted by Al incorporation.

3.2.2. Ferrihydrite. In contrast to Al-goethite and Al-lepidocrocite bioreduction, both initial Fe(III) reduction and acetate production rates significantly decreased with increasing Al-substitution of ferrihydrite. Fe(III) reduction and acetate production rates of 13% Al-ferrihydrite assays were approximately half of those for 0% Al-ferrihydrite (Fig. 5, Table 3). With declining Fe(III) reduction rates, the amount (%) of Fe(III) (hydr)oxide reduced by _S. putrefaciens_ grown on Al-substituted ferrihydrite also sequentially decreased (Fig. 6, Table 3). Cultures grown on 13% Al-ferrihydrite reduced only 27% of the Fe(III) (hydr)oxide present (Fig. 6, Table 3), which is comparable to the amount of lepidocrocite reduced. In contrast to lepidocrocite and goethite, however, the rate of Fe(II) production over time differs between pure and substituted ferrihydrite.

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<tr>
<th>Sample</th>
<th>Fe reduction rate&lt;sup&gt;b&lt;/sup&gt; (mmole Fe(II) L&lt;sup&gt;−1&lt;/sup&gt; d&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th>Fe reduction rate&lt;sup&gt;b&lt;/sup&gt; (mole m&lt;sup&gt;−2&lt;/sup&gt; d&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th>Acetate production rate&lt;sup&gt;b&lt;/sup&gt; (mmole acetate L&lt;sup&gt;−1&lt;/sup&gt; d&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th>Total Fe(II) (mM)</th>
<th>Fe(III) reduced (%)</th>
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<tr>
<td><strong>Bioreduction with slurry Fe oxide</strong></td>
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<tr>
<td>0% Al goethite</td>
<td>0.135 ± 0.016</td>
<td>2.41 × 10&lt;sup&gt;−6&lt;/sup&gt; ± 3.43 × 10&lt;sup&gt;−7&lt;/sup&gt;</td>
<td>0.020 ± 0.002</td>
<td>1.27 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.1 ± 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>2% Al goethite</td>
<td>0.151 ± 0.013</td>
<td>2.49 × 10&lt;sup&gt;−6&lt;/sup&gt; ± 1.95 × 10&lt;sup&gt;−7&lt;/sup&gt;</td>
<td>0.022 ± 0.000</td>
<td>1.21 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.7 ± 0.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4% Al goethite</td>
<td>0.134 ± 0.031</td>
<td>2.71 × 10&lt;sup&gt;−6&lt;/sup&gt; ± 6.06 × 10&lt;sup&gt;−7&lt;/sup&gt;</td>
<td>0.022 ± 0.002</td>
<td>1.09 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.1 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>6% Al goethite</td>
<td>0.129 ± 0.002</td>
<td>2.91 × 10&lt;sup&gt;−6&lt;/sup&gt; ± 8.57 × 10&lt;sup&gt;−8&lt;/sup&gt;</td>
<td>0.021 ± 0.004</td>
<td>0.98 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.5 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>0% Al lepidocrocite</td>
<td>0.198 ± 0.010</td>
<td>1.15 × 10&lt;sup&gt;−6&lt;/sup&gt; ± 2.43 × 10&lt;sup&gt;−8&lt;/sup&gt;</td>
<td>0.058 ± 0.014</td>
<td>4.75 ± 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.5 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>3% Al lepidocrocite</td>
<td>0.179 ± 0.001</td>
<td>1.20 × 10&lt;sup&gt;−6&lt;/sup&gt; ± 6.03 × 10&lt;sup&gt;−8&lt;/sup&gt;</td>
<td>0.063 ± 0.002</td>
<td>4.57 ± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.5 ± 1.3&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>10% Al lepidocrocite</td>
<td>0.150 ± 0.001</td>
<td>7.17 × 10&lt;sup&gt;−7&lt;/sup&gt; ± 1.63 × 10&lt;sup&gt;−8&lt;/sup&gt;</td>
<td>0.063 ± 0.008</td>
<td>4.24 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.7 ± 0.8&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>13% Al lepidocrocite</td>
<td>0.175 ± 0.006</td>
<td>5.81 × 10&lt;sup&gt;−7&lt;/sup&gt; ± 2.80 × 10&lt;sup&gt;−8&lt;/sup&gt;</td>
<td>0.071 ± 0.003</td>
<td>4.71 ± 0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.3 ± 1.2&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0% Al ferrihydrite</td>
<td>0.455 ± 0.046</td>
<td>1.71 × 10&lt;sup&gt;−6&lt;/sup&gt; ± 1.74 × 10&lt;sup&gt;−7&lt;/sup&gt;</td>
<td>0.225 ± 0.039</td>
<td>7.79 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.5 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>3% Al ferrihydrite</td>
<td>0.321 ± 0.055</td>
<td>1.14 × 10&lt;sup&gt;−6&lt;/sup&gt; ± 1.98 × 10&lt;sup&gt;−7&lt;/sup&gt;</td>
<td>0.134 ± 0.000</td>
<td>5.58 ± 0.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>32.5 ± 3.3&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>9% Al ferrihydrite</td>
<td>0.273 ± 0.004</td>
<td>1.07 × 10&lt;sup&gt;−6&lt;/sup&gt; ± 1.74 × 10&lt;sup&gt;−8&lt;/sup&gt;</td>
<td>0.133 ± 0.004</td>
<td>6.02 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33.6 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>13% Al ferrihydrite</td>
<td>0.202 ± 0.016</td>
<td>8.06 × 10&lt;sup&gt;−7&lt;/sup&gt; ± 7.87 × 10&lt;sup&gt;−8&lt;/sup&gt;</td>
<td>0.121 ± 0.009</td>
<td>4.88 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27.1 ± 1.0&lt;sup&gt;c&lt;/sup&gt;</td>
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<th>Sample</th>
<th>Fe reduction rate&lt;sup&gt;b&lt;/sup&gt; (mmole Fe(II) L&lt;sup&gt;−1&lt;/sup&gt; d&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th>Fe reduction rate&lt;sup&gt;b&lt;/sup&gt; (mole m&lt;sup&gt;−2&lt;/sup&gt; d&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th>Acetate production rate&lt;sup&gt;b&lt;/sup&gt; (mmole acetate L&lt;sup&gt;−1&lt;/sup&gt; d&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th>Total Fe(II) (mM)</th>
<th>Fe(III) reduced (%)</th>
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<tr>
<td><strong>Bioreduction with sand-coated Fe oxide</strong></td>
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<tr>
<td>0% Al ferrihydrite</td>
<td>0.136 ± 0.023</td>
<td>N.A.</td>
<td>0.070 ± 0.009</td>
<td>1.66 ± 0.24&lt;sup&gt;d&lt;/sup&gt;</td>
<td>21.6 ± 3.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>3% Al ferrihydrite</td>
<td>0.100 ± 0.012</td>
<td>N.A.</td>
<td>0.056 ± 0.001</td>
<td>1.33 ± 0.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>15.7 ± 2.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>9% Al ferrihydrite</td>
<td>0.070 ± 0.003</td>
<td>N.A.</td>
<td>0.046 ± 0.004</td>
<td>1.00 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.5 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>13% Al ferrihydrite</td>
<td>0.042 ± 0.014</td>
<td>N.A.</td>
<td>0.015 ± 0.005</td>
<td>0.74 ± 0.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.6 ± 0.8&lt;sup&gt;d&lt;/sup&gt;</td>
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N.A.: not available.

<sup>a</sup> Error bars represent the standard deviation of duplicate microbial reduction assays.

<sup>b</sup> Determined from linear production until day 4 for Gt, until day 20 for Lp, until day 13 for Fd, and until day 12 for Fd on sand.

<sup>c</sup> After 20.2 days.

<sup>d</sup> After 12.1 days.

Fig. 6. Percent Fe(III) reduced with unsubstituted and substituted ferrihydrite (Fd), lepidocrocite (Lp), and goethite (Gd) at the end of the experiment, day 20. Error bars represent the standard deviation of duplicate microbial reduction assays.
After 10 days, the rate of Fe(II) production declines for pure ferrihydrite, but does not change for 13% Al ferrihydrite. Thus, given sufficient time and assuming the rate does not change, the extent of Fe(III) reduction may become equivalent between the pure and substituted phases. The decline in ferrihydrite reduction may be in part a consequence of secondary mineralization of the ferrihydrite surface as observed previously (Hansel et al., 2004). While the partitioning of Fe(II) is equivalent between pure and substituted ferrihydrite phases, with only 11% of the total Fe(II) produced associated with the solid-phase, a greater degree of secondary mineralization is observed for pure ferrihydrite (32% ferrihydrite remains) relative to 13% Al-ferrihydrite (49% ferrihydrite remains; LC-EXAFS data not shown).

Although no previous work has determined the effect of Al substitution on bacterial ferrihydrite reduction, Frederickson et al. (2001) found that 5 mol% Ni substitution in ferrihydrite inhibited reduction by S. putrefaciens CN32. In contrast, Kukkadapu et al. (2004) found that 1 and 5 mol% Si substitution in ferrihydrite had no affect on Fe(III) reduction rates by S. putrefaciens CN32. Thus, the reduction of ferrihydrite by S. putrefaciens CN32 is inhibited by Ni and Al but not by Si. It appears, therefore, that the impact of cation substitution on Fe(III) (hydr)oxide reduction is not only a function of the species of microorganism (as illustrated above for goethite) but also the type of cation incorporated into the structure.

While dissolved Al is known to be toxic to bacteria at elevated concentrations (Illmer and Schinner, 1997; Amontette et al., 2003), only a minor fraction of the solid Al became soluble, reaching concentrations of 12–56 μM (Fig. 7B), well below what has been shown to impact bacterial growth (Amontette et al., 2003). Furthermore, while 13% Al substitution within ferrihydrite had the greatest effect on bacterial reduction, the concentration of soluble Al is lowest for this phase (Fig. 7B). In contrast to the chemical dissolution results (Fig. 3), the bacterial induced dissolution of Fe and Al is incongruent (Fig. 7B) as observed previously for microbial reduction of synthetic (Bousserrhine et al., 1999; Dominik et al., 2002) and natural goethites (Kukkadapu et al., 2001). Thus, Al released during bioreduction is likely adsorbing or precipitating on the Fe(III) (hydr)oxide surfaces. Dominik et al. (2002) determined that the majority (80–93%) of Al released during reduction of Al-substituted goethites by C. butyricum was associated with the solid-phase and suggested that this may inhibit further microbial reduction. Here, considering that only ferrihydrite shows an inhibition of microbial reduction in the presence of Al, however, suggests that other factors impacting bioreduction must also be operative.

Given the substantial effect that Al substitution plays in the rate and extent of microbial reduction of ferrihydrite, which is considered the most bioavailable Fe(III) (hydr)oxide in sediments, we wanted to further explore the impact of Al on ferrihydrite reduction rates under conditions more representative of natural environments, where Fe(III) (hydr)oxides typically exist as coatings on soil particles (Cornell and Schwertmann, 2003). Rates of reduction of ferrihydrite-coated quartz sand by S. putrefaciens strain CN32 significantly decreased with increasing Al substitution, consistent with the ferrihydrite slurry experiments (Table 3). The overall rate of Fe(III) reduction of the ferrihydrite-coated sand, however, was lower than those for the slurry incubations, with Fe(III) reduction rates on average 70% lower than those measured in slurry. Although our rates are much lower than those measured in continuous flow conditions (Hansel et al., 2004), Roden et al. (2000) measured comparable Fe(III) reduction rates in batch experiments with Fe(III) (hydr)oxide-coated sand.

3.3. Mineralogical controls on bacterial Fe reduction

Variability in the rates of Fe(III) reduction by DIRB have previously been linked to surface area, solubility, mineral structure, particle size, and crystallinity (Roden and Zachara, 1996; Neal et al., 2003; Glasauer et al., 2003;
Substitution in goethite (Fig. 4 C and D, Supplementary logical differences are observed as a function of Al solution rates (Table 3). Furthermore, structural and morphological differences are observed as a function of Al substitution (Table S1), yet this does not appear to influence the rate and extent of goethite reduction. We also do not see significant differences in the extent and rate of lepidocrocite reduction with increasing Al content, even though acid dissolution rates (Table 1) and surface area (Table 2) increase for every mol% increase in Al substitution (Fig. 9), we can extrapolate the possible effects of Al at higher concentrations. As Al substitution increases, the rates of Fe(III) reduction for ferrihydrite begin to converge with those for lepidocrocite and goethite. Based on projected rates, at molar concentrations above 18%, the rate of ferrihydrite reduction would be less than that of both lepidocrocite and goethite. Also, interestingly, the amount (%) of Fe(III) reduction consistently declines 2% for every mol% increase in Al substitution in ferrihydrite.

4. ENVIRONMENTAL IMPLICATIONS

Faster Fe(III) reduction rates of ferrihydrite compared to more crystalline Fe(III) (hydr)oxides (Roden and Zachara, 1996; Glasauer et al., 2003; Hansel et al., 2004) has implicated ferrihydrite as the most bioavailable, and hence important, Fe(III) phase for microbial respiration (Lovley and Phillips, 1986). However, Fe(III) (hydr)oxides are rarely pure in nature, and are often co-precipitated with Al (Cornell and Schwertmann, 2003). We have found that increasing concentrations of Al in ferrihydrite results in decreasing Fe(III) reduction rates. We do not observe a direct correlation between Fe(III) reduction rates and Al substitution (Table S1), yet this does not appear to influence the rate and extent of goethite reduction. We also do not see significant differences in the extent and rate of lepidocrocite reduction with increasing Al content, even though acid dissolution rates (Table 1) and surface area (Table 2) increase substantially. Also, the increased presence of ferrihydrite and likely structural disorder (Figs. 1, 2 and 4 and Supplementary information Table S1) do not substantially impact the rate and extent of reduction. In contrast to lepidocrocite, however, the dissolution behavior (Table 1), surface area and size (Table 2) of ferrihydrite are not substantially impacted by Al substitution, yet we see significant differences in Fe(III) reduction rates. It is therefore unclear at this point what mineralogical parameters control the differences in Fe(III) reduction among the Fe(III) minerals.

Recently, solubility (Bonneville et al., 2009) and crystallinity (Cutting et al., 2009) have been convincingly linked to the reduction rates of various Fe(III) (hydr)oxides and may be controlling variables here. In fact, Si incorporation in ferrihydrite reduces Fe double corner linkages, which may impact reactivity and be responsible for a lack of ferrihydrite transformation to secondary phases upon reaction with aqueous Fe(II) (Jones et al., 2009). Here, we do not observe changes in the Fe-Fe double corner contribution (~7.5 Å in Fig. 2) with increasing Al content in ferrihydrite. Yet, preliminary EXAFS analysis suggests that the Fe coordination and Fe-O distances are impacted by the incorporation of Al within the structure (data not shown); a detailed structural refinement for the Fe(III) (hydr)oxides is underway. Structural differences may influence electron transfer, for instance, by changing the solubility and (micro) crystallinity of the phases. The impact of Al on the electrical properties (e.g., reduction potential) of the phases also needs to be fully explored.
We have successfully synthesized ferrihydrite containing 24 mol% Al without formation of separate Al phases, which would be far less reactive than both lepidocrocite and goethite (Fig. 9). Given the predominance of Al substitution in natural Fe (hydr)oxides, the bioavailability of ferrihydrite in soils and sediments to DIRB needs to be revisited. Alternatively, since lepidocrocite and goethite reduction is not impacted by Al substitution, these more crystalline Fe (hydr)oxides could play a more important role in sustaining the metabolism of Fe(III)-reducing bacteria in nature than currently thought (van der Zee et al., 2003). Within mature soils and sediments, the preservation of ferrihydrite will be a function of Al (or other cation) substitution, which, as we demonstrate here, decreases its bioavailability and makes more crystalline phases the preferential electron acceptor. In fact, a number of current studies have identified Al-substituted goethite and phyllosilicates as the primary terminal electron acceptors used by Fe(III)-reducing microorganisms in contaminated subsurface sediments (Kukkadapu et al., 2006; Stucki et al., 2007; Komlos et al., 2008). Thus, in situ bioremediation of metals (e.g., Cr, U, Tc) via generation of the reductant Fe(II) (Senko et al., 2002; North et al., 2004; Stucki et al., 2007) may be controlled by microbial reduction of crystalline Fe(III) (hydr)oxides and clays rather than phases routinely considered more bioavailable (e.g., ferrihydrite). Thus, further exploration of the role of Al substitution on microbial Fe(III) reduction will greatly enhance our ability to predict the reducing capacity of sediments and design Fe(II)-based remediation approaches.

ACKNOWLEDGMENTS

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gca.2010.09.008.

REFERENCES


*Associate editor:* Kevin M. Rosso