Role of microbial iron reduction in the dissolution of iron hydroxysulfate minerals

Elizabeth J. P. Jones,[1] Tracie-Lynn Nadeau,[2] Mary A. Voytek,[1] and Edward R. Landa[1]

Received 12 August 2005; revised 30 November 2005; accepted 23 December 2005; published 28 March 2006.

[1] Iron-hydroxysulfate minerals can be important hosts for metals such as lead, mercury, copper, zinc, silver, chromium, arsenic, and selenium and for radionuclides such as ^{226}Ra. These mineral-bound contaminants are considered immobilized under oxic conditions. However, when anoxic conditions develop, the activities of sulfate- or iron-reducing bacteria could result in mineral dissolution, releasing these bound contaminants. Reduction of structural sulfate in the iron-hydroxysulfate mineral jarosite by sulfate-reducing bacteria has previously been demonstrated. The primary objective of this work was to evaluate the potential for anaerobic dissolution of the iron-hydroxysulfate minerals jarosite and schwertmannite at neutral pH by iron-reducing bacteria. Mineral dissolution was tested using a long-term cultivar, *Geobacter metallireducens* strain GS-15, and a fresh isolate *Geobacter* sp. strain ENN1, previously undescribed. ENN1 was isolated from the discharge site of Shadle Mine, in the southern anthracite coalfield of Pennsylvania, where schwertmannite was the predominant iron-hydroxysulfate mineral. When jarosite from Elizabeth Mine (Vermont) was provided as the sole terminal electron acceptor, resting cells of both *G. metallireducens* and ENN1 were able to reduce structural Fe(III), releasing Fe^{+2}, SO_4^{-2}, and K^+ ions. A lithified jarosite sample from Utah was more resistant to microbial attack, but slow release of Fe^{+2} was observed. Neither bacterium released Fe^{+2} from poorly crystalline synthetic schwertmannite. Our results indicate that exposure of jarosite to iron-reducing conditions at neutral pH is likely to promote the mobility of hazardous constituents and should therefore be considered in evaluating waste disposal and/or reclamation options involving jarosite-bearing materials.

Citation: Jones, E. J. P., T.-L. Nadeau, M. A. Voytek, and E. R. Landa (2006), Role of microbial iron reduction in the dissolution of iron hydroxysulfate minerals, *J. Geophys. Res.*, *111*, G01012, doi:10.1029/2005JG000089.

1. Introduction

[2] The formation and dissolution of iron-hydroxysulfate minerals such as jarosite and schwertmannite can influence the mobility of metals and radionuclides in the environment. Jarosite can incorporate lead, mercury, copper, zinc, silver, and radium by substitution for structural K^{+1} or Fe^{+3}, and anions such as chromate, arsenate, and selenate by substitution for SO_4^{-2} [*Dutrizac and Jambor*, 1987]. Schwertmannite may accumulate metals such as copper, zinc, nickel, selenium and arsenic by substitution into the crystalline structure or adsorption [*Sidenko and Sherriff*, 2004; *Waychunas et al.*, 1995]. Although metals and radionuclides may be immobilized by coprecipitation and/or adsorption with iron-hydroxysulfate minerals under acidic and oxidizing conditions, transport or burial of the materials or changes in the local redox environment could lead to conditions favoring remobilization by dissolution or desorption.

[3] Jarosite [$KFe_3(SO_4)_2(OH)_6$] is a ferric sulfate mineral that forms under acidic conditions; it occurs naturally, such as in acid sulfate soils [*Fanning et al.*, 1993] and outcrops and spoil banks of sulphidic rocks [*Parnell*, 1983], and is a common feature of streams impacted by acid mine drainage [*Nordstrom and Alpers*, 1999]. Jarosite was recently identified on the surface of Mars by means of Mössbauer spectra obtained by the Mars Exploration Rover *Opportunity*, providing evidence for aqueous, acid sulfate conditions [*Klinghöfer et al.*, 2004]. Jarosite precipitates requiring environmental disposal are also produced in connection with energy and mineral production. For example, precipitation of jarosite can be used to remove iron and sulfate from acid waste streams generated in coal-cleaning operations aimed at producing low-sulfur coals [*Norton et al.*, 1991]. Jarosite precipitation is used to remove iron and sulfate from extraction solutions during the recovery of zinc, copper and cobalt from ores [*Dutrizac and Jambor*, 1984]. Jarosite is also formed from sulfuric acid–leached uranium mill tailings (UMT), the ground rock residues of uranium ore extraction, to which iron is often added during processing. During the acid leaching of uranium ore,

[1]U.S. Geological Survey, Reston, Virginia, USA.
[2]Office of Wetlands, Oceans, and Watersheds, U.S. Environmental Protection Agency, Washington, DC, USA.

jarosite tends to precipitate, and the incorporation of ^{226}Ra can result in significant enrichment relative to the bulk UMT. A jarosite fraction separated from UMT at the Elliot Lake region of Ontario, Canada, demonstrated a concentration of ^{226}Ra that was 275 times greater than the bulk UMT [*Kaiman*, 1977].

[4] Schwertmannite [$Fe_8O_8(OH)_6(SO_4)$], informally known as "yellowboy", is a poorly crystalline mineral with a high specific surface area, structurally similar to either akaganeite [*Bigham et al.*, 1994] or ferrihydrite [*Loan et al.*, 2004]. It occurs as an ochreous precipitate from acid, sulfate-rich waters [*Bigham et al.*, 1994; *Hammarstrom et al.*, 2000], such as acidic mine-drainage environments, often coprecipitating with jarosite or goethite. Few studies of metal accumulation by schwertmannite have been done. However, in a study of acid mine- drainage waters with mixtures of goethite, schwertmannite, and jarosite [*Sidenko and Sherriff*, 2004], it was demonstrated that schwertmannite was an efficient scavenger of Ni, Zn and Cu.

[5] Few studies have addressed the long-term stability of iron-hydroxysulfate minerals. Both Fe(III) and SO_4^{-2} can serve as terminal electron acceptors for anaerobic bacteria, and either of these processes could promote mineral dissolution. The sulfate-reducing bacterium *Desulfovibrio desulfuricans* was shown to reduce jarosite structural SO_4^{-2} to S^{-2} [*Ivarson et al.*, 1976], however Fe was not released, but rather bound by S^{-2}, forming mackinawite (FeS). Many of the metals associated with jarosite, such as Pb, Hg and Cd, are also likely to form insoluble sulfides and remain immobilized under sulfate-reducing conditions. Iron-reducing bacteria not only have the potential to release metals (such as Pb, Hg, Cu, Zn, Ag, Cr, As, Se) bound in the jarosite structure, but can also prevent the formation of S^{-2} by sulfate-reducing bacteria as a result of microbial competitive inhibition [*Lovley and Phillips*, 1987a.]. Iron-reducing bacteria therefore potentially have a greater impact on the release of metals than sulfate-reducing bacteria.

[6] Dissolution of jarosite [*Bridge and Johnson*, 2000] and schwertmannite [*Küsel et al.*, 2002] by iron-reducing bacteria under acidic (pH <2.5) conditions has been demonstrated previously. Experimental evidence indicates that at low pH, *Acidiphilium* spp. can accelerate the dissolution of ferric iron minerals by way of an indirect mechanism, in which bacterial reduction of dissolved Fe^{+3} results in a shift in equilibrium between solid phase Fe(III) and dissolved Fe^{+3}, thereby driving dissolution of the mineral phase. At neutral pH, the concentration of dissolved Fe^{+3} in equilibrium with solid Fe(III) phases is too low to support dissimilatory iron-reducing bacteria. Studies of ferric oxide phases have demonstrated that anaerobic iron-reducing bacteria can reduce structural Fe(III) by direct contact with the mineral surface under neutral pH conditions [*Nevin and Lovley*, 2002a]. The susceptibility of ferric oxide-bound Fe(III) to microbial reduction varies among mineral phases, both at acidic [*Bridge and Johnson*, 2000] and neutral [*Lovley and Phillips*, 1988] pH. Although reduction of iron hydroxysulfate by bacteria at acidic pH has also been demonstrated [*Bridge and Johnson*, 2000; *Küsel et al.*, 2002], susceptibility of structural Fe(III) in iron hydroxysulfate phases to reductive dissolution by bacteria at neutral pH has not been previously investigated.

[7] The primary objective of this work was to evaluate the potential for anaerobic dissolution of iron-hydroxysulfate minerals by microbial reduction. Experimental conditions were selected to specifically assess the susceptibility of iron hydroxysulfate to bacterial reduction of structural Fe(III) rather than Fe^{+3} in solution. Experiments were conducted using resting cells of *Geobacter,* which have been shown to require direct contact, in a nonchelating buffer, at pH 7. We examined the capabilities of two *Geobacter* species to release structural ions from iron-hydroxysulfate minerals, including an unconsolidated mine drainage jarosite, a lithified jarosite, and a laboratory-synthesized schwertmannite. The bacteria were able to reduce the structural Fe(III) in jarosite, releasing Fe^{+2}, SO_4^{-2} and K^{+1} to solution, but did not reduce Fe(III) bound in schwertmannite.

2. Materials and Methods

2.1. Mineral Sources

[8] Jarosite material from two sources was used for this study. One sample came from a mine drainage site of Elizabeth Copper Mine, Orange County, Vermont [sample LIZM6, *Hammarstrom et al.*, 2000]. A second sample of jarosite, collected in Utah from a lithified geologic unit, was obtained from Ward's Natural Science (Rochester, New York). Prior to chemical analysis and use as a substrate, the Utah jarosite was pulverized using a ceramic ball mill. Schwertmannite in the environment commonly co-occurs with other Fe(III) phases, such as the microbially reducible ferrihydrite. In order to ensure that the schwertmannite in our study was not mixed with other Fe(III)-bearing phases, schwertmannite was synthesized using the abiotic protocol of *Cornell and Schwertmann* [1996], slightly modified by replacing $FeCl_3 \cdot 6H_2O$ with $Fe(NO_3)_3 \cdot 9H_2O$ to avoid possible akaganeite contamination (D. Williams, personal communication, 1999). Briefly, 6 g Na_2SO_4 was dissolved in 2 L deionized water (60°C), and 16.2 g $Fe(NO_3)_3 \cdot 9H_2O$ was added. The solution was maintained at 60°C for 12 min with gentle agitation. The resulting suspension was dialyzed for several days against deionized water, collected via centrifugation, and washed twice with deionized water. The material was air dried at 25°C.

[9] Mineralogy was determined by X-ray diffraction (XRD) on an automated diffractometer using CuKα radiation, as described by *Hammarstrom et al.* [2000]. Both the Utah and Elizabeth Mine jarosite spectra were dominated by the following diagnostic peaks (reported as d spacing (and Miller index of plane *hkl*)) 2.29 Å (*107*), 3.08 Å (*021*), 3.11 Å (*113*) [*Brown*, 1961]. The XRD pattern for synthetic schwertmannite was similar to schwertmannite patterns [*Robbins et al.*, 2000] of material from Shadle Pond in the Southern Anthracite Coalfield of Pennsylvania and a reference pattern of schwertmannite. Characteristically, the XRD pattern of the synthetic schwertmannite contained broad diffraction maxima, an indication of very small crystal size [*Li and Kutal*, 2003].

2.2. Source of Bacteria and Culture Maintenance

[10] The bacteria used in this study were maintained on liquid medium to facilitate the harvesting and washing of cells for use in experiments. *G. metallireducens* strain GS-

15 was isolated from the iron oxide-rich sediment of the Potomac River [*Lovley and Phillips*, 1988] and has been maintained primarily in liquid culture for over ten years. The temperature optimum of *G. metallireducens* is 30 to 35°C, with no detectable Fe(III) reduction at temperatures of 10°C or lower [*Lovley and Phillips*, 1988]. A more recent *Geobacter* isolate was obtained for comparative study from ferric hydroxysulfate-rich sediment collected from a ditch that receives significant mine drainage water from the Shadle Mine, an underground mine in the Southern Anthracite Coalfield of Pennsylvania. The Shadle Mine acid coal mine drainage site has been described by *Robbins et al.* [2000]. At the time of sediment collection, the overlying water had a pH ~3, and the ditch bottom was covered with the ochreous precipitate characteristic of acid mine discharge. Iron-reducing bacteria were enriched by inoculating Shadle Mine sediment into anaerobic bicarbonate-buffered (pH 6.8) freshwater medium [*Lovley and Phillips*, 1988] at 30°C with amorphous FeOOH as the sole terminal electron acceptor and acetate (5 mM) as the electron donor. Iron-reducing bacteria were isolated following established protocols [*Coates et al.*, 1996]. Briefly, successful enrichments in which the rust-colored Fe(III) became black were transferred (10%) to liquid medium with Fe^{+3}-nitrilotriacetic acid (Fe-NTA; 10 mM) as the electron acceptor. Following growth, liquid cultures were serially diluted and added to anaerobic agar shake tubes as described by *Coates et al.* [1996]. Isolated colonies (pink and white) formed in the agar matrix, and pink colonies (characteristic of iron-reducing bacteria) were transferred to liquid medium with Fe-NTA to test for iron-reducing ability. One of these isolates, designated ENN1, was selected for further investigation, and was cultivated for three months in liquid medium prior to use in this study.

[11] Both strains of iron-reducing bacteria used in this study were maintained at 30°C in anaerobic bicarbonate-buffered (pH 7) freshwater medium [*Lovley and Phillips*, 1988] in tubes or bottles sealed with gas tight butyl rubber stoppers. *G. metallireducens* was maintained in culturing medium with acetate (20 mM) and ferric citrate (50 mM); ENN1 was maintained in medium with acetate (10 mM) and Fe-NTA (10 mM).

2.3. Characterization of ENN1

[12] The cell density of ENN1 in culture was limited by the low concentration of Fe^{+3} in Fe-NTA medium. Therefore, in order to facilitate the growth and harvesting of cells for mineral dissolution tests, the ability of ENN1 to grow at higher concentration of Fe-NTA or with an alternative soluble electron acceptor was tested. All electron acceptor tests were performed by adding a 10% inoculum from Fe-NTA grown culture to sterile, anaerobic, bicarbonate-buffered medium [*Lovley and Phillips*, 1988], with acetate (5 mM) and a soluble electron acceptor. Electron acceptors tested included Fe-NTA (20 mM), ferric citrate (50 mM), ferric pyrophosphate (10 mM), fumarate (15 mM) or nitrate (10 mM). A visible increase in cell density and the production of Fe^{+2} in treatments where Fe^{+3} was added were considered to be positive indicators of cell growth.

[13] ENN1 was identified as a *Geobacter* sp. by sequencing the 16S rDNA. ENN1 culture was pelleted, then extracted using the Bio101 Fast DNA Spin Kit for Soil (MP Biomedicals, Irvine, California). 16S rDNA was amplified using the polymerase chain reaction with primers (46f and 519r), and cycle sequencing was performed on both DNA strands using big dye v3.1 (Applied Biosystems). DNA sequences of the two strands were determined using an ABI-310 genetic analyzer, and the two strands were assembled using Autoassembler (Applied Biosystems). The closest phylogenetic relatives were determined by BLASTn search of the NCBI website (NCBI, Rockville, Maryland). The sequence was then aligned with two closely related *Geobacter* 16S rDNA sequences using Clustal W (MacVector) to determine the number of shared bases in a 450 bp region. ENN1 16S rRNA gene sequences were deposited in GenBank (accession numbers AY034484 – 5). The isolate, ENN1 is 95% identical to *G. metallireducens* and 94% identical to *G. akaganeitreducens*, placing it in the *Geobacter* subgroup of the *Geobacteraceae* family of iron- and sulfur-reducing bacteria.

2.4. Mineral Dissolution Measurement

[14] The mineral dissolution studies were carried out using resting (stationary phase) cells of *G. metallireducens* or *Geobacter* sp. strain ENN1 that had been grown in culturing medium, harvested, and resuspended in pH 7 buffer with particles of the test mineral. Early stationary phase cells were harvested by low-speed centrifugation and washed twice with bicarbonate buffer (2.5 g/L $NaHCO_3$ sparged with N_2/CO_2 [80:20]). The cell pellet was checked for any carryover of Fe(III) from the growth medium using the rapid assay for microbially reducible Fe(III) [*Lovley and Phillips*, 1987b]. The cell pellet was resuspended in serum bottles containing bicarbonate buffer, with acetate (10 mM) as electron donor and jarosite or schwertmannite (40 mg/mL) as sole terminal electron acceptor. Prior to addition, mineral materials were sieved through a #200 (74 μm) mesh. Cell suspension experiments were carried out at 30°C in the dark. Two controls, one with no bacteria and one with bacteria incubated at 4°C, were included. Filtered samples (Acrodisc, 0.2 μm pore size), collected anaerobically over time, were analyzed colorimetrically using ferrozine (1 g/L in 50 mM HEPES) to assay dissolved Fe^{+2} [*Stookey*, 1970]. Dissolved SO_4^{-2} and K^{+1} were determined using anion chromatography with conductivity detection (Dionex model DX-100) and direct current plasma atomic emission spectroscopy (ARL Spectraspan 5), respectively. The concentration of cells (as cell protein) was determined before addition to experimental treatments, using the colorimetric Folin reaction developed by Lowry [*Hanson and Phillips*, 1981]. Cell suspension experiments with Utah jarosite and schwertmannite were monitored for unusually long periods (up to 96 hours). Cell viability and physiological health were verified at the termination of these extended experiments both by microscopic examination and by growth upon transfer to fresh, replete medium.

3. Results

[15] *G. metallireducens* incubated at 30°C reduced the Fe(III) of Elizabeth Mine jarosite material, as indicated by the production of Fe^{+2} (Figure 1a). An increase in Fe^{+2} was measured after 2 h of incubation, and the rate of release was constant over a 24 h period, as expected for resting cells.

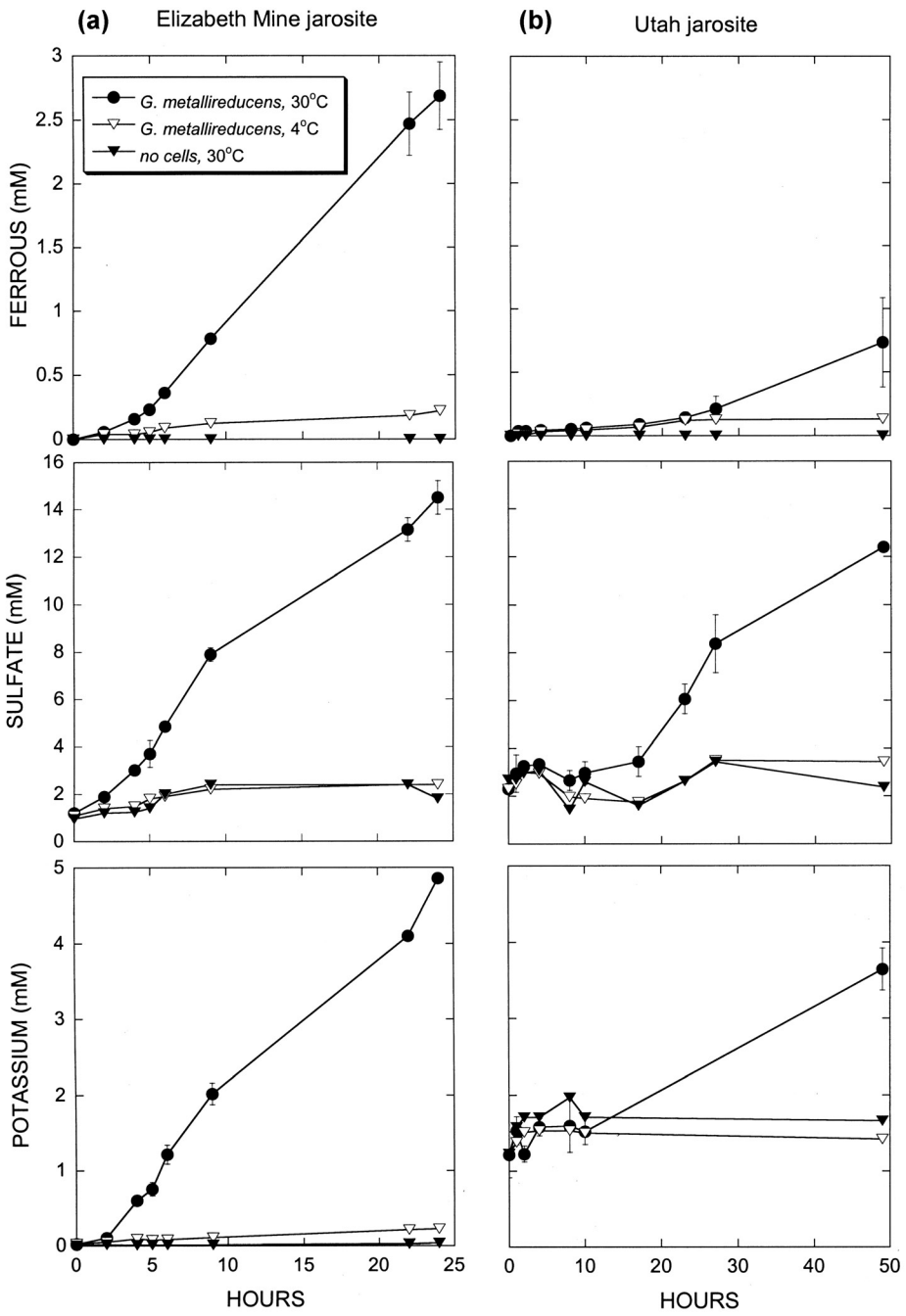

Figure 1. Fe^{+2}, SO_4^{-2}, and K^{+1} produced from (a) Elizabeth Mine and (b) Utah jarosites by resting cells of *G. metallireducens* strain GS-15, 0.15 mg (Figure 1a) and 0.16 mg (Figure 1b) of protein/mL at two temperatures and in controls without cells.

Sulfate and K^{+1} concentrations also increased over time in solution. A distinct color change occurred in treatments with cells at 30°C after 24 h; the solid material, originally golden yellow, became a deep rust-colored orange. The stoichiometry of Fe^{+3}:SO_4^{+2}:K^{+1} in the starting material was 3:2:0.6 on the basis of chemical analyses by *Hammarstrom et al.* [2000], while the release to solution calculated after incubation with *G. metallireducens* was 0.37:2:0.59. The control treatment with cells incubated at 4°C produced comparatively little Fe^{+2}. No Fe^{+2} was produced in the absence of cells; dissolved Fe^{+3} was not measured. The release of SO_4^{-2} and K^{+1} from Elizabeth Mine jarosite in the absence of metabolically active cells was relatively low (Figure 1a). No color change occurred in treatments without cells or with cells at 4°C.

[16] Reduction of Fe(III) in milled Utah jarosite by *G. metallireducens* at 30°C was evident after 24 h (Figure 1b), but the rate of Fe^{+2} release was more than six times slower than from Elizabeth Mine jarosite, with releases of 0.126 ± 0.068 and 0.843 ± 0.081 mM Fe h^{-1}

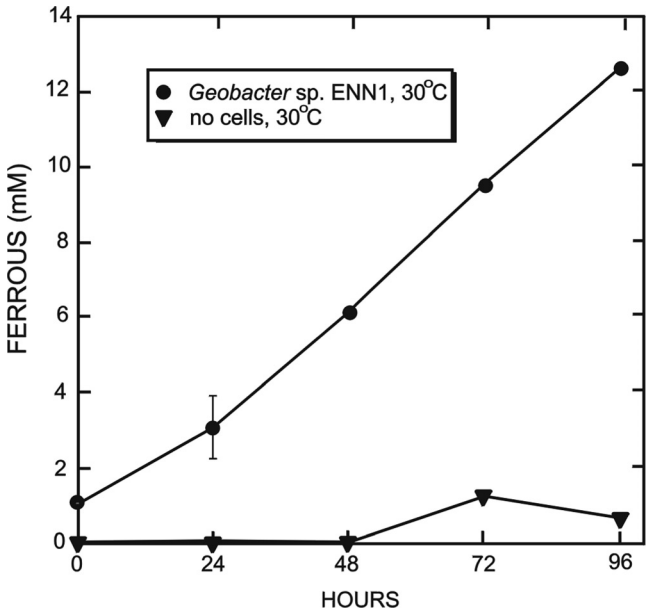

Figure 2. Production of Fe^{+2} from Fe-NTA by resting cells of ENN1 (0.1 mg of protein/mL) with acetate as the electron donor.

(mg cell protein) $^{-1}$ for Utah and Elizabeth Mine jarosites, respectively. There was greater variability between duplicate treatments of Utah jarosite than between Elizabeth Mine duplicates (Figure 1), perhaps reflecting heterogeneity of particle size in the milled material. The cells appeared healthy upon microscopic examination at the end of the experiment, with no indication of lysis, and grew readily when transferred to fresh medium. There was a slight increase in SO_4^{-2} and K^{+1} in all treatments during the first two hours. However, only the treatment with *G. metallireducens* at 30°C had significant release of ions to solution after the first two hours. No Fe^{+2} was produced from unmilled (hand-crushed) Utah jarosite during three days of incubation with *G. metallireducens* (data not shown).

[17] Unlike *G. metallireducens, Geobacter* sp. strain ENN1 displayed a narrow range of electron acceptor utilization. *Geobacter* sp. strain ENN1 reduced Fe^{+3} supplied as Fe-NTA (10 mM) with acetate (5mM) as electron donor (Figure 2), but did not grow with alternative soluble electron acceptors fumarate, nitrate, ferric pyrophosphate, ferric citrate or when a higher concentration of Fe-NTA (20 mM) was provided as the terminal electron acceptor. As a result of this limitation, *Geobacter* sp. strain ENN1 cells were grown with a low concentration of electron acceptor (10 mM Fe^{+3}), and fewer cells were harvested for use in cell suspensions. *Geobacter* sp. strain ENN1 (0.1 mg protein per mL) was able to reduce the Fe(III) in Elizabeth Mine jarosite (Figure 3). Reduction of Fe(III) by *G. metallireducens* at two cell concentrations (0.075 and 0.15 mg protein per mL) are shown for comparison. *Geobacter* sp. strain ENN1 produced almost twice as much Fe^{+2} as *G. metallireducens* when normalized to cell protein [35 and 18 mM Fe^{+2} (mg cell protein)$^{-1}$, respectively, over a 24 h period]. The average concentration of dissolved Fe^{+2} in samples from the 4°C treatment with *Geobacter* sp. strain ENN1 was 0.4 mM Fe^{+2} (mg cell protein)$^{-1}$ and did not increase over time.

[18] Treatments with either type of *Geobacter* cells at 30°C in which schwertmannite was provided as the source of Fe(III) did not release significantly more Fe^{+2} than the control treatments. When *G. metallireducens* was incubated at 30°C with synthetic schwertmannite, there was a slight initial increase in soluble Fe^{+2}, but no increase in Fe^{+2} over time (Figure 4a). The slight initial increase in Fe^{+2} observed with *G. metallireducens* was attributable to a slight carry-over of soluble Fe^{+3} from the growth medium observed in this instance. Treatments were observed for 96 hours without further increase. The release of Fe^{+2} from schwertmannite by active *Geobacter* sp. strain ENN1 cells was less than in the control treatments with cells at 4°C or with no cells (Figure 4b). The *Geobacter* spp. cells appeared healthy upon microscopic examination at the end of the experiments, with no indication of lysis, and grew readily when transferred to fresh medium.

4. Discussion

[19] The release of the jarosite structural ions (iron, sulfate, and potassium) observed in this study (Figure 1) indicates that metals or radionuclides substituted at these structural positions might also be released by microbial Fe(III) reduction under anoxic conditions at neutral pH. By their physical nature, jarosite and related iron minerals precipitating in tailings ponds [*Schuiling and van Gaans*, 1997] and streambeds [*Hammarstrom et al.*, 2000] can form crusts or hardpans that seal the sediment-water interface and limit oxygen penetration to underlying materials, thus promoting the development of anoxic conditions. Although jarosite may be formed and perhaps initially disposed of in oxic environments, it may be subsequently transported to anoxic environments by erosion and burial; for example, dewatered UMT containing jarosite-bound ^{226}Ra can be

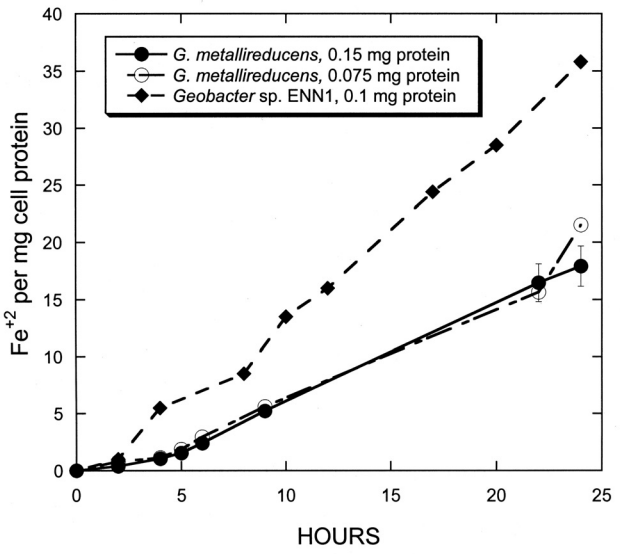

Figure 3. Production of Fe^{+2} from Elizabeth Mine jarosite by resting cells of *Geobacter* sp. ENN1 and *G. metallirducens* at two cell concentrations, normalized to cell protein.

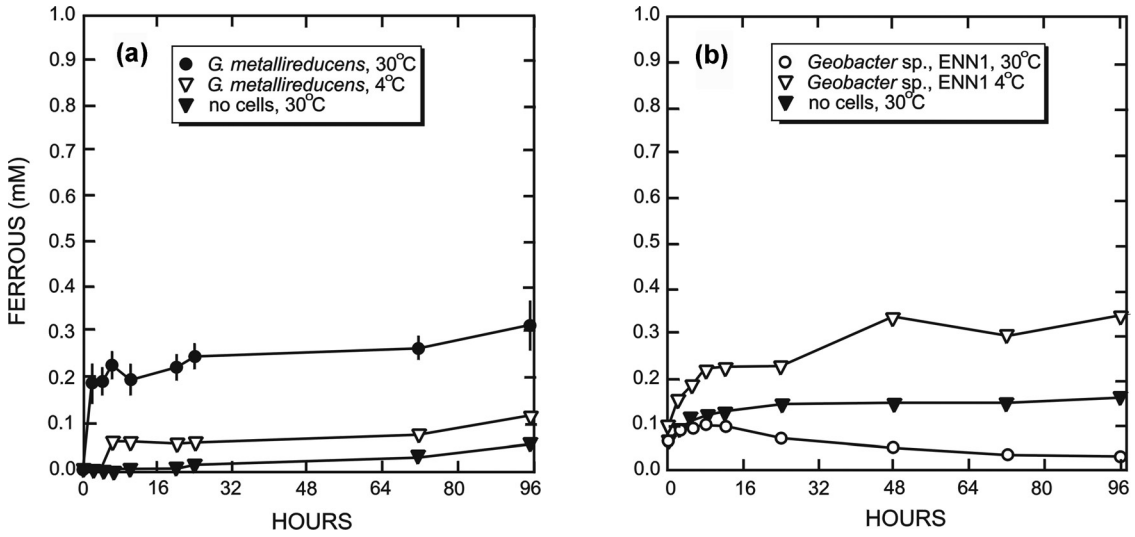

Figure 4. Fe^{+2} produced during incubation of synthetic schwertmannite with resting cells of (a) *G. metallireducens* (0.18 mg of protein/mL) and (b) ENN1 (0.1 mg of protein/mL) at 30°C and 4°C and in no-cell controls.

eroded from surface impoundments and redeposited in anoxic, downstream wetlands. Additionally, waste management practices can enhance the development of anoxic conditions in disposal environments. For example, pyritic UMT may be disposed in deep lakes or covered with organic matter to inhibit oxygen infiltration and pyrite oxidation. Also, the growth of massive crops of submerged aquatic vegetation in UMT disposal ponds can create reducing conditions in underlying tailings [*Landa*, 2005]. Thus the fate of jarosite and its associated contaminants in anoxic environments should be considered in hazard assessments dealing with jarosite-bearing waste materials.

[20] The nonstoichiometric release of Fe^{+2} compared to the other structural ions, SO_4^{-2} and K^{+1}, indicates that measurement of Fe^{+2} alone is not adequate to quantify the extent of the reduction of structural Fe(III). The tendency for Fe^{+2} to associate with oxide surfaces during enzymatic reduction at neutral pH [*Roden*, 2003], and to form secondary minerals such as siderite, magnetite, and vivianite [*Lovley*, 1987], is well known from previous studies of Fe(III) oxide reduction. For example, dissolved Fe^{+2} accounted for only 2% of the ferrous iron in samples of Potomac River sediment [*Lovley*, 1987]. Color changes, similar to those observed in this study, have also been noted in relation to the transformation of ferric oxyhydroxides to more reduced phases [for example, *Lovley et al.*, 1990].

[21] The susceptibility of crystalline Fe(III) to microbial iron reduction varies with the mineral properties. For example, the microbial reducibility of Fe(III) oxides vary, in the order Fe(III)-coated clay > amorphous ferric oxyhydroxide (ferrihydrite) > akaganeite > goethite > hematite, with goethite and hematite being relatively unavailable for microbial reduction [*Lovley and Phillips*, 1988]. In addition, our results indicate that there is a potential for dissolution of jarosite by iron-reducing bacteria, although the lithified jarosite sample was reduced more slowly than the unconsolidated jarosite, suggesting that mineral identification alone is not sufficient to predict microbial dissolution. Crystal size may have been a factor influencing the difference in rates of reduction between the Utah and Elizabeth Mine jarosites. Studies of Fe(III) oxides have demonstrated that surface area, a function of crystal size, exerts a fundamental influence on the rate and extent of bacterial Fe(III) reduction [*Roden*, 2003]. In the current study, the Utah jarosite was not microbially reduced prior to processing in a ceramic ball mill. Even after milling, the Utah jarosite, which contained larger crystals, observed microscopically, than the Elizabeth Mine jarosite, was reduced relatively slowly.

[22] Although Fe(III) in the iron hydroxysulfate mineral jarosite was reduced, schwertmannite Fe(III) was not. It has previously been suggested by other investigators that schwertmannite might be easily degraded by microbial activity due to its poorly crystalline structure [*Küsel et al.*, 1999]. As noted earlier, the structure of schwertmannite has been characterized as being similar to either akaganeite [*Bigham et al.*, 1994] or ferrihydrite [*Loan et al.*, 2004]. Both akaganeite and ferrihydrite have been shown to be reducible by *G. metallireducens* [*Lovley and Phillips*, 1988]. However, in this study schwertmannite was not reduced under the experimental conditions. Thus physiologically discernible, presumably structural, differences between schwertmannite and these other poorly crystalline materials must exist. The nature of these undefined properties appears to be a subject worthy of further study. Mineralogic properties need to be considered when assessing the potential for microbial release of hazardous substituents from these various substrates.

[23] Although well characterized and used in many mineral-microbe studies, *G. metallireducens* may not represent the ideal candidate for measuring the potential for iron-hydroxysulfate mineral dissolution, because it was isolated from the Potomac River, an environment in which iron oxides are the predominant form of Fe(III). Furthermore, long-term cultivation on soluble Fe^{+3} can affect the ability of *Geobacter* spp. to utilize solid Fe(III) [*Childers et al.*, 2002]. Therefore a new iron-reducing bacterium, *Geobacter* sp. strain ENN1, was isolated from an iron-hydroxysulfate

environment for use in this study. *Geobacter* spp. have been reported to be the most readily isolated acetate-oxidizing iron reducers from a variety of iron-reducing sediments [*Coates et al.*, 1996], but to our knowledge, this is the first *Geobacter* isolated from acid mine drainage sediments. *Geobacter* sp. strain ENN1 displayed a narrow range of electron acceptor utilization similar to the aquifer-derived *G. chapellei* [*Lovley et al.*, 1990]; Fe-NTA was the only dissolved electron acceptor tested that supported the growth of ENN1. The failure of ENN1 and some other bacteria to grow at a higher concentration of Fe-NTA is not well understood, but may result from interaction between the chelator and the cell membrane.

[24] ENN1 was able to reduce some iron-hydroxysulfate structural Fe(III), and produced twice as much Fe^{+2} (per mg cell protein) when incubated with Elizabeth mine jarosite than did *G. metallireducens.* This difference was not an artifact of the low cell concentration (reported as cell protein) in the ENN1 experiment, as a lower concentration of *G. metallireducens* did not result in a higher calculated rate. The results obtained using the recent *Geobacter* isolate, ENN1, indicate that the potential for jarosite dissolution determined using *G. metallireducens* may underestimate the potential for jarosite dissolution by environmental bacteria.

[25] The experiments presented here address the question of whether iron hydroxysulfate structural Fe(III) is susceptible to microbial reduction, and demonstrate the reductive dissolution of two out of three samples tested. In this study, we have demonstrated that two Geobacter-type iron-reducing bacteria are able to reduce structural Fe(III) in jarosite and not schwertmannite. It is likely that in the environment other factors will influence the reductive dissolution of Fe(III)-bearing minerals and remobilization of metals. Factors to consider include the capabilities of the specific bacteria present, and environmental conditions or processes influencing the concentration of dissolved Fe^{+3} in the environment. As illustrated above, the ability of bacteria to reduce structural Fe(III) can vary. In addition, there is evidence that some anaerobic bacteria (e.g., *Shewanella* and *Geothrix*) produce compounds that act as extracellular electron shuttles, enabling them to reduce structural Fe(III) without direct contact [*Nevin and Lovley*, 2002a, 2002b]. Factors influencing the solubility of Fe^{+3}, such as pH and chelators in the environment, can also influence the mechanism of reductive dissolution. Under conditions that allow a high concentration of dissolved Fe^{+3}, iron-reducing bacteria can drive Fe(III) mineral dissolution by removing Fe^{+3} from solution. For example, at low pH, acidophilic bacteria such as *Acidiphilium* spp. reduced Fe(III) provided as jarosite or schwertmannite [*Bridge and Johnson*, 2000; *Küsel et al.*, 2002]. However, the reported rates of Fe(III) reduction for jarosite and natrojarosite by *Acidiphilium* at pH 2 [*Bridge and Johnson*, 2000] were two orders of magnitude lower than the rate reported here for Elizabeth mine jarosite by *G. metallireducens*. Organic compounds capable of chelating metals can also affect the reduction of solid phase Fe(III) by bacteria by increasing Fe(III) in solution [*Lovley et al.*, 1996a]. In addition, humic substances can act as electron shuttles for the indirect reduction of Fe(III) [*Lovley et al.*, 1996b]; for example iron-reducing bacteria have been shown to reduce largely unavailable Fe(III) minerals such as goethite and hematite in the presence of humic substances [*Lovley et al.*, 1998]. On the basis of pore water data, *Nordstrom et al.* [1999] noted that considerable reductive dissolution of iron (with little or no indication of sulfate reduction) had occurred in the schwertmannite-bearing sediments (pH 5.5–6.5) of the Keswick Reservoir on the Sacramento River, downstream of the Iron Mountain Superfund Site, the largest producer of acid mine drainage in California. While this might appear to contradict our study, the Keswick sediments included a mixture of ferrihydrite, schwertmannite, and goethite. Ferrihydrite would appear to be the most likely substrate for microbial reduction of iron in this system, although factors such as sediment organic matter and specific microbial capabilities as discussed above may influence environmental schwertmannite-Fe(III) reduction. The limited studies done to date highlight the complexity of processes controlling the reductive dissolution of iron-hydroxysulfate minerals, and further investigation is needed to fully understand the physical, chemical and biological factors likely to control Fe(III)-mineral reduction and the remobilization of bound contaminants in the environment.

5. Summary and Implications

[26] Jarosite is an iron-hydroxysulfate mineral found in a variety of hazardous waste environments. Our results indicate that there is a potential for the dissolution of jarosite by iron-reducing bacteria with concomitant remobilization of metals and radionuclides under anoxic, neutral pH conditions. Microbial Fe(III) reduction is likely to have more impact on the remobilization of hazardous materials from iron-hydroxysulfate minerals than sulfate reduction. Although the focus of this paper is on the reduction of structural Fe^{+3} in jarosite, microbial metal reduction might also impact the incorporated contaminants, for example chromate. The chromate analog of jarosite [$KFe_3(CrO_4)_2(OH)_6$] has been identified in a soil at a chrome-plating facility in Oregon [*Baron et al.*, 1996], and bacteria capable of Fe^{+3} reduction are also known to reduce soluble Cr^{+6} [e.g., *Lovley and Phillips*, 1994]. As the toxicity and solubility of Cr^{+6} are much greater than that of the reduced product Cr^{+3} [*Lloyd*, 2002], microbial reduction of Cr^{+6} in jarosite may mitigate its remobilization due to microbial activity.

[27] The potential for development of iron-reducing conditions and resultant contaminant mobilization has significant implications for hazard assessments dealing with iron-hydroxysulfate-bearing waste materials. For example, the ocean disposal of a total of about 4 million tons of jarosite-bearing waste from a zinc refinery was carried out at a deep water (~2000 m) site off southern Tasmania for 24 years (1973–1997). The waste contained elevated levels of zinc (up to 6.5%), lead (2.4%), arsenic (0.95%), copper (0.24%), cadmium (0.035%), mercury (0.00015%) [*Harris et al.*, 1999]. The potential for jarosite dissolution and contaminant releases to the water column and biota from material deposited with the seafloor sediments exist, and the contaminant inventories are obviously large.

[28] **Acknowledgments.** The authors gratefully acknowledge the following individuals for their technical assistance: T. Councell, C. Cravotta,

J. Hammarstrom, G. Nord, R. Seal, D. Webster, D. Williams (USGS), and D. Lovley (University of Massachusetts); J. Weiss and J. Moreau provided valuable review of the manuscript. Any use of trade, product, or firm names in this report is for descriptive purposes only and does not imply endorsement by the U.S. government.

References

Baron, D., C. D. Palmer, and J. T. Stanley (1996), Identification of two iron-chromate precipitates in a Cr(VI)-contaminated soil, *Environ. Sci. Technol.*, *30*, 964–968.

Bigham, J. M., L. Carlson, and E. Murad (1994), Schwertmannite, a new iron oxyhydroxy-sulphate from Pyhasalmi, Finland, and other localities, *Mineral. Mag.*, *58*, 641–648.

Bridge, T. A. M., and D. B. Johnson (2000), Reductive dissolution of ferric iron minerals by Acidiphilium SJH, *Geomicrobiol. J.*, *17*, 193–206.

Brown, G. (1961), Other minerals, in *The X-Ray Identification and Crystal Structures of Clay Minerals*, edited by G. Brown, pp. 467–488, Mineral. Soc., London.

Childers, S. E., S. Ciufo, and D. R. Lovley (2002), *Geobacter metallireducens* accesses insoluble Fe(III) oxide by chemotaxis, *Nature*, *416*, 767–769.

Coates, J. D., E. J. P. Phillips, D. J. Lonergan, H. Jenter, and D. R. Lovley (1996), Isolation of *Geobacter* species from a variety of sedimentary environments, *Appl. Environ. Microbiol.*, *62*, 1531–1536.

Cornell, R. M., and U. Schwertmann (1996), *The Iron Oxides: Structure, Properties, Reactions, Occurrence and Uses*, 491 pp., John Wiley, Hoboken, N. J.

Dutrizac, J. E., and J. L. Jambor (1984), Formation and characterization of argentojarosite and plumbojarosite and their relevance to metallurgical processing, in *Proceedings of the Second International Congress on Applied Mineralogy in the Minerals Industry*, edited by W. C. Park, D. M. Hausen, and R. D. Hagni, pp. 507–530, Metal. Soc. of AIME, Littleton, Colo.

Dutrizac, J. E., and J. L. Jambor (1987), The behaviour of arsenic during jarosite precipitation: Arsenic precipitation at 97°C from sulphate or chloride media, *Can. Metal. Q.*, *26*, 91–101.

Fanning, D. S., M. C. Rabenhorst, and J. M. Bigham (1993), Colors of acid sulfate soils, in *Soil Color*, SSSA *Spec. Publ. 31*, pp. 91–108, Soil Sci. Soc. of Am., Madison, Wis.

Hammarstrom, J. M., A. L. Meier, J. C. Jackson, R. Barden, P. J. Wormington, J. D. Wormington, and R. R. Seal II (2000), Characterization of mine waste at the Elizabeth Copper Mine, Orange County, Vermont, *U.S. Geol. Surv. Open File Rep. 99-564*, 74 pp.

Hanson, R. S., and J. A. Phillips (1981), Chemical composition, in *Manual of Methods for General Bacteriology*, edited by P. Gerhardt and W. A. Wood, pp. 328–364, Am. Soc. for Microbiol., Washington, D. C.

Harris, P. T., P. E. O'Brien, P. Quilty, A. McMinn, D. Holdway, N. F. Exon, P. J. Hill, and C. W. Wilson (1999), Sedimentation and continental slope processes in the vicinity of an ocean waste-disposal site, southeastern Tasmania, *Aust. J. Earth Sci.*, *46*, 577–591.

Ivarson, K. C., R. O. Hallberg, and T. Wadsten (1976), The pyritization of basic ferric sulfates in acid sulfate soils: A microbiological interpretation, *Can. J. Soil Sci.*, *56*, 393–406.

Kaiman, S. (1977), Mineralogical examination of old tailings from the Nordic Lake mine, Elliot Lake, Ontario, *Miner. Sci. Lab. Rep. ERP/MSL*, pp. 77–190, Can. Cent. for Miner. and Energy Technol., Ottawa.

Klinghöfer, G., et al. (2004), Jarosite and hematite at Meridiani Planum from Opportunity's Mössbauer spectrometer, *Science*, *306*, 1740–1745.

Küsel, K., T. Dorsch, G. Acker, and E. Stackebrandt (1999), Microbial reduction of Fe (III) in acidic sediments: Isolation of *Acidiphilium cryptum* JF-5 capable of coupling the reduction of Fe (III) to the oxidation of glucose, *Appl. Environ. Microbiol.*, *65*, 3633–3640.

Küsel, K., U. Roth, and H. L. Drake (2002), Microbial reduction of Fe(III) in the presence of oxygen under low pH conditions, *Environ. Microbiol.*, *4*, 414–421.

Landa, E. R. (2005), Microbial biogeochemistry of uranium mill tailings, *Adv. Appl. Microbiol.*, *57*, 113–130.

Li, X., and C. Kutal (2003), Synthesis and characterization of superparamagnetic $Co_xFe_{3-x}O_4$ nanoparticles, *J. Alloys Compd.*, *349*, 264–268.

Lloyd, J. R. (2002), Bioremediation of metals: The application of microorganisms that make and break minerals, *Microbiol. Today*, *29*, 67–69.

Loan, M., J. M. Cowley, R. Hart, and G. M. Parkinson (2004), Evidence on the structure of synthetic schwertmannite, *Am. Mineral.*, *89*, 1735–1742.

Lovley, D. R. (1987), Organic matter mineralization with the reduction of ferric iron: A review, *Geomicrobiol. J.*, *5*, 375–399.

Lovley, D. R., and E. J. P. Phillips (1987a), Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments, *Appl. Environ. Microbiol.*, *53*, 2636–2641.

Lovley, D. R., and E. J. P. Phillips (1987b), Rapid assay for microbially reducible ferric iron in aquatic sediments, *Appl. Environ. Microbiol.*, *53*, 1536–1540.

Lovley, D. R., and E. J. P. Phillips (1988), Novel mode of microbial energy metabolism: Organic carbon oxidation coupled to dissimilatory reduction of iron or manganese, *Appl. Environ. Microbiol.*, *54*, 1472–1480.

Lovley, D. R., and E. J. P. Phillips (1994), Reduction of chromate by *Desulfovibrio vulgaris* and its c_3 cytochrome, *Appl. Environ. Microbiol.*, *60*, 726–728.

Lovley, D. R., F. H. Chapelle, and E. J. P. Phillips (1990), Fe(III)-reducing bacteria in deeply buried sediments of the Atlantic Coastal Plain, *Geology*, *18*, 954–957.

Lovley, D. R., J. C. Woodward, and F. H. Chapelle (1996a), Rapid anaerobic benzene oxidation with a variety of chelated Fe(III) forms, *Appl. Environ. Microbiol.*, *62*, 288–291.

Lovley, D. R., J. D. Coates, E. L. Blunt-Harris, E. J. P. Phillips, and J. C. Woodward (1996b), Humic substances as electron acceptors for microbial respiration, *Nature*, *382*, 445–448.

Lovley, D. R., J. L. Fraga, E. L. Blunt-Harris, L. A. Hayes, E. J. P. Phillips, and J. D. Coates (1998), Humic substances as a mediator for microbially catalyzed metal reduction, *Acta Hydrochim. Hydrobiol.*, *26*, 152–157.

Nevin, K. P., and D. R. Lovley (2002a), Mechanisms for Fe(III) oxide reduction in sedimentary environments, *Geomicrobiol. J.*, *19*, 141–159.

Nevin, K. P., and D. R. Lovley (2002b), Mechanisms for accessing insoluble Fe(III) oxide during dissimilatory Fe(III) reduction by *Geothrix fermentens*, *Appl. Environ. Microbiol.*, *68*, 2294–2299.

Nordstrom, D. K., and C. N. Alpers (1999), Geochemistry of acid mine waters, in *The Environmental Geochemistry of Mineral Deposits*, *Rev. Econ. Geol.*, vol. 6A, edited by G. S. Plumlee and M. J. Logsdon, pp. 133–160, Soc. of Econ. Geol., Littleton, Colo.

Nordstrom, D. K., C. N. Alpers, J. A. Coston, H. E. Taylor, R. B. McCleskey, J. W. Ball, S. A. Ogle, J. S. Cotsifas, and J. A. Davis (1999), Geochemistry, toxicity, and sorption properties of contaminated sediments and pore waters from two reservoirs receiving acid mine drainage, in *U.S. Geological Survey Toxic Substances Hydrology Program—Proceedings of the Technical Meeting, Charleston, South Carolina, March 8–12, 1999*, *U.S. Geol. Surv. Water Resour. Invest. Rep. 99-4018A*, vol. 1, 289–296.

Norton, G. A., R. G. Richardson, R. Markuszewski, and A. D. Levine (1991), Precipitation of jarosite compounds as a method for removing impurities from acidic wastes from chemical coal cleaning, *Environ. Sci. Technol.*, *25*, 449–455.

Parnell, R. A. (1983), Weathering processes and pickeringite formation in sulfidic schist: A consideration in acid precipitation neutralization studies, *Environ. Geol.*, *4*, 209–215.

Robbins, E. I., J. E. Anderson, C. A. Cravotta III, G. L. Nord Jr., and E. T. Slonecker (2000), Remotely-sensed multispectral reflectance variations in acidic versus near-neutral contaminated coal mine drainage in Pennsylvania, paper presented at Fifth International Conference on Acid Rock Drainage (ICARD 2000), Soc. for Min., Metal., and Explor., Denver, Colo., 21–24 May.

Roden, E. E. (2003), Fe(III) oxide reactivity toward biological versus chemical reduction, *Environ. Sci. Technol.*, *37*, 1319–1324.

Schuiling, R. D., and P. F. M. van Gaans (1997), The waste sulfuric acid lake of the TiO_2-plant at Armyansk, Crimea, Ukraine. part I. Self-sealing as an environmental protection mechanism, *Appl. Geochem.*, *12*, 181–186.

Sidenko, N. V., and B. L. Sherriff (2004), Attenuation of metals by iron-phases from surface and ground water of Ni-Cu mine waste, in *Applied Mineralogy*, edited by M. Pecchio et al., pp. 503–506, Int. Counc. for Appl. Mineral., Fed. Inst. for Geosci. and Nat. Resour., Hannover, Germany.

Stookey, L. L. (1970), Ferrozine—A new spectrophotometric reagent for iron, *Anal. Chem.*, *42*, 779–781.

Waychunas, G. A., N. Xu, C. C. Fuller, J. A. Davis, and J. M. Bigham (1995), XAS study of AsO_4^{3-} and SeO_4^{2-} substituted schwertmannites, *Physica B*, *208–209*, 481–483.

E. J. P. Jones, E. R. Landa, and M. A. Voytek, U.S. Geological Survey, 430 National Center, Reston, VA 20192, USA. (ejjones@usgs.gov)

T.-L. Nadeau, Office of Wetlands, Oceans, and Watersheds (4501T), U.S. Environmental Protection Agency, Washington, DC 20460, USA.