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Immobilization of Radionuclides and Heavy Metals through Anaerobic Bio-Oxidation of Fe(II)

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Adsorption of heavy metals and radionuclides (HMR) onto iron and manganese oxides has long been recognized as an important reaction for the immobilization of these compounds. However, in environments containing elevated concentrations of these HMR the adsorptive capacity of the iron and manganese oxides may well be exceeded, and the HMR can migrate as soluble compounds in aqueous systems. Here we demonstrate the potential of a bioremediative strategy for HMR stabilization in reducing environments based on the recently described anaerobic nitrate-dependent Fe(II) oxidation by *Dechlorosoma* species. Bio-oxidation of 10 mM Fe(II) and precipitation of Fe(III) oxides by these organisms resulted in rapid adsorption and removal of 55 μM uranium and 81 μM cobalt from solution. The adsorptive capacity of the biogenic Fe(III) oxides was lower than that of abiotically produced Fe(III) oxides (100 μM for both metals), which may have been a result of steric hindrance by the microbial cells on the iron oxide surfaces. The binding capacity of the biogenic oxides for different heavy metals was indirectly correlated to the atomic radius of the bound element. X-ray absorption spectroscopy indicated that the uranium was bound to the biogenically produced Fe(III) oxides as U(VI) and that the U(VI) formed bidentate and tridentate inner-sphere complexes with the Fe(III) oxide surfaces. *Dechlorosoma suillum* oxidation was specific for Fe(II), and the organism did not enzymatically oxidize U(IV) or Co(II). Small amounts (less than 2.5 μM) of Cr(III) were reoxidized by *D. suillum*; however, this appeared to be inversely dependent on the initial concentration of the Cr(III). The results of this study demonstrate the potential of this novel approach for stabilization and immobilization of HMR in the environment.

The mobility of trace metals and radionuclides released into aquatic and terrestrial environments by mining, industrial processes, and municipal waste disposal practices is an area that deserves significant scientific, public health, and regulatory attention. The U.S. Environmental Protection Agency includes cadmium, chromium, copper, lead, mercury, nickel, silver, and zinc on its priority pollutant list for waste effluents. Geochemical controls that regulate the trace element concentrations in oxic natural waters include adsorption and coprecipitation by hydrous oxides of iron and manganese. These hydrous oxides occur as discrete grains and as coatings on aquifer materials. They have been shown to be the major host minerals for many trace elements in soils and for ^{60}Co and isotopes of plutonium and americium in soils and sediments of a disposal area at Oak Ridge National Laboratory (27).

Adsorption of heavy metals and radionuclides (HMR) onto iron and manganese oxides has long been recognized as an important reaction for immobilization of these compounds (2, 4, 28, 33, 45, 46, 47, 51, 52). However, the adsorptive capacity of the hydrous oxides in some environments may not be sufficient to immobilize all of the HMR present. Many studies have investigated the bioremediative potential of stimulating reducing bacteria to use some of the soluble HMR as electron acceptors and thus precipitate them out of solution (10, 35, 42, 43). However, there are many unknowns and potential limitations for this technique, including (i) the fate of the reduced immobilized HMR once the bioremediative process is complete and the environment reverts back to an oxic state; (ii) the potential for bio-oxidation and subsequent resolubilization of the reduced immobilized HMR; (iii) the fact that reductive remediation may not be suitable for low-level, long-term contamination as there may be insufficient HMR contaminants available to support a metal-reducing microbial community; and (iv) the fact that many metals are bound and solubilized by natural and anthropogenic organic matter present in most environments regardless of their valence state (12, 16, 50, 59).

An alternative to bioreduction is selective anaerobic bio-oxidation of added Fe(II) under anoxic conditions. This technique, if successful, should result in immobilization of contaminating HMR on newly formed Fe(III) oxides. Anaerobic bio-oxidation of Fe(II) was only recently identified, and very little is known regarding the ubiquity and diversity of organisms capable of this metabolism. Previous studies have shown that Fe(II) oxidation is mediated by anoxygenic phototrophs (32, 61), as well as various nitrate-respiring organisms (8) and perchlorate-respiring organisms (11, 15, 20, 38, 48). The end product of this metabolism is generally amorphous Fe(III) oxide (8, 11, 57, 61). Amorphous Fe(III) oxide [$Fe_2O_3 \cdot H_2O$(am)], or ferrihydrite, has often been used for studies of adsorption of trace metals because it is a uniform material with well-known surface properties that is easily reproduced (2). It is also representative of metal oxides in the natural environment and is a

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precursor of many natural forms of crystalline Fe(III) oxides, such as goethite and hematite (22, 53, 63). Previous studies have shown that metals such as cobalt, chromium, cadmium, lead, uranium, and radium are rapidly adsorbed by this iron form (2, 4, 44, 51), and some of these metals with lower ionic radii (e.g., Co^{2+} and Cd^{2+}) are incorporated into the Fe(III) oxide structure as the amorphous Fe(III) oxides begin to recrystallize with age. Under these conditions these trace metals become tightly bound in the Fe(III) oxide crystals (2) and are thus immobilized.

As part of a study on the metabolic diversity of organisms capable of growth by anaerobic respiration of perchlorate, we isolated a novel organism, *Dechlorosoma suillum* strain PS, from a swine waste lagoon (1, 20, 48). Physiological characterization revealed that *D. suillum* rapidly oxidized Fe(II) with nitrate or chlorate as the electron acceptor under strictly anaerobic conditions (15, 38, 48). Recently, we demonstrated that Fe(II) oxidation by *D. suillum* resulted in the formation of different end products depending on the rate of Fe(II) oxidation (15, 38). In cell suspension experiments, Fe(II) was rapidly oxidized to an amorphous ferrihydrite similar to that formed by other previously described nitrate-dependent Fe(II) oxidizers (38); however, under growth conditions with acetate as a cosubstrate and nitrate as the electron acceptor, Fe(II) oxidation resulted in the production of a broad range of crystalline iron minerals, including magnetite, which accounted for as much as 25% of the original Fe(II) in the culture under the growth conditions tested (15). Here we report on the bioremediation potential of this metabolism for immobilization of HMR in reducing environments as a result of engineered anaerobic bio-oxidation.

MATERIALS AND METHODS

Medium and culture conditions. *D. suillum* strain PS was maintained in an anoxic, defined freshwater medium described previously (11) with acetate (10 mM) as the sole electron donor and chlorate (10 mM) or nitrate (10 mM) as the sole electron acceptor. Standard anaerobic techniques were used throughout this study (34). Anoxic medium (pH 6.8) was prepared by boiling the medium to remove dissolved O_2 before it was dispensed under an N_2-CO_2 (80:20, vol/vol) gas phase into anaerobic pressure tubes or serum bottles that were sealed with thick butyl rubber stoppers.

Alternative electron donors and acceptors were added from sterile stock solutions. Chloride salts of the metals cadmium ($CdCl_2$), cobalt ($CoCl_2$), and uranium (UCl_2O_2) were also added from sterile stock solutions. Reduced U(IV) was produced in culture medium by amending freshly prepared anoxic basal medium with an anoxic uranyl chloride stock to give the desired final U(IV) concentration. The medium was further amended with palladium-coated aluminum chips and gassed out with H_2 to abiotically reduce the UO_2Cl_2. Once the uranium was reduced, the headspace gas was replaced with N_2-CO_2 and the aluminum chips were removed by decantation.

Cell suspension preparation. Cells of *D. suillum* strain PS were grown anaerobically in 500-ml volumes of medium with acetate (10 mM) as the electron donor and chlorate or nitrate (10 mM) as the electron acceptor. After dense growth of *D. suillum*, cells were harvested by centrifugation at 4°C under an N_2-CO_2 headspace. The cell pellets were each washed twice and resuspended in 1 ml of anoxic bicarbonate buffer (2.5 g $liter^{-1}$, pH 6.8) and sealed in a 10-ml serum vial with a thick butyl rubber stopper under an N_2-CO_2 headspace.

Analytical techniques. Acetate concentrations were analyzed by high-performance liquid chromatography with UV detection at 210 nm (model SPD-10A; Shimadzu Scientific Instruments, Columbia, Md.) by using an HL-75H$^+$ cation-exchange column (catalog no. 79476; Hamilton Company, Reno, Nev.). The eluent was 0.016 N H_2SO_4 at a flow rate of 0.4 ml min^{-1}. Chlorate, chloride, nitrate, and nitrite concentrations were analyzed by ion chromatography with conductivity detection (model CDD-6A; Shimadzu Scientific Instruments) by using IonPac AS9-HC with suppressed conductivity by ASRS-II in a recycle mode (catalog no. 51786; Dionex Corporation, Sunnyvale, Calif.). The eluent was 9 mM sodium carbonate at a flow rate of 1.0 ml min^{-1}. Growth of cultures on soluble electron acceptors was determined by direct cell counting and by monitoring the increase in optical density at 600 nm. Concentrations of HCl-extractable Fe(II) were determined colorimetrically by the ferrozine assay at 562 nm (41). For insoluble Fe(II) minerals, the total Fe(II) content was determined by extraction for 24 h in 5 N HCl prior to analysis with the ferrozine assay.

Soluble concentrations of U(VI) were determined by using reverse-phase chromatography coupled to postcolumn derivatization with the dye Arsenazo III. This method is a modification of the method first described by Barkley et al. (7). A detection limit of 0.50 μM can be achieved with this technique. A Dionex DX500 instrument equipped with a Supelcosil LC-18 column (150 by 450 mm), an absorbance detector set to a wavelength of 658 nm, and a pneumatic unit for delivery of the postcolumn reagent was used. The chromatographic conditions included a mobile phase of 85% 0.20 M hydroxyisobutyric acid (pH 4.0) and 15% methanol at a flow rate of 1.4 ml · min^{-1} and 0.125 mM Arsenazo III as a postcolumn reagent at a flow rate of 0.60 ml·min^{-1}.

X-ray absorption spectroscopy. The biogenic Fe(III) oxides containing uranium were centrifuged, and the resulting wet paste was mounted in a hollowed-out Plexiglas (thickness, 1.5 mm) sample holder. Kapton film was used to contain the sample within the hollowed-out region of the Plexiglas holder and to allow penetration of the synchrotron radiation. The centrifuge tube was opened, and the material was mounted in the Plexiglas holder in an anaerobic glove box (Coy Laboratories) to maintain anoxic conditions. Uranium L-III edge fluorescence X-ray absorption fine-structure (XAFS) spectroscopy (37) measurements were obtained for the wet homogeneous paste. All XAFS measurements were made at the Materials Research Collaborative Access Team insertion device beamline (54) at the Advanced Photon Source, Argonne National Laboratories, Argonne, Ill. The energy of the incident X rays was selected by using Bragg reflection from two Si(III) crystals from the third harmonic of the beamline undulator. Higher-order harmonics were rejected by using an Rh mirror. The incident X-ray intensity was sampled by using an ion chamber filled with nitrogen gas, and the fluorescent X-ray intensity was sampled by using a Stern-Heald detector filled with free-flowing Ar gas at atmospheric pressure. An Sr filter of six absorption lengths was used to reduce the elastically scattered radiation contributing to the background signal. Linearity tests (36) indicated that there was less than 0.38% nonlinearity in the experimental setup for a 50% decrease in incident X-ray intensity. The incident X-ray intensity varied by less than 15% throughout the energy range of the XAFS measurements. The transmission XAFS signal of an yttrium-containing X-ray filter was used as an energy reference to accurately align the edge-energy positions of all data, as described elsewhere (23). Three energy scans were collected at six different locations on the sample to reduce radiation-induced chemical effects on the sample. The sample was exposed for approximately 1 min for each of the three measurements at each location. Measuring several spectra at each of the six sample locations allowed determination of radiation-induced chemical effects at the 1-min time scale. No time-dependent change was observed in the X-ray absorption near-edge spectra (XANES) data for any of the samples.

Three experimental U extended X-ray absorption fine-structure standards were measured: hydrated uranium ([U] = 500 ppm in double-distilled deionized water, pH 0.96), uranium in an acetic acid solution ([U] = 500 ppm in acetic acid [ratio of U to acetic acid, 1:100], pH 4.4), and a uranium phosphate solution ([U] = 500 ppm [ratio of U to P, 1:100], pH 1.5). In addition, to determine the average valence state of the U in a bio-oxidized sample, two U XANES powder standards (UO_2 and UO_3) were measured. The theoretical codes contained in the UWXAFS package (55) were used to analyze all data. The program FEFF7 (62) was used to construct the theoretical XAFS data on the basis of uranyl crystal structures, and the results are presented in Table 1. The results of a best-fit analysis of the experimentally obtained data with theoretically generated data indicated that the S02 best-fit values were 1.0 ± 0.2. The value for E0 was determined in the fit. For the standards, two different E0 values were used. The structural parameters determined in a fit to the U-containing biogenic Fe(III) oxide XAFS data included N_{degen} (coordination number), R (distance to the neighboring atoms for a single scattering path), and σ^2 (relative mean square displacement between the absorbing U atom and the neighboring atoms for a single scattering path). Fitting of the XAFS data for the U-containing biogenic Fe(III) oxides had three independent points and 12 variables. Error analysis and goodness-of-fit parameters were calculated with the fitting routine FEFFIT.

RESULTS AND DISCUSSION

Abiotic adsorption of HMR onto ferrihydrite. When the Fe(II) content of anoxic uninoculated basal culture medium

TABLE 1. Best-fit values for the XAFS data for the uranium-containing biogenic iron oxide sample[a]

Path	N_{degen}	R (Å)	σ^2 (10^{-3} Å^2)
U-Oax	2.0	1.797 ± 0.015	0.0 ± 0.2
U-Oeq	4.7 ± 4.5	2.379 ± 0.045	5.1 ± 15.3
U-Oeq	1.7 ± 0.7	2.161 ± 0.143	5.1 ± 15.3
U-Oax1–U-Oax1	2.0	3.594 ± 0.029	0.0 ± 0.7
U-Oax1–U-Oax2	2.0	3.594 ± 0.029	0.0 ± 0.7
U-Oax1–U-Oax2	2.0	3.594 ± 0.029	0.0 ± 0.7
U-Fe1	0.7 ± 0.2	2.921 ± 0.033	0.0 ± 15.4
U-Fe2	0.5 ± 0.4	3.521 ± 0.061	0.0 ± 15.4

[a] Path indicates the atom types in the scattering paths of the photoelectron that are included in the model of the experimental XAFS data. For example, U-Oax represents a single scattering path from a uranium atom to an axial oxygen atom and then back to the original uranium atom. U-Fe1 and U-Fe2 represent two unique single scattering paths with different distances between the U atom and the Fe1 or Fe2 atom. N_{degen} is the degeneracy of the specific path. For single scattering paths, the degeneracy is equal to the average number of atoms at the same average distance from the U atom, commonly referred to as the XAFS coordination number. R is one-half of the photoelectron path length described by the path. The R for a single scattering path is the average distance between the uranium atom and the backscattering atom. σ^2 is the relative mean square displacement about the equilibrium half-path length. σ^2 is commonly referred to as the XAFS Debye-Waller factor.

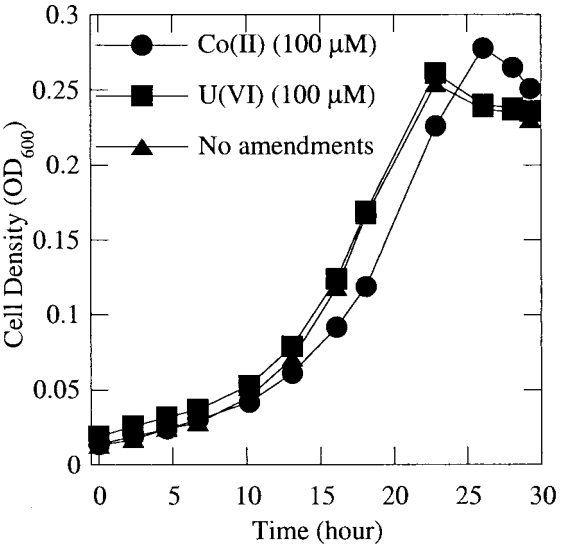

FIG. 2. Growth of *D. suillum* in the absence and in the presence of soluble uranium and cobalt. OD_{600}, optical density at 600 nm.

(11) was abiotically oxidized by a brief (1-min) exposure to air, an orange-brown precipitate, presumably amorphous ferric oxyhydroxide, rapidly formed within 1 h. Addition of Co(III) or U(VI) (100 μM) to the air-treated medium resulted in slow removal of the soluble metals from solution over a period of 120 days (Fig. 1). In contrast, if the anoxic Fe(II)-containing medium was exposed to oxygen after addition of the uranium or cobalt, the HMR were rapidly removed from solution, and complete removal was observed within 2 h (Fig. 1). Previous studies have similarly shown that metals such as cobalt, cadmium, lead, uranium, and radium are rapidly adsorbed by ferric iron mineral phases, especially amorphous ferric oxyhydroxide, and pulled from solution over time (2, 4, 51). In addition, some metals with lower ionic radii (e.g., Co^{2+} and Cd^{2+}) may be incorporated into the Fe(III) oxide structure as the amorphous Fe(III) oxides crystallize with age. These trace metals can become tightly bound into the Fe(III) oxide crystals (2) and thus become immobilized.

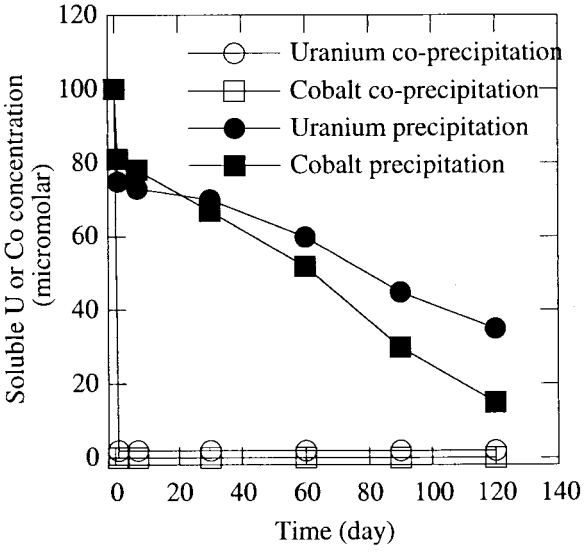

FIG. 1. Adsorption and precipitation of uranium and cobalt by Fe(III) oxides abiotically formed prior to and after addition of soluble U(VI) and Co(III).

Anoxic biological HMR immobilization. Addition of soluble U(VI) or Co(III) at a concentration of 100 μM had no effect on growth or on nitrate-dependent Fe(II) oxidation by *D. suillum* (Fig. 2). Similar to what was observed for abiotic oxidation of the Fe(II)-containing medium (see above), anaerobic biological oxidation of the Fe(II) by *D. suillum* in the presence of either Co(III) or U(VI) also resulted in rapid removal of the HMR from solution (Fig. 3). Interestingly, the removal of the U(VI) or Co(III) by the biogenically produced oxides was not as complete as the removal observed in the abiotic experiments, and only 55% of the initial 100 μM uranium was removed from solution. This difference in binding capacity between the abiotically produced Fe(III) oxides and the biogenic Fe(III) oxides may be the result of microbial cells bound to the reactive surfaces of the biogenically produced Fe(III) oxides, resulting in a decrease in available binding sites for HMR.

More cobalt (81% of the initial 100 μM) than uranium was removed from solution as a result of biological Fe(II) oxidation. When a similar experiment was performed with cadmium, 69% of the initial 100 μM was bound by the biogenically formed iron minerals. Comparison of the atomic radii of these ions (R_U = 1.75 Å, R_{Cd} = 1.55 Å, R_{Co} = 1.35 Å) to the atomic radius of Fe (1.4 Å) suggests that the amounts of U, Cd, and Co removed from solution are inversely related to the similarity of their sizes to the size of Fe.

Valence state and local chemical environment of uranium bound to the biogenic iron oxides. XANES is a useful technique for determination of the average valence state of uranium in samples (3, 9, 24, 37) as the energy position of the edge step (i.e., the increase in adsorption) is directly related to the valence state of the uranium. Comparison of the energy-

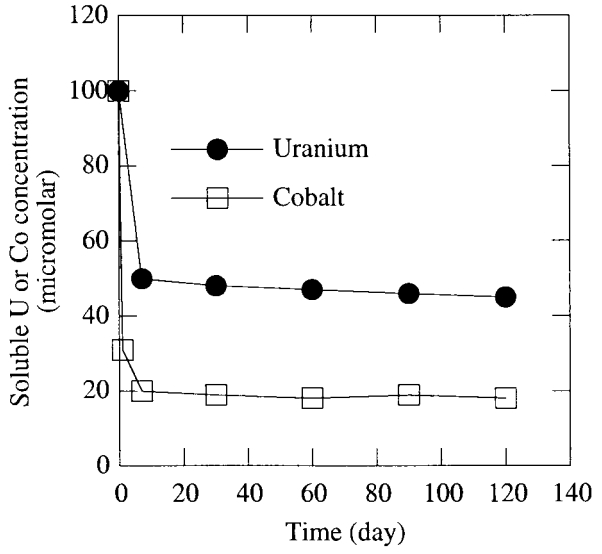

FIG. 3. Adsorption and precipitation of soluble uranium and cobalt by Fe(III) oxides biogenically formed after addition of U(VI) and Co(III).

aligned and step-height-normalized XANES data from the uranium content of the biogenically formed iron oxides produced by *D. suillum* with the data obtained with UO_2 [U(IV)] and UO_3 [U(VI)] standards indicated that the uranium was present only in the oxidized U(VI) state (Fig. 4A).

Theoretical models based on the crystal structure of hydrogen uranyl phosphate tetrahydrate (49) and sodium uranyl(VI) triacetate (58) were generated with the FEFF7 theoretical codes and used as preliminary models for the experimental XAFS data for uranium acetate, uranium phosphate, and hydrated uranyl solutions. The fit to the hydrated uranyl standard showed the importance of multiple scattering paths from the two closely bound axial oxygen atoms of the uranyl. Therefore, these multiple scattering paths were included in the fitting of the XAFS data for the bio-oxidized sample. Two distinct equatorial oxygen paths improved the quality of the fit for the biogenic sample, decreasing the reduced chi-square value by a factor of 2.6. The results of the best fit of the XAFS data indicated that two different equatorial oxygen groups were present in the biogenic uranium-iron oxide solids with approximately 4.7 oxygen atoms at 2.2 Å and 1.7 oxygen atoms at 2.4 Å (Table 1). The data in the Fourier transform region from 2 to 3.5 Å were modeled with two higher coordination shells containing all possible combinations of C, P, U, or Fe at ~2.9 and ~3.5 Å. The model that included two Fe shells was statistically better than any other model, reducing the reduced chi-square value by a factor of 3 to 7. Results of the fit to the experimental magnitude and real part of the Fourier transformed data are shown in Fig. 4B. The sum of the average numbers of Fe atoms in both higher coordination shells is consistent with one, indicating that uranyl was associated with the surface of the iron (hydr)oxides in two different geometries. A U-Fe distance of approximately 3.5 Å is consistent with U(VI) forming bidentate inner-sphere complexes with iron (hydr)oxide surfaces.

Similar uranium XAFS studies of uranium-iron (hydr)oxide

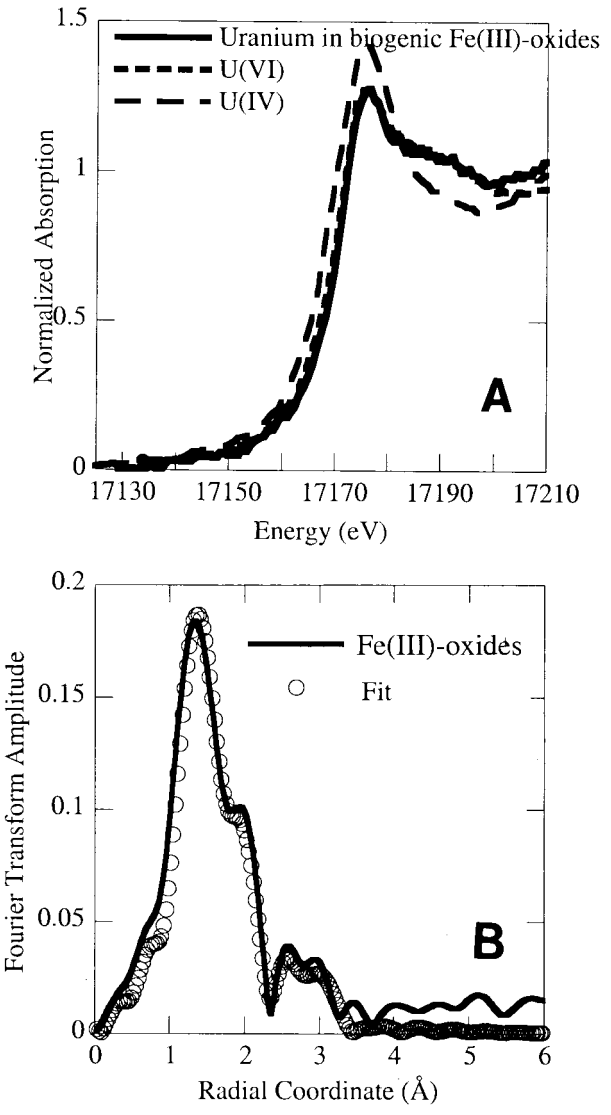

FIG. 4. (A) Energy-aligned and step-height-normalized XANES data from the UO_2 [U(IV)] and UO_3 [U(VI)] standards compared with data from the uranium content of biogenically formed iron oxides. (B) Magnitude and real part of the Fourier transformed $\chi(k)*k$ best-fit model and data from biogenic solids.

interactions have been described previously (6, 44, 60). Although U-C distances of ~2.9 Å have been reported in a previous study (6), the results from the fitting of our experimental data are better described with a U-Fe correlation of ~2.9 Å than with a U-C correlation at ~2.9 Å. It is important that in our experimental system there are a large number of crystallographic iron (hydr)oxide phases. Therefore, the shorter U-Fe distance reported here (to our knowledge, the first observation of its kind) may be due to an as-yet-unknown interaction between uranium and iron (hydr)oxide.

Potential remobilization of uranium as a result of biological Fe(II) oxidation. With a reduction potential (E'_0) of approximately −0.07 V at pH 7.0 for the U(VI)-U(IV) couple (26), it is possible that any insoluble U(IV) in the environment abiotically could react with biogenically produced Fe(III) to form

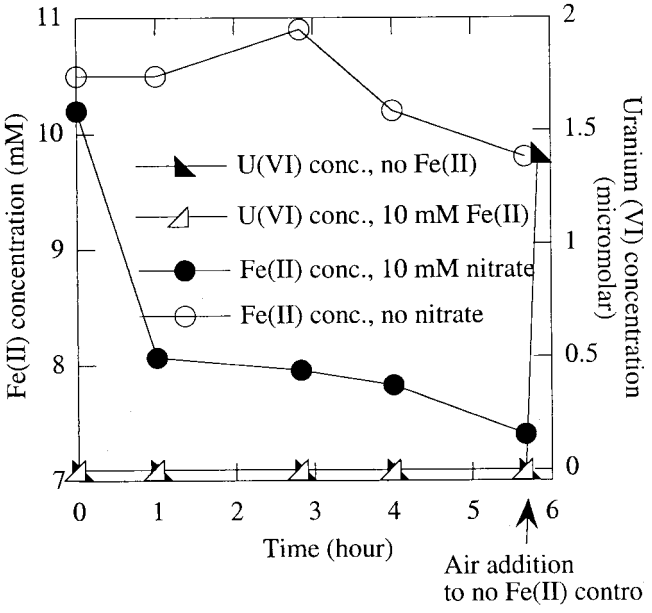

FIG. 5. Fe(II) bio-oxidation and uranium solubilization by an active culture of *D. suillum* with nitrate as the sole electron acceptor. conc., concentration.

soluble U(VI) and Fe(II). If anaerobic biogenically formed Fe(III) (hyd)oxides are to be utilized as a way to attenuate radionuclides in reducing environments, it is very important to ensure that metals such as uranium are not abiotically reoxidized and solubilized by these iron (hydr)oxides. To ensure that the bio-oxidation of Fe(II) did not result in resolubilization of any previously reduced and immobilized U(IV), an anaerobic washed cell suspension of *D. suillum* was amended with insoluble U(IV) and 10 mM $FeCl_2$ and with nitrate (10 mM) as the sole electron acceptor. Cation chromatography analysis indicated that in the absence of Fe(II), no U(VI) was present in solution, demonstrating that *D. suillum* cannot use reduced uranium as an electron donor (Fig. 5). If O_2 was added to the headspace of the control culture that was not amended with Fe(II), the uranium rapidly appeared in solution as U(VI), demonstrating that in the absence of the biogenically produced Fe(III) (hydr)oxides the U(IV) in this system was unstable and could readily be reoxidized and solubilized (Fig. 5). This was expected as U(IV) is notoriously unstable in the presence of oxygen (26). In samples amended with Fe(II), the uranium did not come back into solution during the incubation, although nitrate-dependent Fe(II) oxidation occurred rapidly, indicating that the insoluble uranium remained in an insoluble form regardless of its valence state in the presence of the biogenically formed Fe(III) (hydr)oxides (Fig. 5).

Oxidation of other metals. Many of the organisms known to be capable of dissimilatory Fe(III) reduction have also been shown to be capable of utilizing the oxidized forms of several other transition metals as alternative electron acceptors in place of Fe(III) (13, 14, 17, 18, 19, 21, 39, 40). It is currently not known if nitrate-dependent Fe(II) oxidizers can similarly utilize the reduced forms of other transition metals as surrogates for Fe(II). As outlined above, the results shown in Fig. 5 indicated that U(IV) was not oxidized by *D. suillum* with nitrate as the sole electron acceptor. Similar to the results observed for U(IV) (see above), when an anaerobic washed cell suspension of *D. suillum* in bicarbonate buffer was amended with 100 μM Co(II) as a potential electron donor and nitrate as the sole electron acceptor, no Co(III) was produced during incubation. In contrast, however, when a similar experiment was performed with Cr(III), small amounts of Cr(VI) were produced during the 4-h incubation, and a total of 2.5% of the initial Cr(III) was oxidized (data not shown). No Cr(III) oxidation occurred if the cells or the nitrate was omitted. If the initial Cr(III) concentration was increased to 500 μM, only 0.24% of the Cr(III) was oxidized (data not shown), suggesting that chromium toxicity may have affected enzymatic oxidation of the Cr(III) by the cells.

Significance. Anaerobic bio-oxidation of Fe(II) was only recently identified, and very little is known regarding the ubiquity and diversity of organisms capable of this metabolism. Previous studies have shown that Fe(II) oxidation is mediated by anoxygenic phototrophs (32, 61), as well as by various nitrate-respiring organisms (8, 29, 56, 57). Recent studies have also demonstrated that members of the newly described genera *Dechloromonas* and *Dechlorosoma* (1), isolated for their ability to grow by dissimilatory perchlorate reduction, also oxidize Fe(II) anaerobically with chlorate or nitrate as an alternative electron acceptor (11, 15, 20, 38, 48). Previous investigations demonstrated that nitrate-dependent Fe(II) oxidation by *D. suillum* was not limited to soluble Fe^{2+} ions and that insoluble Fe(II) bound up in mineral matrices was also available for these organisms (15). In addition, these studies also demonstrated that the oxidized iron end product formed [amorphous Fe(III) (hydr)oxide or carbonate-containing green rusts] was dependent on the rate of Fe(II) oxidation by this organism (15, 38). Both of these forms of iron are known to be unstable in the environment and are strong adsorbents for HMR (4, 5, 25, 30, 31).

The results of this study demonstrate that this metabolism offers a unique alternative for immobilization of toxic HMR in affected environments. Thus, selective anaerobic bio-oxidation of Fe(II) added to the environment may be an effective means of capping off and completing the attenuation of HMR in a reducing environment, allowing the system to naturally revert to an oxic state while preventing remobilization of previously reduced and immobilized HMR. This bio-oxidation process may be applied in two ways: (i) by precipitating Fe(III) (hydr)oxides over immobilized HMR in situ, forming an insoluble barrier that crystallizes with time, inhibiting future bioreduction, and adsorbing any leached HMR locally, or (ii) by engineering an Fe(III) oxide wall in situ, downstream of the immobilized HMR, which catches and adsorbs any HMR that may be solubilized and remobilized as a result of environmental fluxes, such as reoxidation (biotically or abiotically) or ligation.

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