Pergamon

Geochimica et Cosmochimica Acta, Vol. 68, No. 12, pp. 2639–2648, 2004

0016-7037/04 $30.00 + .00

doi:10.1016/j.gca.2004.01.005

Reduction of uranium(VI) under sulfate-reducing conditions in the presence of Fe(III)-(hydr)oxides

R. K. Sani,[1] B. M. Peyton,[1,*] J. E. Amonette,[2] and G. G. Geesey[3,4]

[1]Department of Chemical Engineering, Center for Multiphase Environmental Research, Washington State University, Dana Hall Rm 118, Pullman, WA 99164-2710, USA
[2]Fundamental Science Directorate, Pacific Northwest National Laboratory, P.O. Box 999, K8-96, Richland, WA 99352, USA
[3]Department of Microbiology, 109 Lewis Hall, PO Box 173520, Montana State University, Bozeman, MT 59717-3520, USA
[4]Center for Biofilm Engineering, 366 EPS, Montana State University, Bozeman, MT 59717-3980, USA

(*Received June* 3, 2003; *accepted in revised form January* 9, 2004)

Abstract—Hexavalent uranium [U(VI)] dissolved in a modified lactate-C medium was treated under anoxic conditions with a mixture of an Fe(III)-(hydr)oxide mineral (hematite, goethite, or ferrihydrite) and quartz. The mass of Fe(III)-(hydr)oxide mineral was varied to give equivalent Fe(III)-mineral surface areas. After equilibration, the U(VI)-mineral suspensions were inoculated with sulfate-reducing bacteria, *Desulfovibrio desulfuricans* G20. Inoculation of the suspensions containing sulfate-limited medium yielded significant G20 growth, along with concomitant reduction of sulfate and U(VI) from solution. With lactate-limited medium, however, some of the uranium that had been removed from solution was resolubilized in the hematite treatments and, to a lesser extent, in the goethite treatments, once the lactate was depleted. No resolubilization was observed in the lactate-limited ferrihydrite treatment even after a prolonged incubation of 4 months. Uranium resolubilization was attributed to reoxidation of the uraninite by Fe(III) present in the (hydr)oxide phases. Analysis by U L_3-edge XANES spectroscopy of mineral specimens sampled at the end of the experiments yielded spectra similar to that of uraninite, but having distinct features, notably a much more intense and slightly broader white line consistent with precipitation of nanometer-sized particles. The XANES spectra thus provided strong evidence for SRB-promoted removal of U(VI) from solution by reductive precipitation of uraninite. Consequently, our results suggest that SRB mediate reduction of soluble U(VI) to an insoluble U(IV) oxide, so long as a suitable electron donor is available. Depletion of the electron donor may result in partial reoxidation of the U(IV) to soluble U(VI) species when the surfaces of crystalline Fe(III)-(hydr)oxides are incompletely reduced.

1. INTRODUCTION

Solid and liquid wastes containing heavy metals and radionuclides discharged to the ground over a 50-yr period constitute a major environmental problem at more than 120 U.S. Department of Energy (DOE) sites in 36 states and territories (McCullough et al., 1999). These metals and radionuclides may be mobile within groundwater, threaten down-gradient water resources and can pose difficult remediation challenges. Microbial reduction offers a potential mechanism to immobilize these contaminants because many heavy metals and radionuclides [e.g., chromium (Cr), uranium (U), and technetium (Tc)] are less soluble when reduced (Lovley and Coates, 1997). Of these, U is the most common radionuclide in soils, sediments, and groundwater at DOE sites and is therefore of particular environmental concern (Riley et al., 1992).

Bioremediation of U-containing groundwater typically involves the transformation of the uranyl ion (UO_2^{2+}) and its soluble complexes to the highly insoluble mineral uraninite [UO_2(s)] by the creation and maintenance of reducing conditions (Langmuir, 1978; Parks and Pohl, 1988). The U-reduction process has been demonstrated in the laboratory using cultures of *Cellulomonas* sp., *Clostridium* sp., *Desulfovibrio* sp., *Desulfotomaculum* sp., *Geobacter* sp., *Shewanella* sp., and other microorganisms (Lovley et al., 1991; Francis et al., 1994; Ganesh et al., 1997; Abdelouas et al., 1998; Tebo and Obraztsova, 1998; Sani et al., 2002). In addition, microbial processes have been shown to remove U from contaminated groundwater (Lovley and Phillips, 1992a) and from contaminated soil wash through reductive precipitation (Phillips et al., 1995).

The sulfate-reducing bacteria (SRB) can enzymatically reduce Cr(VI), Mn(IV), Fe(III), U(VI), and Tc(VII) (Lovley, 1993; Lovley and Coates, 1997; Lloyd and Lovley, 2001). In addition, SRB are present in many contaminated subsurface sites and carry out dissimilatory reduction of sulfate to sulfide (Barnes et al., 1998; Abdelouas et al., 1998, 2000). The resulting sulfide often reacts to form sulfide precipitates of many heavy metals [e.g., Cd(II), Cu(II), Hg(II), Ni(II), Pb(II), and Zn(II)] (Poulson et al., 1997; Bhattacharya et al., 1981; Sani et al., 2001a, 2003). Thus, SRB offer great potential for treating complex mixtures of heavy metals and radionuclides in the deep subsurface. Further, the formation of Fe-sulfide deposits may help maintain a low redox-potential barrier for in situ bioremediation by scavenging oxygen, thus hindering reoxidation of reduced contaminant precipitates. The benefit of this scavenging action, however, should be further investigated, since it has been reported that FeS minerals in contact with other metal sulfides may be galvanically protected (Holmes and Crundwell, 1995; Attia and El-Zeky, 1990). In these regards, Fe-sulfides are of particular interest, given the abundance of Fe in most soils and sediments (Amonette, 2003).

* Author to whom correspondence should be addressed (bmp@wsu.edu).

In addition, SRB can reduce Fe(III) to Fe(II) enzymatically as well as indirectly by H_2S production (Pyzik and Sommer, 1981; Berner, 1984; Le Gall et al., 1994). Microbial Fe(III)-(hydr)oxide reduction strongly influences the geochemistry of anaerobic soil and sedimentary environments as well as the persistence and mobility of various types of organic and inorganic contaminants (Fredrickson and Gorby, 1996; Fredrickson et al., 2000; Zachara et al., 2001). For example, Zachara et al. (2001) showed that Fe(III)-oxide-entrained trace metals were mobilized during bacterial iron reduction leading to a net increase, in most cases, in aqueous metal concentrations.

U(VI) reduction has been shown in the absence of soil minerals, under nongrowth conditions (Lovley et al., 1991; Lovley and Phillips, 1992b; Ganesh et al., 1997; Spear et al., 1999, 2000); however, data on U(VI) reduction under growth conditions is more sparse. With a better understanding of these processes, in situ stimulation of anaerobic microbial metal-transformation processes may be an effective treatment alternative for heavy metals and radionuclides. More research is needed, however, to better understand interactions of contaminants with bacteria and mineral phases in the subsurface. In this study, we present results from studies of microbial growth and reduction of sorbed and soluble phase U(VI) to less mobile U(IV) in the presence of redox-sensitive and redox-insensitive minerals including hematite, goethite, ferrihydrite, and quartz under sulfate-reducing conditions.

2. MATERIALS AND METHODS

2.1. Bacteria, Media, and Minerals

Desulfovibrio desulfuricans G20 (subsequently referred to as G20) used in the study was a gift of J. Wall, University of Missouri-Columbia (Columbia, MO, USA), derived from *D. desulfuricans* G100A (Wall et al., 1993). G20 was maintained in a lactate-C medium modified for metal toxicity studies (MTM, Sani et al., 2001a), which contained (mM): sodium lactate, 45; sodium sulfate, 15; calcium chloride dihydrated, 0.41; ammonium chloride, 19; magnesium sulfate, 4; and PIPES (1, 4-piperazinediethane sulfonic acid disodium salt monohydrate), 30. In addition, yeast extract and tryptone were added at concentrations of 0.05 and 0.5 g/L, respectively. The pH was adjusted to 7.2 with 6 N HCl. G20 contained the Green Fluorescent Protein (GFP) reporter gene construct (Neal et al., 2001) that was used in another part of a larger project that included direct nondestructive microscopic observations of G20 on mineral surfaces. The GFP was unused in the present study, but to maintain the GFP plasmid, 20 μg/mL chloramphenicol was added to the medium before inoculation.

Medium components were analytical grade purchased from Fisher Scientific (Pittsburgh, PA) with the following exceptions: yeast extract and tryptone were obtained from Difco Chemical Co. (Detroit, MI) and 1, 4-piperazinediethane sulfonic acid disodium salt monohydrate and sodium sulfate were obtained from Aldrich Chemical Co. (Milwaukee, WI). Uranium was purchased as $UO_2Cl_2 \cdot 3H_2O$ from Bodman Industries (Aston, PA). Water was supplied from a Barnstead/Nanopure system and had a nominal resistivity of 17.6 mΩ-cm. All glassware was washed with 2 N HNO_3.

The Fe(III)-(hydr)oxides, hematite (α-Fe_2O_3), goethite (α-FeOOH), and ferrihydrite ($Fe_5O_7OH \cdot 4H_2O$) were synthesized as described by Schwertmann and Cornell (1991). A Coulter SA 3100 BET analyzer (using N_2 sorption) was used to determine the specific surfaces (Gregg and Sing, 1982) of the hematite, goethite, ferrihydrite, and quartz (α-SiO_2, 212–300 μm) and were found to be 30, 52, 180, and 0.02 m^2/g, respectively. The Fe(III)-(hydr)oxides were not autoclaved due to potential phase modifications that can occur during autoclaving (Fredrickson et al., 1998). However, before use, the Fe(III)-(hydr)oxides were heat-treated in pre-sterilized culture tubes in an oven at 80°C for 16 h. No measurable change in the mineral specific surface areas occurred as a result.

2.2. Cell Preparation and U(VI) Reduction Experiments

Cells for inoculation were prepared as described previously (Sani et al., 2001b). In brief, H_2S initially present in an active inoculum was removed by flushing with ultra-pure N_2 for 1 h. The cells were centrifuged in the presence of ultra-pure N_2 at 10,000 $\times$ *g* for 10 min. The supernatant was discarded and the cell pellets were suspended in 0.89% NaCl. This process was repeated twice and washed cells were used as inoculum. Volumes of 100 mL of MTM in 150-mL serum bottles were autoclaved separately. A filtered (0.2 μm, Gelman Acrodisc) stock solution (42 mM) of $UO_2Cl_2 \cdot 3H_2O$ was aseptically added by syringe and needle to the serum bottles to give 90 μM U(VI) before inoculation. After inoculation (3 mg/L, total cell protein), serum bottles were then incubated at 25°C at 125 rpm and sampled for cell growth as measured by total cell protein and soluble concentration of U(VI).

2.3. U(VI) Reduction with Fe(III)-(hydr)oxides

Two electron donor/acceptor ratios, one lactate-limited and one sulfate-limited, were used in the study. The respective concentrations of lactate and sulfate were 30 and 20 mM for the lactate-limited medium (LLM) and 45 and 9 mM for the sulfate-limited medium (SLM). With each set of experiments, mineral-, sulfate-, and lactate-free controls were also used. Volumes of 120 mL of medium in 150-mL serum bottles were autoclaved separately. A filtered (0.2 μm, Gelman Acrodisc) stock solution (42 mM) of $UO_2Cl_2 \cdot 3H_2O$ was aseptically added to the serum bottles to give the desired U(VI) concentrations. Samples were withdrawn aseptically to measure initial concentrations of U(VI), lactate, and sulfate. Heat-treated Fe(III)-(hydr)oxides were then added to serum bottles containing U(VI) and medium. The total surface area for each redox-sensitive mineral was chosen to be constant at 4.16 m^2; thus 139, 80, and 23.1 mg were added for hematite, goethite, and ferrihydrite, respectively. In addition, each serum bottle was supplemented with 1.52 g of quartz having a total surface area of 0.03 m^2. The serum bottles were then purged with ultra-pure N_2(g) for 6 min, sealed with butyl rubber septa, capped and crimped with aluminum seals, and pressurized with ultra-pure N_2 at 68.9 kPa (10 lb/in^2) above atmospheric pressure. Uninoculated serum bottles were incubated at 25°C on a rotamix operated at 20 rpm to monitor abiotic sorption of U(VI) to minerals over a 5–9 d period to establish equilibrium of U(VI) between the soluble and solid phases.

After abiotic sorption of U(VI) onto the minerals, washed cells of G20 were injected into the serum bottles to give a final concentration of 3 mg/L protein. After inoculation, serum bottles were incubated at 25°C on an orbital shaker (Lab-Line Instruments, Inc., IL) at 125 rpm. Periodically, 3-mL samples were aseptically removed by syringe and needle and were analyzed for total cell protein and soluble concentrations of U, lactate, sulfate, acetate, sulfide, and Fe(II).

2.4. Analytical methods

2.4.1. Soluble Uranium

To monitor abiotic U(VI) sorption onto minerals, samples were taken before inoculation and centrifuged at 5000 $\times$ *g* for 5 min in a microcentrifuge. The supernatant was filtered (0.2 μm, Gelman Acrodisc) and U(VI) concentrations were measured as previously described (Sani et al., 2002) with a kinetic phosphorescence analyzer (KPA-11, Chemcheck Instruments Inc., Richland, WA). KPA uses a pulsed laser and a complexing agent (Uraplex) to measure U(VI) concentrations in solution (Brina and Miller, 1992). Calibration was done using $UO_2Cl_2 \cdot 3H_2O$ solutions of 0 to 160 nM. Because the KPA-11 allows detection of U(VI) concentrations as low as 0.04 nM with a precision of $\pm$5%, the estimated detection limit in this study using 1000-fold dilutions was 40 nM.

After appearance of biotic activity, samples were filtered (0.2 μm) and analyzed for U(VI) and total U using the KPA-11. The U(VI) was measured immediately after filtration to minimize oxidation of U(IV) in solution (Anderson, 1984). Total U was determined by the KPA-11 after oxidizing the samples by mixing 0.1 mL of sample with 0.3 mL

concentrated HNO_3 and exposing to air for 1 h (Lovley and Phillips, 1992b; Ganesh et al., 1997). The concentration of reduced U was calculated as the difference between total U and filtered U(VI).

2.4.2. Total Cell Protein, Anions, Soluble Sulfide, and Fe(II)

Total cell protein was determined using a quantitative colorimetric Coomassie assay method (Pierce, Rockford, IL). Samples (1 mL) were mixed with 1 mL of 1 N NaOH and incubated in an oven at 99°C for 10 min. Afterwards, samples were mixed again and cooled for 1 h to reach room temperature (24°C) and settle mineral precipitates. Then, 1-mL subsamples were taken into another set of culture tubes, and mixed with 0.1 mL of 6 N HCl. Coomassie reagent (1 mL) was added to each tube, mixed, and left for 15 min. The absorbance of each solution was measured at 595 nm and compared to a standard curve generated for bovine serum albumin.

Samples for lactate, sulfate, and acetate were filtered (0.2 μm), and concentrations were determined using a Dionex DX-500 ion chromatograph equipped with conductivity detector-20 with an IonPac AS11-HC 4-mm column (Dionex, Sunnyvale, CA) with an estimated error of $\pm$5%. Elution was carried out using an NaOH gradient (1–100 mM). The detection limit was 3 mg/L for each anion. Final soluble sulfide and Fe(II) concentrations were determined spectrophotometrically (Milton Roy Co. Spectronic GENESYS 5, Rochester, NY) using the methylene blue and 1,10 phenanthroline methods (Hach Co., Loveland, CO), respectively.

2.5. XANES Spectroscopy of U Solids

At the end of the experiments, mineral solids were separated by centrifugation at 10,000 $\times$ *g* for 10 min under anoxic conditions. After decanting the supernatant, the minerals were dried at ambient temperature in a glove box (Model 1025, Forma Scientific Inc., OH) under an O_2-free N_2:CO_2:H_2 (90:5:5) atmosphere. Dried solid subsamples were packed between Kapton windows in a multi-specimen Al sample holder modified to fit into a Lytle detector chamber. To avoid sample oxidation, the specimen-mounting process was carried out in a glove box, and the sample holder was placed inside a BBL Gas-Pak (Becton Dickinson, Franklin Lakes, NJ) container for shipping and storage at the synchrotron before analysis.

XANES spectra at the U L_3-edge were collected in fluorescence mode at the Pacific Northwest Consortium–Collaborative Access Team (PNC-CAT) bending-magnet beamline (20-BM) at the Advanced Photon Source (Argonne, IL) using a scintillation detector with a single-channel analyzer to define the energy window. A natural uraninite (nominally UO_2) sample was also analyzed. During XANES analysis, the Lytle detector chamber was fitted with a Sr filter and was continuously flushed with $He_{(g)}$ to prevent air oxidation of the samples. Resulting spectra were normalized for comparative purposes.

2.6. Statistical Analysis

Each set of experiments was carried out in duplicate and repeated four times. In each set of experiments, duplicate treatment profiles were similar in total cell protein, U(VI), lactate, sulfate, and acetate; however, the length of the lag time was somewhat variable. One-way analysis of variance (ANOVA) was used to determine if there were statistically significant differences in G20 lag times among controls and treatments containing U with and without Fe(III)-(hydr)oxides. The threshold level of statistical significance for this study was $\alpha = 0.05$.

3. RESULTS

3.1. Reduction of U(VI) by *D. desulfuricans* G20 under Growth Conditions

Figure 1A shows that in the absence of solid mineral phase, the addition of 90 μM U(VI) increased the lag time of G20 from 1 to 17 d. However, the final cell protein concentration was essentially the same as the U(VI)-free control. Figure 1B shows that soluble U(VI) concentrations decreased initially, but then remained nearly constant until growth began. Once G20 growth was observed, U(VI) concentrations decreased from 67 to 4 μM over a 9-d period. In contrast G20-free controls showed only a slight decrease in U(VI) concentration that might have resulted from the U(VI) adsorption to the glass serum bottles.

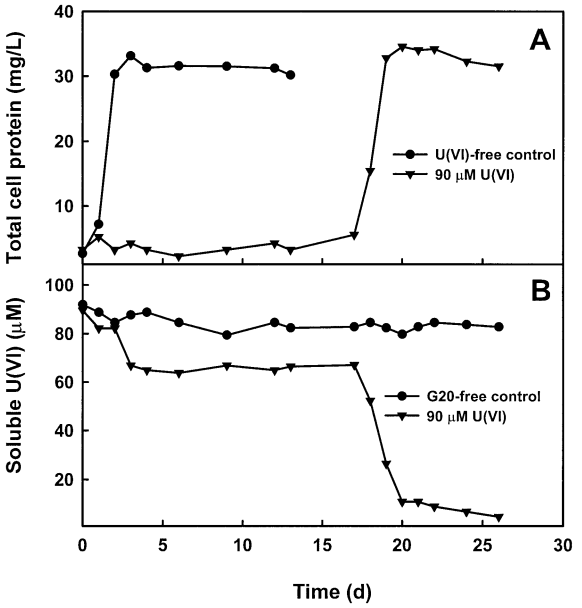

Fig. 1. A. The effect of U(VI) (90 μM) on the growth of *D. desulfuricans* G20 as measured by total cell protein. B. Profiles of U(VI) concentrations with and without G20. Symbols show the mean of duplicate analyses.

3.2. Reduction of U(VI) by *D. desulfuricans* G20 in the Presence of Hematite

3.2.1. Sulfate-Limited Medium

The first 9 d of experiment consisted of abiotic equilibration of U(VI) in sulfate-limited medium (SLM) with hematite and quartz. As shown in Figure 2A, three distinct trends in U(VI) concentration profiles were observed. In the absence of minerals and G20, U(VI) concentration decreased slightly during the first 2 d and then remained stable at ~130 μM. When the minerals were present, U(VI) concentrations during the first 2 d decreased significantly, depending on the presence or absence of lactate in the SLM. In these treatments, the decrease in U(VI) concentration likely stemmed from adsorption of U(VI) to mineral surfaces or possibly precipitation of U(VI) solid phases.

After 9 d, four of the five mineral treatments were inoculated with washed G20. With SLM, U(VI) concentrations decreased gradually (Fig. 2A), but once growth began, U(VI) concentrations decreased to 5 μM over a 6-d period and remained low throughout the remainder of the experiment. With the sulfate-free SLM, the U(VI) reduction rate was very slow, whereas for the lactate-free SLM, no decrease in soluble concentration of U(VI) was observed even after prolonged incubation. This indicates that no abiotic reductive processes were occurring and that lactate was required for biotic removal of U(VI) from

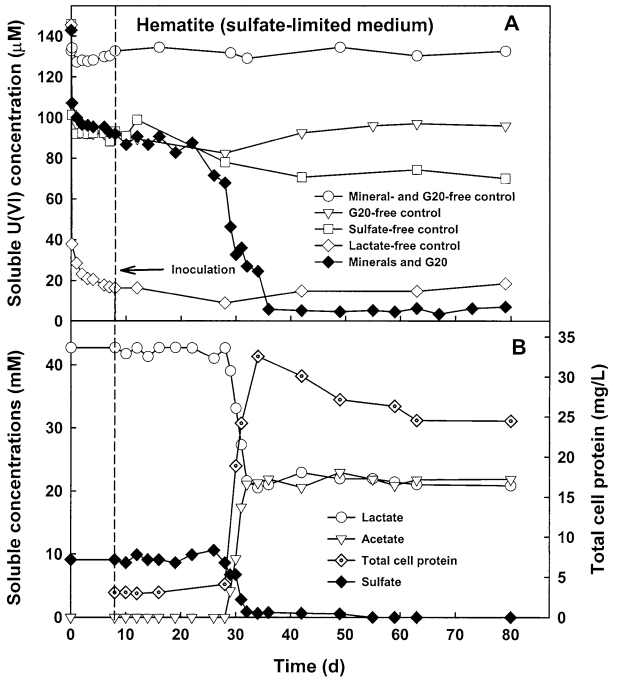

Fig. 2. A. Concentration profiles of soluble U(VI) in SLM during abiotic sorption onto hematite and quartz, and reduction by G20 under growth conditions. B. Concentration profiles of total cell protein, and soluble lactate, sulfate, and acetate, in the presence of hematite, quartz, U(VI), and G20 in SLM.

solution. The decrease in soluble U(VI) concentration with the full and sulfate-free SLM treatments after G20 inoculation was likely a combination of active microbial reduction and sorption of U(VI) onto bacterial surfaces (both freely suspended G20 and G20 that were attached to minerals).

As measures of biotic activity, concentration profiles for total cell protein, lactate, sulfate, and acetate were also monitored. The profile for total cell protein in the full SLM-mineral treatment (Fig. 2B, right axis) shows that significant growth of G20 occurred after a lag period of about 18 d. During the growth period of 5 d, the soluble concentration of lactate decreased from 43 to 21 mM, and that of sulfate from 9 to 0.6 mM (Fig. 2B, left axis). These decreases occurred during the same period that U(VI) concentrations decreased (Fig. 2A). The sulfate concentration continued to decrease and dropped below the detection limit 40 d after inoculation, whereas the lactate concentrations remained at 21 mM for the remainder of the experiment, thus confirming that the medium was sulfate-limited. The soluble acetate concentrations increased from 0 to 21 mM during the same 5 d that growth was observed and then remained approximately constant for the duration of the experiment.

During the course of the experiments, the color of the solids changed from red, characteristic of hematite, to black, characteristic of iron sulfides. In contrast, no black precipitates formed in the lactate-, sulfate-, and G20-free treatments. Moreover, no growth was observed in these treatments, and neither the sulfate concentration in the lactate-free treatment nor the lactate concentration in the sulfate-free treatment changed during incubation periods as long as 90 d (data not shown).

3.1.2. Lactate-Limited Medium

The trends in the U(VI) concentration profiles for the LLM (Fig. 3A) were similar to those observed for the SLM (Fig. 2A), with one exception. After inoculation, the U(VI) concentration in the full LLM-mineral treatment decreased to ~25 μM and then increased slowly over the next 30 d to ~64 μM. Addition of lactate (10-mM) to the system at day 79 resulted in a rapid decrease in the U(VI) concentration.

Concentration profiles of total cell protein, lactate, sulfate, and acetate for the LLM (Fig. 3B) were analogous to those for the SLM (Fig. 2B), with the main differences being in the profiles for sulfate and lactate. Growth lagged inoculation by ~18 d, and resulted in significant decreases in sulfate and lactate concentrations during a 6-d period of high biotic activity, with concomitant increases in total cell protein and acetate. Sulfate concentrations decreased to ~7 mM and then remained constant until further addition of lactate. However, lactate concentrations decreased to 2 mM 5 d after initiation of growth and then to below detection after another 10 d of incubation, as would be expected for a LLM. Addition of lactate at day 79 stimulated further biotic activity that resulted in increases in total cell protein and acetate, and decreases in sulfate. As in the SLM, visible black precipitates characteristic of iron sulfides formed in the LLM-mineral serum bottles that were inoculated with G20.

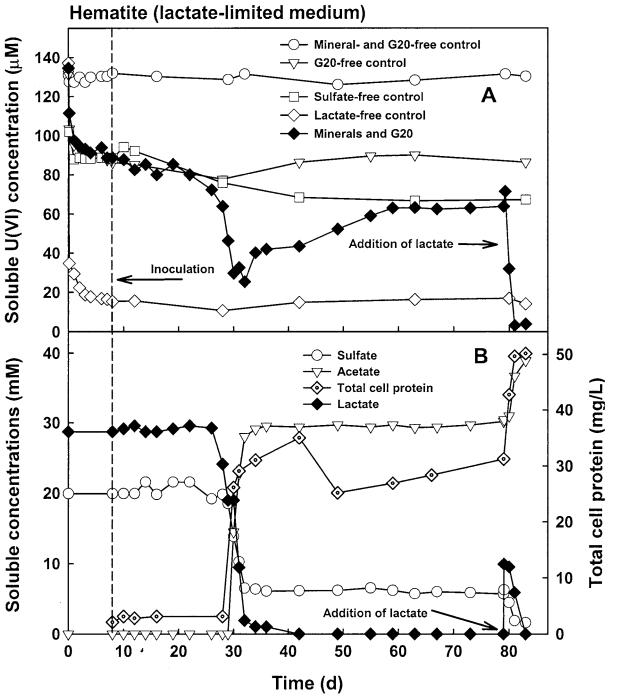

Fig. 3. A. Concentration profiles of soluble U(VI) in LLM during abiotic sorption onto hematite and quartz, and reduction by G20 under growth conditions. B. Concentration profiles of total cell protein, and soluble lactate, sulfate, and acetate, in the presence of hematite, quartz, U(VI), and G20 in LLM.

3.3. Reduction of U(VI) by *D. desulfuricans* G20 in the Presence of Goethite and Ferrihydrite

Concentration profiles for the SLM and LLM involving goethite were similar to those obtained with hematite as the iron mineral and, for this reason, only results for the full SLM are shown (Fig. 4A). In the SLM, slightly greater sorption of U(VI) was observed before inoculation, but otherwise the concentrations of total cell protein, lactate, sulfate, and acetate followed the same trends seen with the hematite system. Once G20 growth was initiated, U(VI) and sulfate reduction occurred concomitantly. In the LLM, the same general trends in concentration profiles were observed with goethite (Fig. 4B) as for hematite (Fig. 3B). However, in contrast to the hematite results, further addition of lactate (10-mM) at day 79 resulted in no change in U(VI), acetate, or sulfate concentrations during the ensuing week.

For the ferrihydrite system, to achieve similar solution-phase concentrations of U(VI) as the hematite and goethite experiments after abiotic sorption, an initial soluble U(VI) concentration of 110 μM was used rather than 140 μM. Again, only results for the full SLM and LLM treatments are shown (Figs. 5A,B). Before inoculation, the concentration profiles for the sulfate- and lactate-limited ferrihydrite treatments behaved the same as in the hematite and goethite experiments. After inoculation, although the stoichiometric relationships between the concentrations of the soluble constituents were similar to those in the other experiments, the ferrihydrite profiles differed in several respects. First, the lag period between inoculation and the onset of growth was much shorter; a couple of days rather than 3 weeks. Second, at the end of the experiment, lower U(VI) concentrations (4–9 μM) were reached; and third, no increase in U(VI) concentrations was observed after the minimum values were achieved.

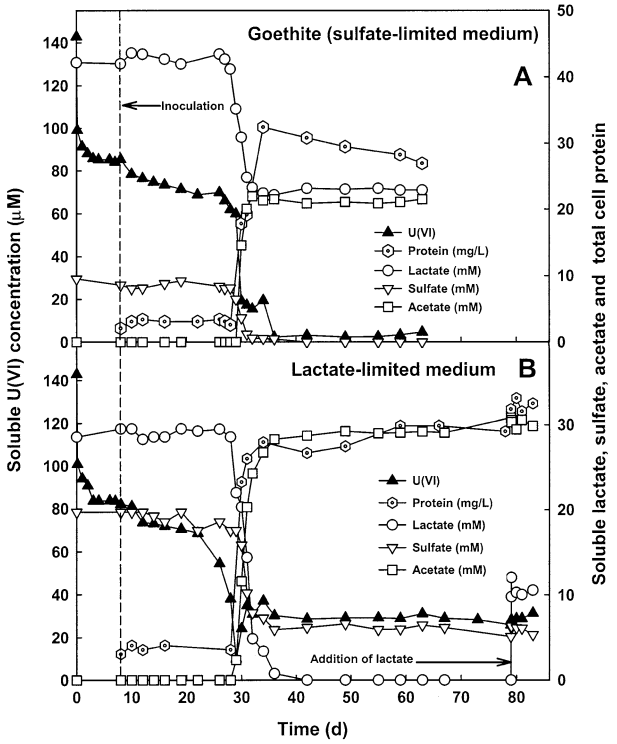

Fig. 4. Concentration profiles of total cell protein, and soluble U(VI), lactate, sulfate and acetate, in the presence of goethite and quartz during abiotic sorption and bacterial reduction of U(VI) in SLM (A) and LLM (B).

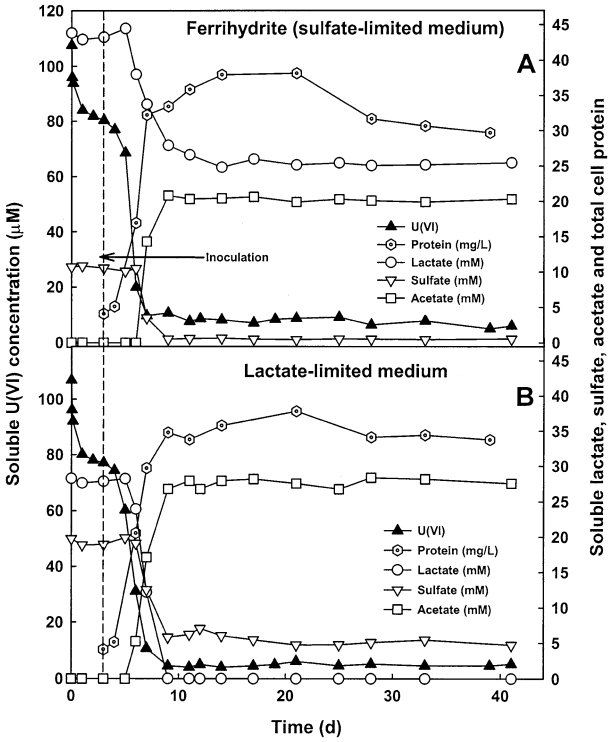

Fig. 5. Concentration profiles of total cell protein, and soluble U(VI), lactate, sulfate, and acetate, in the presence of ferrihydrite and quartz during abiotic sorption and bacterial reduction of U(VI) in SLM (A) and LLM (B).

3.4. XANES Spectra of U in Solids

U L_3-edge XANES spectra for hematite and goethite samples incubated with U(VI) in the absence of G20 were typical of U(VI) solids. These spectra were easily distinguishable from that of an untreated natural uraninite (UO_2) by a slightly higher edge energy, a slightly less intense white line just above the edge, and a greater intensity in the region 15–35 eV above the edge (Fig. 6A). Spectra for hematite and goethite samples incubated in the presence of G20 were similar to that of uraninite, but had distinct features, notably a much more intense and slightly broader white line (Figs. 6B,C). Thus, the spectra for the G20-treated samples clearly show that U(VI) was reduced during the treatment. Due to inadvertent exposure of the samples to air while awaiting analysis, spectra for ferrihydrite samples are not available.

The unique feature of the biotically reduced spectra, notably the intense white line, most likely indicates that the reduced-U particles are very small, on the order of a few nanometers (Suzuki et al., 2002). The small particle size apparently eliminates some of the destructive interference in the electron waves giving rise to the absorption spectrum. Alternative explanations include either a different bonding environment for

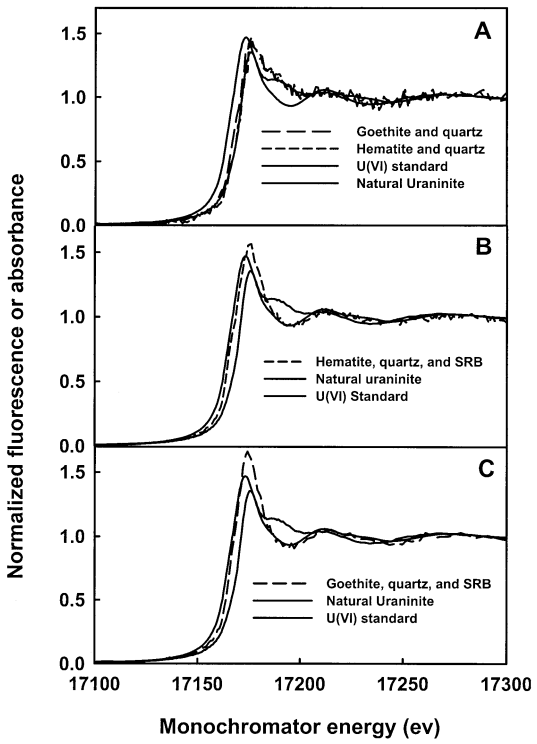

Fig. 6. A. U L_3-edge XANES spectra for natural uraninite, U(VI) standard, hematite/quartz and goethite/quartz treated with U(VI) solutions under abiotic conditions. B. U L_3-edge XANES spectra for natural uraninite, U(VI) standard, and U(VI) treated with hematite/quartz and G20. C. U L_3-edge XANES spectra for natural uraninite, U(VI) standard, and U(VI) treated with goethite/quartz and G20.

U(IV) that results in a lower degree of screening of the core hole and a stronger white line (Hudson et al., 1995), or the presence of some U in a lower oxidation state, such as U(III). Based on U M_5-edge XANES spectra, Francis et al. (1994) concluded that some U(III) was produced along with U(IV) when U(VI) was reduced by *Clostridium* sp.

4. DISCUSSION

4.1. Abiotic Removal of U(VI)

The removal of U(VI) from solution under abiotic conditions occurred as a result of adsorption to, or possibly, precipitation onto, Fe(III)-(hydr)oxide mineral surfaces. From a kinetic perspective, most of the abiotic removal of U(VI) occurred within 3 h of incubation, and apparent equilibrium between the solution and solid phase was achieved within 5 d. When interpreted strictly in terms of sorption processes, our results corroborate previous reports showing less U(VI) sorption to hematite than goethite per unit surface (Hsi and Langmuir, 1985). As a different concentration of U(VI) was used for the ferrihydrite treatments, a direct comparison of sorption with that for hematite and goethite is not possible. However, since a lower initial concentration of U(VI) was required to achieve roughly the same bulk equilibrium concentration as with the crystalline minerals, a qualitative interpretation suggests that sorption of U(VI) per unit surface of ferrihydrite was less than that of hematite and goethite.

Mineral-free abiotic treatments showed no evidence for U precipitation. These observations were consistent with the results of Fredrickson et al. (2000), who showed that in 30 mM PIPES buffer (pH 7), 125 μM U(VI) remained in solution with a computed $U(VI)_{aq}$ equilibrium speciation that was dominated by the hydroxo complexes $UO_2OH^+_{(aq)}$ or $UO_2(OH)_{2(aq)}$. Slight decreases in U(VI) concentration in the absence of minerals likely stemmed from adsorption of U(VI) to the serum bottles (Arnold et al., 1998; Franklin et al., 2000).

4.2. G20-Mediated Reduction of U(VI) in the Presence of Fe(III)-(hydr)oxides

4.2.1. Lag Times

In the absence of minerals, 90 μM soluble U(VI) increased the lag times of *D. desulfuricans* significantly ($p < 7.9 \times 10^{-6}$) compared to U(VI)-free control. In each set of experiments, the duplicate treatment profiles were similar in total cell protein and U(VI); however, the length of the lag time was somewhat variable. Similar variability in lag times of *D. desulfuricans* among different experiments has been observed for metals such as Cu(II), Ni(II), and Zn(II) (Poulson et al., 1997; Sani et al., 2001a,b, 2003). The increase in lag time may be the result of U(VI) toxicity to *D. desulfuricans*. Other investigators studying U reduction in 30 mM bicarbonate have not observed a lag time (Caccavo et al., 1992; Lovley et al., 1993; Truex et al., 1997). Under these conditions, U(VI) was almost entirely complexed in the $UO_2(CO_3)^{2-}_2$ and $UO_2(CO_3)^{4-}_3$ forms (Fredrickson et al., 2000), and would likely have been less toxic to target microorganisms. Spear et al. (1999) reported that the lag-time before enzymatic U(VI) reduction occurred was inversely correlated to cell concentration. They hypothesized that the transfer of a small inoculum volume into a large medium volume can cause the cells to lose vitamins and cofactors into the bulk medium and thus reduce the concentration of critical reactants required for a threshold value. However, they observed lag times on the order of 4 h in contrast to 21 d observed in the present study. To help resolve these issues, further studies are ongoing in our laboratory.

As in the mineral-free U(VI) treatments, average lag times of *D. desulfuricans* obtained from four sets of experiments in the presence of hematite, goethite, and ferrihydrite were 20 $\pm$ 6, 21 $\pm$ 6, and 6 $\pm$ 4 d, respectively. As compared to U(VI)-free controls, lag times of *D. desulfuricans* in SLM and LLM increased significantly due to the presence of U(VI) with *p*-values of 0.00033, 0.00033, and 0.008431, respectively. Although aqueous phase U(VI) concentrations after abiotic sorption were approximately equal, the shorter lag times obtained with ferrihydrite may have resulted from an initial U(VI) concentration of 110 μM compared to 140 μM with hematite and goethite.

4.2.2. Reaction Stoichiometry

Under both sulfate- and lactate-limited conditions, concomitant U(VI) and sulfate reduction occurred in the presence of G20. The observed U(VI) reduction may be the result of enzymatic activity (Lovley et al., 1991), reaction with biogenic sulfide (Eqns. 1 and 2), or both. It has been reported that in the presence of bicarbonate buffer, sulfide cannot reduce U(VI)

Table 1. Reactions involving reduction of U(VI), SO_4^{2-}, and Fe(III), and re-oxidation of UO_2 by hematite.

$8\ UO_2OH^+ + 8\ HS^- \rightarrow 8\ UO_{2(S)} + S_{8(s)} + 8\ H_2O$	(1)
$8\ UO_2(OH)_2 + 8\ HS^- + 8\ H^+ \rightarrow 8\ UO_{2(S)} + S_{8(s)} + 16\ H_2O$	(2)
$2\ CH_3CHOHCOO^- + SO_4^{2-} + H^+ \rightarrow 2\ CH_3COO^- + 2\ CO_2 + 2H_2O + HS^-$	(3)
$8\ Fe_2O_3(s) + 8\ HS^- + 40\ H^+ \rightarrow 16\ Fe^{2+} + S_{8(s)} + 24\ H_2O$	(4)
$16\ FeOOH(s) + 8\ HS^- + 40\ H^+ \rightarrow 16\ Fe^{2+}{}_{(aq)} + S_{8(s)} + 32\ H_2O$	(5)
$4\ Fe_5O_74H_2O(s) + 8\ HS^- + 52\ H^+ + 4\ e^- \rightarrow 20\ Fe^{2+} + S_{8(s)} + 48\ H_2O$	(6)
$Fe_2O_3(s) + 6\ H^+ + 2\ e^- \rightarrow 2\ Fe^{2+} + 3\ H_2O$	(7)
$2\ FeOOH(s) + 6\ H^+ + 2\ e^- \rightarrow 2\ Fe^{2+} + 4\ H_2O$	(8)
$Fe_5O_7OH{\cdot}4H_2O_{(s)} + 15\ H^+ + 5\ e^- \rightarrow 5\ Fe^{2+} + 12\ H_2O$	(9)
$UO_{2(s)} + Fe_2O_{3(s)} + 6\ H^+ \rightarrow UO_2^{2+} + 2\ Fe^{2+} + 3\ H_2O$	(10)

(Lovley et al., 1991). We have shown, however, that in the absence of bicarbonate buffer, sulfide can reduce U(VI) (unpublished data). Therefore, once growth began, where sulfide and carbon dioxide were produced together, it was difficult to determine whether U(VI) reduction was enzymatic or sulfide-mediated. Our results showed that sulfate up to 30 mM had no effect on U(VI) reduction, and U(VI) additions up to 140 μM did not influence the rate of sulfate reduction. Similar results with *D. desulfuricans* were observed in the absence of a mineral phase under nongrowth conditions by Ganesh et al. (1999) and under growth conditions by Tucker et al. (1996). Spear et al. (2000) also showed that under nongrowth conditions (10 mM lactate in 20 mM sodium bicarbonate), *D. desulfuricans* reduced U(VI) and sulfate simultaneously and exhibited faster U(VI) reduction in the presence of sulfate. Recently, Senko et al. (2002), using mixed culture sediment amendments, reported that U(VI) and sulfate reduction occurred concomitantly in situ as well as in laboratory incubations.

After growth, the molar lactate/sulfate utilization ratio averaged 2.08 $\pm$ 0.08, in close agreement with the theoretical value of 2 shown in Eqn. 3 (Table 1) (Thauer et al., 1977). Similarly, the molar lactate consumption to acetate production ratio averaged 0.98 $\pm$ 0.03, in excellent agreement with the theoretical value of 1 from Eqn. 3. These results clearly suggest that sulfate reduction proceeded according to Eqn. 3 in the presence of Fe(III)-(hydr)oxides, and that most of the Fe(III) was reduced indirectly by hydrogen sulfide (Eqns. 4–6; Table 1) (Pyzik and Sommer, 1981; Neal et al., 2001). Although G20 can enzymatically reduce Fe(III) (Robert and Berthelin, 1986; Coleman et al., 1993; Le Gall et al., 1994) (Eqns. 7–9; Table 1), such activity appeared to be very low in these tests. From a thermodynamic standpoint (Francis et al., 1994; Amonette, 2003) and due to its much greater solubility and thus, bioavailability, soluble U(VI) and sulfate should be reduced preferentially over solid-phase Fe(III).

The initial concentrations of solid-phase Fe(III) in the hematite, goethite, and ferrihydrite treatments were 14.4, 7.5, and 2 mM, respectively (Table 2). If the Fe sulfide phase formed is assumed to be greigite (Fe_3S_4; Herbert et al., 1998), 1.33 mol of sulfide would be consumed for each mole of Fe(III), leading to a maximum theoretical consumption of 19.2, 10.0, and 2.7 mM sulfide by reaction with hematite, goethite, and ferrihydrite, respectively. From the final concentrations of sulfate and the stoichiometry of Eqn. 3, the theoretical maximum concentration of sulfide, assuming no reaction with Fe(III), should be 9 mM in the SLM and 19, 19, and 14 mM in the LML with hematite, goethite, and ferrihydrite, respectively. Thus one would predict that all the sulfide would be consumed in the treatments for which available Fe(III) exceeded sulfide production, and that measurable amounts of sulfide would remain in those treatments for which potential sulfide production exceeded available Fe(III).

Table 2 shows that the final measured soluble sulfide concentration in the SLM was 5.65 mM for the ferrihydrite treatment, suggesting that ~3.35 mM sulfide had been consumed by reaction with Fe(III) (consumption of 2.7 mM was predicted). Low levels of sulfide were measured in the hematite and goethite sulfate-limited treatments, as expected from the available Fe(III) concentrations being greater than 9 mM (Table 2). Similarly, in the lactate-limited treatments, only the goethite and ferrihydrite treatments showed large amounts of sulfide, as the amount of Fe(III) in the hematite treatment was still slightly greater than the amount of sulfide that could be generated. These results thus support the lactate/acetate stoichiometric data suggesting that Fe(III) reduction occurred primarily by reaction with biogenic sulfide (Eqns. 4–6) and that minimal enzymatic reduction of Fe(III) occurred (Eqns. 7–9). While these predictions are supported by the data shown in Table 2, it is known that the stoichiometry of Fe and S mineralization is rarely homogeneous. Neal et al. (2001), in a system with hematite and *D. desulfuricans* Essex 6, observed the presence of intermediate S species including S^{2-}, S_2^{2-}, and S_n^{2-}. Similar

Table 2. Initial concentrations of Fe(III) present in the solid phase and final soluble sulfide and Fe(II) concentrations in microcosms.

	Initial Fe(III) amount used (mM)	Sulfate-limited system		Lactate-limited system	
		Sulfide (mM)	Fe(II) (mM)	Sulfide (mM)	Fe(II) (mM)
Hematite	14.4	0.003	0.56	0.92	—[a]
Goethite	7.5	0.013	0.14	4.74	—[a]
Ferrihydrite	2	5.65	—[a]	6.92	—[a]

[a] Fe(II) concentrations were below the detection limit (0.11 μM).

S species may also have formed in our experiments, which would be an additional complication to the stoichiometric analysis presented above.

4.2.3. Remobilization of Solid-Phase U

After depletion of all lactate in the LLM, a slow and unexpected increase in soluble U(VI) concentration was observed. This phenomenon was greater in the hematite treatment than in the goethite treatment, and was completely absent in the ferrihydrite treatment. The slow increase in U(VI) concentration in the hematite and goethite treatments after depletion of lactate could be the result of one or more of the following processes: (i) an increase in soluble carbonate concentration stemming from G20 activity (Eqn. 3), leading to greater stability for U(VI)-carbonate complexes; (ii) release of U(VI) sorbed onto insoluble Fe(III) minerals when Fe(III) is reduced by biogenic H_2S (Lovley, 1995); and (iii) reoxidation of U(IV) to U(VI) by Fe(III) remaining in the system (Bruynesteyn, 1989; Panak et al., 1998; Nevin and Lovley, 2000) Eqn. 10 (Table 1). With respect to the first mechanism, it is known that carbonates in solution strongly decrease the sorption of U(VI) onto soil particles (Ho and Miller, 1986; Jung et al., 1999) through the formation of negatively charged uranyl-carbonate complexes such as $UO_2(CO_3)_2^{2-}$ (Dementeyev and Syromyatnikow, 1968) or $UO_2(CO_3)_2^{4-}$ (Giblin et al., 1981).

To test the first two mechanisms, we equilibrated goethite and hematite with LLM containing 145 μM U(VI) under anoxic and sterile conditions using the same serum bottles and volumes as previously used. After equilibration, serum bottles were pressurized by CO_2 at 137.8 kPa (20 lb/in^2) or amended with 10 mM Na_2S, and U(VI) concentrations in solution were measured over time (data not shown). In both instances, soluble U(VI) concentrations increased significantly within 2 d of the addition of CO_2 or Na_2S; however, with Na_2S treatment, soluble U(VI) concentrations again slightly decreased possibly due to U(VI) reduction by sulfide. These results suggested that in our system, CO_2 and Na_2S could mobilize sorbed U(VI) back into solution. However, ferrihydrite inoculated with G20 under lactate-limited conditions showed no increase in soluble U(VI) concentration even though CO_2 and sulfide were produced, suggesting that these two mechanisms, if operable, were not the only mechanisms at work.

The third mechanism [oxidation by Fe(III)] is consistent with the results obtained for the hematite and ferrihydrite treatments. Incomplete reduction of Fe(III) likely occurred in the hematite treatment and thus, Fe(III) reoxidation of U(IV) was possible. On the other hand, complete reduction of Fe(III) in the ferrihydrite seems likely given its high surface area, high reduction potential, and low concentration. With no Fe(III) available, no reoxidation of U(IV) would occur. With the goethite treatment, however, the data are in conflict. Reoxidation clearly occurred (Fig. 4), but high levels of sulfide were observed at the end of the experiment (Table 2), suggesting that no Fe(III) was available. It seems likely then that the first or second mechanisms were operational for the goethite treatment, and cannot be excluded in the hematite treatment either.

Nevin and Lovley (2000) showed that addition of U(VI) to bicarbonate-buffered cell suspensions of *Geobacter metallireducens* in the presence of synthetic poorly crystalline Fe(III) oxide stimulated the reduction of Fe(III) oxide. Their results suggested that under anaerobic bicarbonate-buffered conditions, reduced uranium [U(IV)] could act as an electron shuttle for synthetic, poorly crystalline Fe(III) oxide. Similar reactions could have occurred in our studies under sulfate reducing conditions.

4.3. Environmental Implications

The precipitation of U from contaminated waters as a result of microbial reduction of U(VI) to U(IV) is a potentially viable method for in situ plume stabilization and has possible advantages over other methods of U removal (Lovley et al., 1991; Gorby and Lovley, 1992). Our results have strong implications for field application of in situ biologic reduction of U(VI), since they suggest that complete reduction of all available Fe(III) may be required to avoid Fe(III) oxidation of reduced U in situ. Using data from MINTEQA2 and NBS databases (Wagman et al., 1982; USEPA, 1991; Grenthe et al., 1992), the calculated value of log K for the Eqn. 10 at pH 7.2, is 13.715, which suggests that even at low Fe(III) to Fe(II) ratios, the U(IV) oxidation reaction may proceed with unfavorable consequences for the reduced U.

In addition to complete reduction of Fe(III) to better stabilize reduced U, effective treatment of U from contaminated groundwater depends on the subsequent removal of reduced U from solution. In our experiments, as much as 98% U was reduced when hematite and ferrihydrite were present, and 88% was reduced in the presence of goethite. However, when filtered samples (0.2-μm membrane filter) were oxidized by acid treatment to determine the total U present, the amount of U measured increased by as much as 42% (data not shown) suggesting substantial amounts of dissolved or suspended reduced U were present in the filtrate. Similar observations have been reported previously (Gorby and Lovley, 1992; Ganesh et al., 1997; Robinson et al., 1998). Our results indicate that significant amounts of reduced U passed through the 0.2-μm membrane filter, either as soluble complexes with organic ligands or as nm-sized particles. We favor the latter explanation, based on the interpretation of the XANES spectra, which indicated the presence of nm-sized uraninite particles in reduced samples. Recently, Suzuki et al. (2002) reported that uraninite particles formed in sediments by bacterial reduction were typically less than 2 nm across. Taken together, our results suggest that if lactate were injected to stimulate SRB activity in the subsurface, reduction of U(VI) would likely depend not only on the levels of electron donor and electron acceptor, but also on the type of redox-sensitive Fe(III)-(hydr)oxides present.

5. CONCLUSIONS

Our results show that sulfate and U(VI) were reduced concomitantly in the presence of G20; however, in lactate-limited systems, partial reoxidation of U(VI) was observed after depletion of all lactate. Results indicate that after depletion of all electron donor, some of the Fe(III), which was not reduced either enzymatically or with biogenic H_2S, may have been responsible for oxidizing reduced U to U(VI) in solution. Under lactate-limited conditions, reoxidation was dependent on the concentration of Fe(III) and type of Fe(III)-(hydr)oxide. These

results suggest that complete reduction of all available Fe(III) may be required to avoid Fe(III) oxidation of reduced U. Analysis by U L_3-edge XANES spectroscopy of the G20-promoted reduced-U mineral precipitates yielded spectra consistent with the formation of nanometer-sized particles. It appears that U(VI) in ground and surface waters containing U(VI) can be successfully reduced using G20, but that care must be taken to account for the complexity of the biogeochemical interactions that may occur. The small size of the U particles may contribute to their reactivity in the presence of Fe(III) when electron donor becomes limiting. These factors are likely to influence the stability of U reduced and precipitated by SRB in surface and subsurface environments.

Acknowledgments—The authors gratefully acknowledge the financial support provided by the Natural and Accelerated Bioremediation Research program (NABIR), Office of Biologic and Environmental Research, U.S. Department of Energy (DOE), USA (Grants DE-FG03-98ER62630/A001 and DE-FG03-01ER63270). The support of the Center for Multiphase Environmental Research and the Department of Chemical Engineering also contributed significantly to this research. The Pacific Northwest National Laboratory is operated for DOE by Battelle Memorial Institute under contract DE-AC06-76RL0 1830. This research was also supported by Inland Northwest Research Alliance (grant no. MSU003). Finally, the authors wish to thank the four anonymous reviewers for their insightful review comments.

Associate editor: C. M. Eggleston

REFERENCES

Abdelouas A., Lu Y., Lutze W., and Nuttall H. E. (1998) Reduction of U(VI) to U(IV) by indigenous bacteria in contaminated ground water. *J. Contam. Hydrol.* **35,** 217–233.

Abdelouas A., Lutze W., Gong W., Nuttall H. E., Strietelmeier B. A., and Travis B. J. (2000) Biological reduction of uranium in groundwater and subsurface soil. *Sci. Total Environ.* **250,** 21–35.

Amonette J. E. (2003) Iron redox chemistry of clays and oxides: Environmental applications. *In Electrochemical Properties of Clays, CMS Workshop Lectures,* Vol. 10 (ed. A. Fitch), pp. 89–146. The Clay Minerals Society.

Anderson R. F. (1984) A method for determining the oxidation state of uranium in natural waters. *Nucl. Inst. Phys. Res.* **223,** 213–217.

Arnold T., Zorn T., Bernhard H., and Nitsche H. (1998) Sorption of U(VI) onto phyllite. *Chem. Geol.* **151,** 129–141.

Attia Y. A. and El-Zeky M. (1990) Effects of galvanic interactions of sulfides on extraction of precious metals from refractory complex sulfides by biobleaching. *Int. J. Mineral Proc.* **30,** 99–111.

Barnes S. P., Bradbrook S. D., Cragg B. A., Marchesi J. R., Weightam A. J., Fry J. C., and Parkes R. J. (1998) Isolation of sulfate-reducing bacteria from deep sediment layers of the Pacific Ocean. *Geomicrobiol. J.* **15,** 67–84.

Berner R. A. (1984) Sedimentary pyrite formation: An update. *Geochim. Cosmochim. Acta* **48,** 605–615.

Bhattacharya D., Jumawan A. B., Sun G., Sund-Hegelburg C., and Schwitzgebel K. (1981) Precipitation of heavy metals with sodium sulfide: Bench-scale and full-scale experimental results. *AIChE Symp. Ser.* **209,** 31–38.

Brina R. and Miller A. G. (1992) Direct detection of trace levels of uranium by laser induced kinetic phosphoremeter. *Anal. Chem.* **64,** 1415–1418.

Bruynesteyn A. (1989) Mineral biotechnology. *J. Biotechnol.* **11,** 1–10.

Caccavo F., Blakemore R. P., and Lovley D. R. (1992) A hydrogen oxidizing, Fe(III)-reducing microorganism from the Great Bay Estuary, New Hampshire. *Appl. Environ. Microbiol.* **58,** 3211–3216.

Coleman M. D., Hedrick D. B., Lovley D. R., White D. C., and Pye K. (1993) Reduction of Fe(III) in sediments by sulfate-reducing bacteria. *Nature* **36,** 436–438.

Dementeyev V. S. and Syromyatnikow N. G. (1968) Conditions of formation of a sorption barrier to the migration of uranium in an oxidizing environment. *Geokhimiya* **4,** 459–465.

Francis A. J., Dodge C. J., Lu F., Halada G. P., and Clayton C. R. (1994) XPS and XANES studies of uranium reduction by *Clostridium* sp. *Environ. Sci. Technol.* **28,** 636–639.

Franklin N. M., Stauber J. L., Markich S. J., and Lim R. P. (2000) pH-dependent toxicity of copper and uranium to a tropical freshwater alga (*Chlorella* sp.). *Aquat. Toxicol.* **48,** 275–289.

Fredrickson J. K. and Gorby Y. A. (1996) Environmental processes mediated by iron-reducing bacteria. *Curr. Opin. Biotechnol.* **7,** 287–294.

Fredrickson J. K., Zachara J. M., Kennedy D. W., Dong H., Onstott T. C., Hinman N. W., and Li S. (1998) Biogenic iron mineralization accompanying the dissimilatory reduction of hydrous ferric oxide by a groundwater bacterium. *Geochim. Cosmochim. Acta* **62,** 3239–3257.

Fredrickson J. K., Zachara J. M., Kennedy D. W., Duff M. C., Gorby Y. A., Li S. W., and Krupka K. M. (2000) Reduction of U(VI) in goethite (α-FeOOH) suspensions by a dissimilatory metal-reducing bacterium. *Geochim. Cosmochim. Acta* **64,** 3085–3098.

Ganesh R., Robinson K. G., Reed G. D., and Sayler G. (1997) Reduction of hexavalent uranium from organic complexes by sulfate- and iron-reducing bacteria. *Appl. Environ. Microbiol.* **63,** 4385–4391.

Ganesh R., Robinson K. G., Chu L., Kucsmas D., and Reed G. D. (1999) Reductive precipitation of uranium by *Desulfovibrio desulfuricans*: Evaluation of co-contaminant effects and selective removal. *Water Res.* **33,** 3447–3458.

Giblin A. M., Batta B. D., and Swaine D. J. (1981) Laboratory simulation studies of uranium mobility in natural waters. *Geochim. Cosmochim. Acta* **45,** 699–709.

Gorby Y. A. and Lovley D. R. (1992) Enzymatic uranium precipitation. *Environ. Sci. Technol.* **26,** 205–207.

Gregg S. J. and Sing K. S. W. (1982) Adsorption, Surface Area and Porosity. Academic Press.

Grenthe I., Fuger J., Konings R. J. M., Lemire R. J., Muller A. B., Nguyen-Trung C., and Wanner H. (1992) *Chemical Thermodynamics of Uranium.* Chemical Thermodynamics Series, Vol. 1. Elsevier Science.

Herbert R. B., Benner S. G., Pratt A. R., and Blowes D. W. (1998) Surface chemistry and morphology of poorly crystalline iron sulfides precipitated in media containing sulfate-reducing bacteria. *Chem. Geol.* **144,** 87–97.

Ho C. H. and Miller N. H. (1986) Adsorption of uranyl species from bicarbonate solution onto hematite particles. *J. Coll. Interf. Sci.* **110,** 165–171.

Holmes P. R. and Crundwell F. K. (1995) Kinetic aspects of galvanic interactions between minerals during dissolution. *Hydrometallurgy* **39,** 353–375.

Hsi C.-K. D. and Langmuir D. (1985) Adsorption of uranyl onto ferric oxyhydroxides: Application of the surface complexation site-binding model. *Geochim. Cosmochim. Acta* **49,** 1931–1941.

Hudson E. A., Rehr J. J., and Bucher J. J. (1995) Multiple-scattering calculations of the uranium L(3)-edge X-ray-absorption near-edge structure. *Phys. Rev. B* **52,** 13815–13826.

Jung J., Hyun S. P., Lee J. K., Cho Y. H., and Hahn P. S. (1999) Adsorption of UO_2^{2+} on natural composite materials. *J. Radioanal. Nucl. Chem.* **242,** 405–412.

Langmuir D. (1978) Uranium solution-mineral equilibria at low temperatures with applications to sedimentary ore deposits. *Geochim. Cosmochim. Acta* **42,** 547–569.

Le Gall J., Payne W. J., Chen L., Liu M. Y., and Xavier A. V. (1994) Localization and specificity of cytochromes and other electron transfer proteins from sulfate-reducing bacteria. *Biochimie* **76,** 655–665.

Lloyd J. R. and Lovley D. R. (2001) Microbial detoxification of metals and radionuclides. *Curr. Opinion. Biotechnol.* **12,** 248–253.

Lovley D. R. (1993) Dissimilatory metal reduction. *Annu. Rev. Microbiol.* **47,** 263–90.

Lovley D. R. (1995) Microbial reduction of iron, manganese, and other metals. *Adv. Agron.* **54,** 175–231.

Lovley D. R., Phillips E. J. P., Gorby Y. A., and Landa E. R. (1991) Microbial reduction of uranium. *Nature* **350,** 413–416.

Lovley D. R. and Phillips E. J. P. (1992a) Bioremediation of uranium contamination with enzymatic uranium reduction. *Environ. Sci. Technol.* **26,** 2228–2234.

Lovley D. R. and Phillips E. J. P. (1992b) Reduction of uranium by *Desulfovibrio desulfuricans*. *Appl. Environ. Microbiol.* **58,** 850–856.

Lovley D. R., Widman P., Woodward J., and Phillips E. J. P. (1993) Reduction of uranium by cytochrome c3 of *Desulfovibrio vulgaris*. *Appl. Environ. Microbiol.* **59,** 3572–3576.

Lovley D. R. and Coates J. D. (1997) Bioremediation of metal contamination. *Curr. Opin. Biotechnol.* **8,** 285–289.

McCullough J., Hazen T. C., Benson S. M., Metting F. B., and Palmisano A. C. (1999) Bioremediation of Metals and Radionuclides. . .What It Is and How It Works: A NABIR Primer. U.S. Department of Energy, LBNL-42595. Lawrence Berkeley National Laboratory, Berkeley, CA.

Nevin K. P. and Lovley D. R. (2000) Potential for nonenzymatic reduction of Fe(III) via electron shuttling in subsurface sediments. *Environ. Sci. Technol.* **34,** 2472–2478.

Neal A. L., Techkarnjanaruk S., Dohnalkova A., McCready D., Peyton B. M., and Geesey G. G. (2001) Iron sulfides and sulfur species produced at hematite surfaces in the presence of sulfate-reducing bacteria. *Geochim. Cosmochim. Acta* **65,** 223–235.

Panak P., Hard B. C., Pietzsch K., Kutschke S., Röske K., Selenska-Pobell S., Bernhard G., and Nitsche H. (1998) Bacteria from uranium mining waste pile: Interactions with U(VI). *J. Alloys Comp.* **271-273,** 262–266.

Parks G. A. and Pohl D. C. (1988) Hydrothermal solubility of uraninite. *Geochim. Cosmochim. Acta* **52,** 863–875.

Phillips E. J. P., Landa E. R., and Lovley D. R. (1995) Remediation of uranium contaminated soils with bicarbonate extraction and microbial U(VI) reduction. *J. Indus. Microbiol.* **14,** 203–207.

Poulson S. R., Colberg P. J. S., and Drever J. I. (1997) Toxicity of heavy metals (Ni, Zn) to *Desulfovibrio desulfuricans*. *Geomicrobiol. J.* **14,** 41–49.

Pyzik A. J. and Sommer S. E. (1981) Sedimentary iron monosulfides: Kinetics and mechanism of formation. *Geochim. Cosmochim. Acta* **45,** 687–698.

Riley R. G., Zachara J. M. and Wobber F. J. (1992) *Chemical Contaminants on DOE Lands and Selection of Contaminant Mixtures for Subsurface Research.* DOE/ER-0547T. U.S. Department of Energy.

Robert M. and Berthelin J. (1986) *Role of biological and biochemical factors in soil mineral weathering* (eds. P. M. Huang and M. Schnitzer), pp. 453–495. Soil Science Society of America Special Publication 17.

Robinson K. G., Ganesh R., and Reed G. (1998) Impact of organic ligands on uranium removal during anaerobic biological treatment. *Water Sci. Technol.* **37,** 73–80.

Sani R. K., Geesey G., and Peyton B. M. (2001a) Assessment of lead toxicity to *Desulfovibrio desulfuricans* G20: Influence of components of lactate C medium. *Adv. Environ. Res.* **5,** 269–276.

Sani R. K., Peyton B. M., and Brown L. T. (2001b) Copper-induced inhibition in Growth of *Desulfovibrio desulfuricans* G20: Assessment of its toxicity and correlation with zinc and lead. *Appl. Environ. Microbiol.* **67,** 4765–4772.

Sani R. K., Peyton B. M., Smith W. A., Apel W. A., and Petersen J. N. (2002) Dissimilatory reduction of Cr(VI), Fe(III), and U(VI) by *Cellulomonas* isolates. *Appl. Microbiol. Biotechnol.* **60,** 192–199.

Sani R. K., Peyton B. M., and Jandhyala M. (2003) Toxicity of lead in aqueous medium to *Desulfovibrio desulfuricans* G20. *Environ. Toxicol. Chem.* **22,** 252–260.

Schwertmann U. and Cornell R. M. (1991) Iron Oxides in the Laboratory: Preparation and Characterization. VSH.

Senko J. M., Istok J. D., Suflita J. M., and Krumholz L. R. (2002) In-situ evidence for uranium immobilization and remobilization. *Environ. Sci. Technol.* **36,** 1491–1496.

Spear J. R., Figueroa L. A., and Honeyman B. D. (1999) Modeling the removal of uranium U(VI) from aqueous solutions in the presence of sulfate-reducing bacteria. *Environ. Sci. Technol.* **33,** 2667–2675.

Spear J. R., Figueroa L. A., and Honeyman B. D. (2000) Modeling reduction of uranium U(VI) under variable sulfate concentrations by sulfate-reducing bacteria. *Appl. Environ. Microbiol.* **66,** 3711–3721.

Suzuki Y., Kelly S. D., Kenneth M. K., and Banfield J. F. (2002) Nanometer-size products of uranium bioreduction. *Nature* **419,** 134.

Tebo B. M. and Obraztsova A. Y. (1998) Sulfate-reducing bacterium grows with Cr(VI), U(VI), Mn(IV) and Fe(III) as electron acceptors. *FEMS Microbiol. Lett.* **162,** 193–198.

Thauer R. K., Jungerman K., and Decker K. (1977) Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev.* **41,** 100–180.

Truex M. J., Peyton B. M., Valentine N. B., and Gorby Y. A. (1997) Kinetics of U(VI) reduction by a dissimilatory Fe(III)-reducing bacterium under non-growth conditions. *Biotechnol. Bioengin.* **55,** 490–496.

Tucker M. D., Barton L. L., and Thomson B. M. (1996) Kinetic coefficients for simultaneous reduction of sulfate and uranium by *Desulfovibrio desulfuricans*. *Appl. Microbiol. Biotechnol.* **46,** 74–77.

USEPA. (1991) *Equilibrium Metal Speciation Model (MINTEQA2) Version 3.11*. Center for Exposure Assessment Modeling. U.S. Environmental Protection Agency.

Wagman D. E., Evans W. H., Parker V. B., Schumm R. H., Halow I., Bailey S. M., Churney K. L. and Nuttall R. L. (1982) The NBS tables of chemical thermodynamic properties. Selected values for inorganic and C1 and C2 organic substances in SI units. *J. Phys. Chem. Ref. Data* **11**(Suppl. 2), 1–392.

Wall J. D., Rapp-Giles B. J., and Rousset M. (1993) Characterization of a small plasmid from *Desulfovibrio desulfuricans* and its use for shuttle vector construction. *J Bacteriol.* **175,** 4121–4128.

Zachara J. M., Fredrickson J. K., Smith S. C., and Gassman P. L. (2001) Solubilization of Fe(III) oxide-bound trace metals by a dissimilatory Fe(III) reducing bacterium. *Geochim. Cosmochim. Acta* **65,** 75–93.