

Role for Fe(III) Minerals in Nitrate-Dependent Microbial U(IV) Oxidation

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Microbiological reduction of soluble U(VI) to insoluble U(IV) is a means of preventing the migration of that element in groundwater, but the presence of nitrate in U(IV)-containing sediments leads to U(IV) oxidation and remobilization. Nitrite or iron(III) oxyhydroxides may oxidize U(IV) under nitrate-reducing conditions, and we determined the rate and extent of U(IV) oxidation by these compounds. Fe(III) oxidized U(IV) at a greater rate than nitrite (130 and 10 μ M U(IV)/day, respectively). In aquifer sediments, Fe(III) may be produced during microbial nitrate reduction by oxidation of Fe(II) with nitrite, or by enzymatic Fe(II) oxidation coupled to nitrate reduction. To determine which of these mechanisms was dominant, we isolated a nitrate-dependent acetate- and Fe(II)-oxidizing bacterium from a U(VI)- and nitrate-contaminated aquifer. This organism oxidized U(IV) at a greater rate and to a greater extent under acetate-oxidizing (where nitrite accumulated to 50 mM) than under Fe(II)-oxidizing conditions. We show that the observed differences in rate and extent of U(IV) oxidation are due to mineralogical differences between Fe(III) produced by reaction of Fe(II) with nitrite (amorphous) and Fe(III) produced enzymatically (goethite or lepidocrocite). Our results suggest the mineralogy and surface area of Fe(III) minerals produced under nitrate-reducing conditions affect the rate and extent of U(IV) oxidation. These results may be useful for predicting the stability of U(IV) in aquifers.

Introduction

The reduction of soluble U(VI) to insoluble U(IV) has been proposed as a strategy for preventing the migration of that toxic element with groundwater (1, 2). The strategy entails the injection of physiological electron donors to stimulate U(VI) reduction by microbial communities native to contaminated aquifers (3, 4). U(VI) reduction should occur under Fe(III)- and/or sulfate-reducing conditions (4–6). Nitrate (a frequent cocontaminant with U(VI)), if present, must be reduced before U(VI), Fe(III), and sulfate reduction will commence (4, 6–8). Recent laboratory and field-scale studies

have shown the potential of microbial communities at contaminated sites to reduce nitrate and U(VI) if an exogenous electron donor is supplied (3, 4).

While nitrate reduction must be complete before U(VI) will be reduced, the addition of nitrate to U(IV)-containing sediments leads to oxidation and subsequent remobilization of U (4, 7, 8). Therefore, nitrate inputs to aquifers where uranium has been reductively immobilized could seriously threaten U(IV) stability. Fe(III) oxidizes U(IV) under environmentally relevant conditions (8–12), and may play a role in U(IV) oxidation under nitrate-reducing conditions. Finneran et al. (8) hypothesized that Fe(III) produced by nitrate-dependent Fe(II)-oxidizing (lithotrophic) bacteria oxidizes U(IV). Alternatively, intermediates in dissimilatory nitrate reduction are quite reactive and have been shown to abiotically oxidize U(IV) in sediments (7). Under this latter scenario, as organotrophic, dissimilatory nitrate reduction proceeds, these intermediates (especially nitrite) transiently accumulate (7, 13–15) and react with U(IV), oxidizing it to U(VI). It is unclear which of these is the predominant mechanism of U(IV) oxidation. Finneran et al. (8) suggested that U(IV) oxidation by nitrite in heat-killed sediment incubations proceeds slowly compared to U(IV) oxidation that occurs in nonsterile sediment incubations, but in studies by Senko et al. (7), nitrite (5 μ mol/g of sediment) completely oxidized U(IV) (75 nmol/g of sediment) in heat-killed sediments within 3 days.

To establish the predominant mechanism of U(IV) oxidation under nitrate-reducing conditions, we examined the rates and extents of “abiotic” U(IV) oxidation by nitrite and Fe(III), and show here that nitrate-dependent U(IV) oxidation is likely to proceed via abiogenic or biogenic Fe(III). We propose a new mechanism of U(IV) oxidation, in which U(IV) is oxidized by Fe(III) produced by nitrite oxidation of Fe(II). This mechanism of U(IV) oxidation appears to be more effective than U(IV) oxidation mediated by biogenic Fe(III) due to differences in mineralogy and surface area between the Fe(III) phases formed by the two processes.

Materials and Methods

Abiotic Incubations. All incubations to assess abiotic U(IV) oxidation were performed in anoxic bicarbonate buffer (40 mM, pH 6.8) under a headspace of 80% N₂ and 20% CO₂ contained in serum bottles that were sealed with thick rubber stoppers (16). Incubations were performed under static conditions, but shaken by hand once at each sampling time. Where appropriate, biogenic U(IV), NaNO₂, FeCl₂, or Fe(III) minerals (described below) were added from sterile or pasteurized concentrated stock solutions. Briefly, biogenic U(IV) was produced by incubating resting cells of *Desulfovibrio vulgaris* Hildenborough (approximately 4 mg of protein/mL) with 4 mM uranyl acetate and 5 mM sodium lactate in anoxic 40 mM bicarbonate buffer (pH 6.8, 80:20 N₂/CO₂ headspace) (2, 17). U(IV) was harvested and purified as described by Elias et al. (17) and Fredrickson et al. (18). This procedure included washing pellets with 1 M NaOH to remove the cell mass, followed by washing with water to remove excess NaOH. Concentrated U(IV) stock suspensions were sterilized by autoclaving. U(IV) concentration in the stock suspensions was determined by sequential extraction of soluble U(VI), solids-associated U(VI), and U(IV) as described below, and confirmed by X-ray absorption near-edge spectroscopy (XANES). The XANES spectrum for biogenic U(IV) was consistent with that of U(IV) (not shown). U(VI) represented no more than 4% of the total U in the biogenic U(IV) stock suspension.

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Bacterial Culturing Techniques. *D. vulgaris* Hildenborough was grown on lactate–sulfate medium described by Rapp and Wall (19). An autotrophic, anaerobic, nitrate-dependent, FeS-oxidizing enrichment culture was obtained from a 10^{-8} dilution from an MPN series to enumerate nitrate-dependent FeS-oxidizing bacteria in a landfill leachate-impacted aquifer (Senko et al., submitted for publication). The enrichment culture was maintained in a bicarbonate-buffered (3.5 g/L, pH 6.8) liquid medium containing 0.8 g/L NaCl, 1 g/L NH_4Cl , 0.1 g/L KCl, 0.03 g/L KH_2PO_4 , 0.2 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.04 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, vitamins, and trace metals (20) in serum tubes or bottles containing a headspace of 80% N_2 and 20% CO_2 and sealed with thick rubber stoppers (16). FeS (5 mM) was the sole electron donor in this medium, and NaNO_3 (10 mM) was the sole electron acceptor.

An acetate- and Fe(II)-oxidizing pure culture was isolated from nitrate- and U(IV)-contaminated groundwater at the U.S. Department of Energy's Field Research Center (FRC) in Oak Ridge, TN. During tests to stimulate microbiological nitrate- and U(VI)-reducing bacterial activity, successive electron donor additions were made to the aquifer (4), and extensive biomass was observed in water extracted from injection wells. Biomass was recovered from a well (FW33) that received glucose stimulation (4). Water was collected in sterile centrifuge tubes, refrigerated, and shipped to the laboratory, where the samples were serially diluted and spread onto a solid medium. An organism (designated FW33AN) was isolated on the minimal medium described above with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (50 mM, pH 7) instead of bicarbonate under a N_2 headspace and agar (20 g/L) as a solidifying agent (20). Acetate (30 mM) was the sole electron donor, and nitrate (20 mM) was the sole electron acceptor. Plates were prepared under oxic conditions before transfer to an anoxic glovebag (Coy Laboratory Products, Grass Lake, MI), where they equilibrated overnight under anoxic conditions. Colonies were picked and transferred to anoxic HEPES-buffered acetate–nitrate liquid medium (as above but with no agar) in serum tubes with a N_2 headspace (16). The purity of the isolate was assessed microscopically.

Biologically Mediated Nitrate-Dependent U(IV) Oxidation. The pH of FRC groundwater (from well FW21, starting pH 3.7, approximately 130 mM nitrate) was adjusted to approximately 7.0 with sodium bicarbonate (approximately 10 g/L) as described by Istok et al. (4). Neutralization resulted in the formation of extensive precipitates that were removed by filtration. Oxygen was removed from neutralized FRC groundwater by boiling under a stream of 80:20 N_2/CO_2 . Groundwater (50 mL) was transferred to 120 mL serum bottles with a headspace of 80% N_2 and 20% CO_2 , and the bottles were sealed with rubber stoppers (16). Acetate (95 mM CH_3COONa), Fe(II) (0.4 mM FeCl_2), and U(IV) (0.4 mM biogenic U(IV)) were added by syringe from sterile stocks. Strain FW33AN was grown to mid-log phase in HEPES-buffered acetate–nitrate medium described above, harvested by centrifugation, washed three times with anoxic bicarbonate buffer (40 mM, pH 6.8), and resuspended in bicarbonate buffer before addition to groundwater to a final concentration of 0.1 mg of protein/mL. During groundwater incubations, samples were periodically removed by needle and syringe and analyzed as described below.

Fe(III) Mineral Preparation. Poorly crystalline iron(III) oxyhydroxide and magnetite were prepared as described by Lovley and Phillips (21) and Schwertmann and Cornell (22), respectively. The precipitates were washed three times to remove ions, bubbled with N_2 to remove O_2 , and pasteurized. Lepidocrocite and goethite were purchased from Alfa Aesar (Alfa Aesar, Ward Hill, MA), and stock suspensions were prepared similarly. Fe(III) from oxidation of Fe(II) by nitrite was prepared by reacting 5 mM FeCl_2 with 5 mM NaNO_2 in

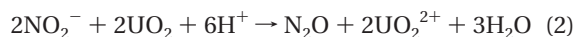
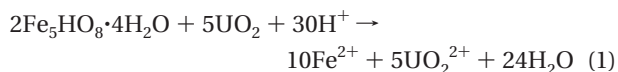
bicarbonate buffer. Biogenic Fe(III) (later identified as goethite) was prepared by incubating strain FW33AN (0.1 mg of protein/mL) with 5 mM FeCl_2 and 5 mM NaNO_3 in bicarbonate buffer. Another biogenic Fe(III) mineral (later identified as lepidocrocite) was obtained from the nitrate-dependent, FeS-oxidizing culture (described above). All anaerobically prepared Fe(III) minerals were harvested by centrifugation under anoxic conditions, washed three times, and resuspended in water. This final suspension served as a stock suspension that was added to U(IV)-containing bicarbonate-buffered incubations.

Sampling and Analytical Techniques. Samples were periodically removed from the incubations by syringe in an anoxic glovebag. Solids were removed by centrifugation, and U(VI), Fe(II), nitrate, and nitrite were quantified in the soluble fraction. Fe(II) was preserved in 0.5 M HCl. Solid-phase U and Fe species were extracted from pellets and quantified as described below. Solids-associated U(VI) and U(IV) were extracted with 100 mM sodium bicarbonate and nitric acid, respectively, as described by Elias et al. (17). Solids-associated U(VI) was solubilized using anoxic 100 mM sodium bicarbonate (7, 17, 23, 24). Concentrated nitric acid was used to oxidize the remaining U(IV) under oxic conditions (7, 17). U(VI) was quantified by kinetic phosphorescence analysis (KPA) on a KPA-11 (ChemChek Instruments, Richland, WA) (25). Solid-phase Fe(II) was extracted with 0.5 M HCl (21).

Nitrate and nitrite were quantified by ion chromatography with conductivity detection (Dionex DX 500 fitted with an AS-4A column; Dionex Corp., Sunnyvale, CA) (7). Fe(II) was quantified by ferrozine assay (21). Due to the poor extractability of crystalline Fe(III) minerals (21), Fe(III) minerals from stock suspensions were dried in an anoxic glovebag and Fe(III) concentration was determined gravimetrically. Protein was quantified using the bicinchoninic assay (Pierce Biotechnology, Inc., Rockford, IL). X-ray diffraction of powdered mineral samples was determined on an automated Rigaku diffractometer (Rigaku/MS, The Woodlands, TX).

Results and Discussion

Abiotic Oxidation of U(IV) under Anoxic Conditions. We compared rates of U(IV) oxidation by chemically prepared poorly crystalline iron(III) oxyhydroxide (21) (eq 1) and nitrite (eq 2).



Nitrous oxide has not been shown experimentally to be the product of U(IV) oxidation by nitrite (eq 2), but it has been proposed as the most likely product of nitrite reduction by Fe(II) (26–30). In our experiments, U(IV) was rapidly but incompletely oxidized by poorly crystalline iron(III) oxyhydroxide (130 μM U(IV) oxidized/day), while U(IV) oxidation by nitrite proceeded slowly (10 μM U(IV) oxidized/day) (Figure 1A). Magnetite did not oxidize U(IV) (not shown). The pH was never influenced by the reactions, likely due to the buffering capacity of the bicarbonate. We hypothesized that incomplete oxidation of U(IV) by iron(III) oxyhydroxide occurred as a result of Fe(II) accumulation during reduction of Fe(III) by U(IV) and subsequent passivation of the reaction between Fe(III) and U(IV). Similar passivation has been reported for microbiological Fe(III) reduction, where it has been hypothesized that free Fe(II) thermodynamically “self-inhibits” Fe(III) reduction (31–38). While we could detect the release of Fe(II) into solution as Fe(III) oxidized U(IV) (Figure 1B), an electron equivalent amount of Fe(II) was not released into solution during Fe(III) reduction by U(IV),

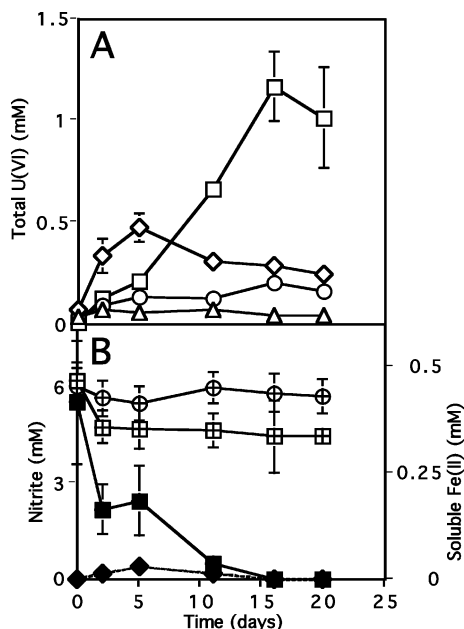
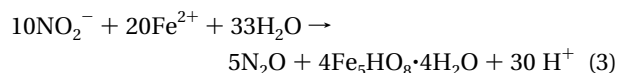


FIGURE 1. Anaerobic U(IV) oxidation in the presence of no oxidant (triangles), 6 mM nitrite (circles), 5 mM poorly crystalline iron(III) oxyhydroxide (tilted squares), or 6 mM nitrite and 0.4 mM Fe(II) (squares). Total U(VI) concentrations are shown in panel A, and soluble Fe(II) and nitrite are shown in panel B. Open shapes represent total U(VI) concentrations, crossed shapes represent nitrite concentrations, and closed shapes represent soluble Fe(II) concentrations. Bars represent 1 standard deviation.

suggesting that Fe(II) was adsorbed to the Fe(III) surfaces, thus inhibiting further U(IV) oxidation by Fe(III).

We then determined whether accumulation of Fe(II) inhibited U(IV) oxidation, by incubating iron(III) oxyhydroxide with various ratios of Fe(II) to Fe(III). Less U(IV) oxidation occurred in the presence of progressively higher ratios of Fe(II)/Fe(III) (Figure 2A). U(IV) was oxidized by 5 and 3 mM Fe(III) at similar rates and to similar extents (not shown), indicating that Fe(III) concentration did not limit U(IV) oxidation in these incubations. Rather, U(IV) oxidation proceeded until the system reached a thermodynamic limit (Figure 2C), suggesting that U(IV) oxidation was, like bacterial Fe(III) reduction (31–38), thermodynamically limited by Fe(II) accumulation. The addition of nitrite to incubations rapidly oxidized Fe(II) (Figure 2B), and alleviated the inhibition of U(IV) oxidation by Fe(II) (Figure 2A).

While nitrite alone was a poor oxidant of U(IV), the addition of 400 μ M Fe(II) to U(IV)- and nitrite-containing incubations stimulated complete U(IV) oxidation (Figure 1A). Fe(II) was oxidized by nitrite in these incubations (Figure 1B), suggesting that Fe was acting as a catalyst for U(IV) oxidation by nitrite, where nitrite oxidized Fe(II) to Fe(III), which in turn oxidized U(IV) (eqs 3 and 1, respectively). Fe(II)



reduces nitrite to ammonium or nitrous oxide (26–30, 39), and we hypothesized that Fe acts as an “electron shuttle” from U(IV) to nitrite. If this hypothesis is correct, the extent of U(IV) oxidation by nitrite should be independent of Fe(II) concentration, if Fe(II) is present at adequate concentration to react at the maximum rate and provided nitrite is present at levels sufficient to oxidize all Fe(II) and U(IV). U(IV) oxidation by nitrite was stimulated at all Fe(II) concentrations tested (Figure 3), suggesting that nitrite oxidized Fe(II) to Fe(III), which in turn oxidized U(IV). Fe(II) in excess of nitrite

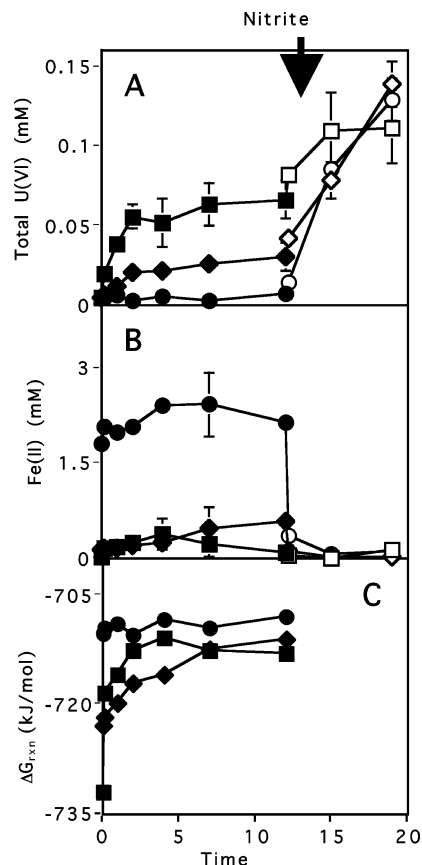


FIGURE 2. Anaerobic U(IV) oxidation by Fe(III) at Fe(II)/Fe(III) concentrations of 0 mM/5 mM (squares), 0.2 mM/4.8 mM (tilted squares), and 2 mM/3 mM (circles) with changes in free energy available as the reactions proceeded. Total U(VI) concentrations are shown in panel A, total Fe(II) concentrations are shown in panel B, and ΔG_{rxn} values are shown in panel C. Open shapes represent total U(VI) or Fe(II) concentrations after the addition of 5 mM nitrite. Bars represent 1 standard deviation. ΔG_{rxn} values were calculated using U and Fe data from this figure and eq 1 in the text ($\Delta G_R^\circ = 704$ kJ/mol of U(IV)) with values obtained from Dean (77).

(100 mM) inhibited U(IV) oxidation (not shown). We use the term “electron shuttling” because Fe-mediated U(IV) oxidation by nitrite appears to proceed in a manner similar to that of the shuttling of electrons by humic material from iron-reducing bacteria to Fe(III) minerals (12, 40). Like this latter process, the extent of U(IV) oxidized by nitrite is largely independent of the amount of electron shuttle (Fe) present. Nitrite oxidizes Fe(II) to Fe(III) (eq 3), which in turn oxidizes U(IV) (eq 1). The Fe(II) produced by this reaction can then be oxidized again by the remaining nitrite. Work by Weber et al. (41) showed that nitrite is a poor oxidant of Fe(II), but experiments conducted by these workers used nitrite concentrations at a nitrite:Fe(II) molar ratio of 1:4. In all of our experiments and most likely in aquifers undergoing bioremediation (4), nitrite:Fe(II) ratios were not lower than 1:1, which almost certainly enhances the reaction rate.

Evaluation of Mechanisms of Nitrate-Dependent U(IV) Oxidation by a Pure Culture Isolated from the Oak Ridge National Laboratory Field Research Center (FRC). While it was clear from the preceding chemical experiments that U(IV) was ultimately oxidized by Fe(III), it was not clear whether biogenic Fe(III) and abiogenic Fe(III) (i.e., produced by oxidation of Fe(II) by nitrite) were equally effective oxidants of U(IV). To establish this, we isolated an acetate- and Fe(II)-oxidizing, nitrate-reducing bacterium from FRC groundwater. We could then compare rates and extents of U(IV)

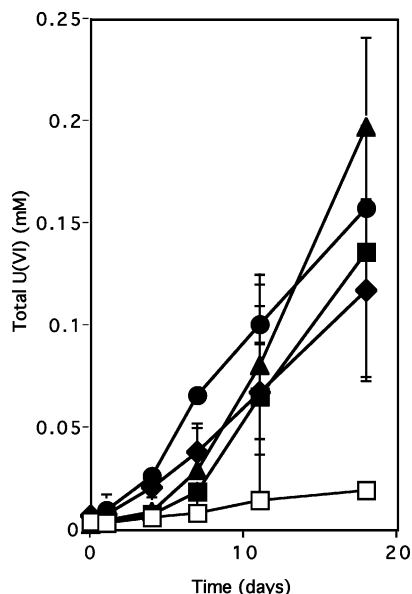


FIGURE 3. Anaerobic U(IV) oxidation by 5 mM nitrite in the presence of 0 mM ($\square$), 0.01 mM ($\blacklozenge$), 0.1 mM ($\bullet$), 1 mM ($\blacktriangle$), and 10 mM ($\blacksquare$) Fe(II). Bars represent 1 standard deviation.

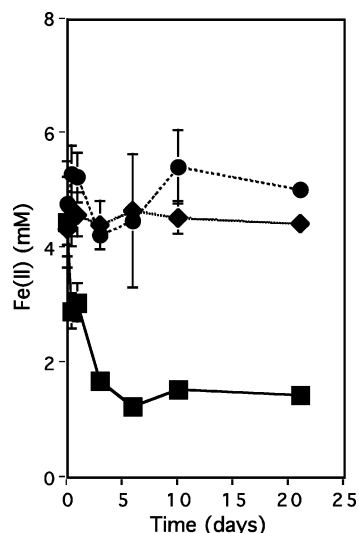


FIGURE 4. Nitrate-dependent Fe(II) oxidation by strain FW33AN: ($\blacksquare$) cells + nitrate; ($\blacklozenge$) no nitrate; ($\bullet$) no cells. Bars represent 1 standard deviation.

oxidation under organotrophic conditions (where Fe(II) is oxidized by nitrite produced during acetate oxidation) and lithotrophic conditions (where Fe(II) is oxidized by microbial respiration).

Strain FW33AN was isolated from U(VI)- and nitrate-contaminated groundwater at the Oak Ridge FRC, and shares 97% 16S rDNA sequence identity with *Klebsiella oxytoca* (Senko et al., manuscript in preparation). FW33AN was able to couple the oxidation of Fe(II) to the reduction of nitrate (Figure 4), a mode of metabolism that is widespread among diverse dissimilatory nitrate-reducing bacteria (8, 42–51). Molecular microbial community analyses of FRC sediment and groundwater revealed the presence of microorganisms whose relatives have been shown to be capable of dissimilatory nitrate reduction and nitrate-dependent Fe(II) oxidation (52–55).

We compared U(IV) oxidation by this organism under acetate-oxidizing (organotrophic) and Fe(II)-oxidizing (lithotrophic) conditions. Strain FW33AN was incubated in neutralized FRC groundwater (which contains approximately

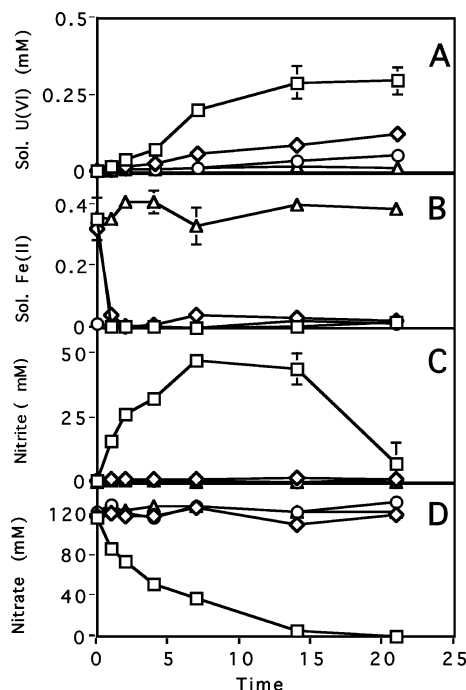


FIGURE 5. U(IV) oxidation in FRC groundwater by strain FW33AN with ($\circ$) no amendment, ($\square$) acetate and Fe(II) amendment, and ($\diamond$) Fe(II) amendment and ($\triangle$) in uninoculated groundwater. Soluble U(VI) concentrations are shown in panel A, soluble Fe(II) concentrations in panel B, nitrite concentrations in panel C, and nitrate concentrations in panel D. Bars represent 1 standard deviation.

130 mM nitrate) with U(IV) and amended with 0.4 mM Fe(II) or 0.4 mM Fe(II) and 95 mM acetate. We hypothesized that if U(IV) oxidation were catalyzed by Fe(III) produced by lithotrophic activity, the addition of Fe(II) alone would stimulate U(IV) oxidation. Alternatively, if U(IV) were oxidized predominantly by the shuttling of electrons from U(IV) to biologically produced nitrite through Fe, we would expect to see U(IV) oxidation only in the presence of both Fe(II) and acetate.

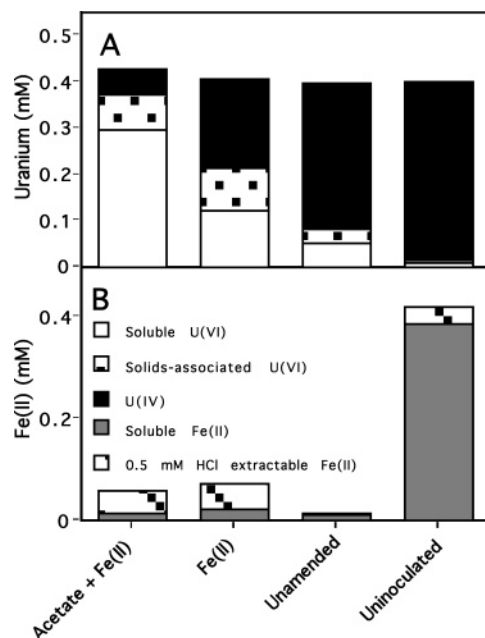
Resting cells of FW33AN rapidly and nearly completely oxidized U(IV) in neutralized FRC groundwater with acetate and Fe(II) amendment (Figures 5A and 6A). U(IV) oxidation occurred concurrently with nitrate reduction (Figure 5D), nitrite accumulation (Figure 5C), and rapid Fe(II) oxidation (Figures 5B and 6B). Nitrite transiently accumulated to 50 mM, a concentration consistent with in situ tests at the FRC where nitrite accumulated up to 130 mM (4). Fe(II) was also rapidly oxidized in acetate-free incubations (Figures 5B and 6B). In these incubations, U(IV) was oxidized to a greater extent than in acetate- and Fe(II)-unamended controls, but at a lower rate and to a lesser extent than in acetate- and Fe(II)-amended incubations (Figures 5A and 6A). Due to the low levels of electron donor relative to available nitrate in acetate-free incubations, nitrate reduction could not be assessed (Figure 5D). Nitrite accumulation was not detected in acetate-free incubations (Figure 5C), suggesting that Fe(II) oxidation in acetate-free incubations was due to the enzymatic oxidation of Fe(II) by strain FW33AN, and not to reaction of Fe(II) with nitrite.

These results are consistent with in situ results where organic electron donor was required for nitrate-dependent U(IV) oxidation; no U(IV) oxidation occurred when nitrate-containing, electron-donor-free groundwater was injected (4). While U(IV) was clearly oxidized to a greater extent under organotrophic conditions than lithotrophic conditions, it was unclear why this was occurring, since Fe(III) was generated in both cases. We hypothesized that differences in Fe(III)

TABLE 1. Characteristics of Fe(III) Minerals Used in U(IV)-Oxidizing Incubations

method of Fe(III) mineral preparation and XRD spectrum in Figure 7	XRD identity	E_H^f ^a (V)	surface area (m ² /g)
hydrolysis of FeCl ₃ solution (20) (C)	hydrous ferric oxide (HFO) ^b	+0.34	87.9
Fe(II) oxidation by nitrite (D)	HFO	+0.34	113.8
nitrate-dependent Fe(II) oxidation by strain FW33AN (B)	goethite	-0.14	26.1
nitrate-dependent FeS oxidation by enrichment culture (A)	lepidocrocite	-0.04	109.3

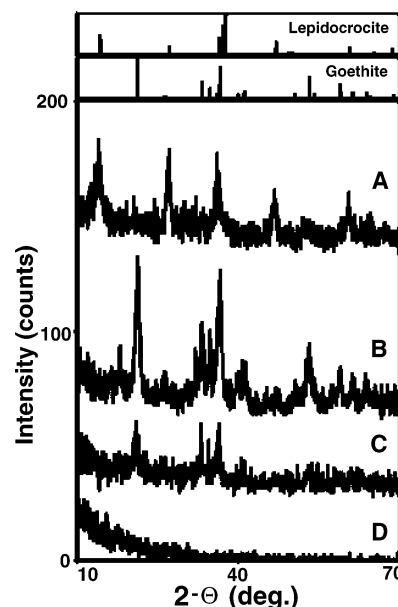
^a From Roden (56). ^b A weak goethite signal could be detected in this Fe(III) phase by XRD, but the method of synthesis should yield HFO (21, 56); therefore, we use the E_H^f value for HFO.


FIGURE 6. Extraction of solid-phase U (A) and Fe(II) (B) species in incubations of strain FW33AN in FRC groundwater at $t = 21$ days.

mineralogy between “abiotically” formed and biologically formed Fe(III) could explain this observation.

Characterization of Biogenic Fe(III) Minerals and Their Reactivity toward U(IV). During the above experiments and others, we have noted that different methods of biological and abiotic Fe(III) production yielded Fe(III) solid phases of different shades of red/orange/brown. Fe(III) mineralogy (e.g., crystal structure, surface area) is known to strongly influence its susceptibility to both biological and chemical reduction (56–63), which might explain the differences in rate and extent of U(IV) oxidation that we observed in the preceding experiments. Furthermore, a comparison of U(IV) oxidation by the manganese(III,IV) oxides bixbyite (Mn₂O₃) and pyrolusite (β -MnO₂) revealed that bixbyite oxidized U(IV) at a greater rate and to a greater extent than pyrolusite (18).

To test the hypothesis that Fe(III) mineralogy was responsible for the observed differences in U(IV) oxidation rates, we produced Fe(III) minerals by (1) hydrolysis of FeCl₃ under oxic conditions (the poorly crystalline Fe(III) oxyhydroxide used for previous experiments) (21), (2) oxidation of Fe(II) by nitrite under anoxic conditions, (3) nitrate-dependent oxidation of soluble Fe(II) by resting cells of strain FW33AN, and (4) nitrate-dependent oxidation of FeS by an enrichment culture obtained from a landfill leachate-impacted aquifer (Senko et al., submitted for publication). Fe(III) produced by nitrate-dependent oxidation of FeS by an enrichment culture was lepidocrocite (Figure 7A), Fe(III) produced by nitrate-dependent Fe(II) oxidation by strain FW33AN was goethite (Figure 7B), Fe(III) produced by hydrolysis of FeCl₃ was identified as hydrous ferric oxide (HFO), though a goethite signal could be detected, probably


FIGURE 7. X-ray diffraction spectra for Fe(III) minerals: Fe(III) produced by nitrate-dependent FeS-oxidizing enrichment culture (A), Fe(III) produced by nitrate-dependent Fe(II) oxidation by strain FW33AN (B), Fe(III) prepared by hydrolysis of FeCl₃ (21) (C), Fe(III) produced by oxidation of Fe(II) by nitrite (D). Reference plots for goethite and lepidocrocite are from JADE 3.1.

due to dissolution/precipitation of Fe(III) as the suspension aged (22) (Figure 7C), and Fe(III) produced by oxidation of Fe(II) by nitrite was amorphous HFO (Figure 7D). The surface areas of these minerals were determined and are shown in Table 1. HFO produced by reaction of Fe(II) with nitrite and poorly crystalline iron(III) oxyhydroxide goethite were the most effective oxidants of U(IV), while the more crystalline biogenic lepidocrocite and goethite were the least effective oxidants of U(IV) (Figure 8). The extent of U(IV) oxidation by Fe(III) minerals corresponded with the predicted effects of the redox potentials of the individual minerals and their surface areas (Figure 7 and Table 1). Commercially available lepidocrocite was a more effective U(IV) oxidant than commercially available goethite (not shown).

These results provide an explanation for why U(IV) was oxidized by FW33AN to a greater extent under organotrophic conditions than under lithotrophic conditions. Fe(III) was generated under both conditions, but due to its amorphous nature and higher surface area, the Fe(III) produced by abiotic Fe(II) oxidation by nitrite (HFO) was more reactive than biogenic Fe(III) (goethite). These results are consistent with the observation by Roden (56) that the susceptibility of Fe(III) minerals to chemical reduction by ascorbate is dependent on the surface area and the redox potential as determined by mineralogy.

The biogenic production of crystalline Fe(III) phases is well-known. Goethite (61, 62, 64, 65), lepidocrocite (62, 64, 66), green rust (47), akaganeite (67), schwertmannite (68),

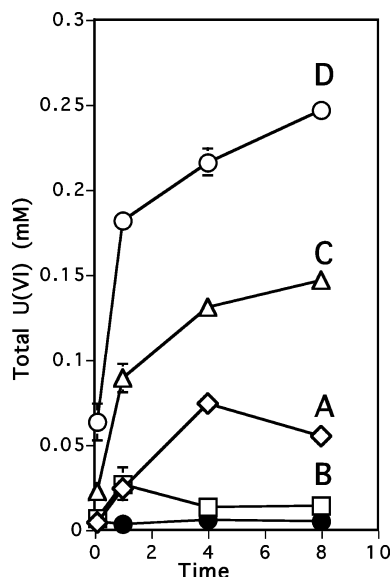


FIGURE 8. Anaerobic U(IV) oxidation with no oxidant (●), with anoxically prepared hydrous ferric oxide (○), with poorly crystalline iron(III) oxyhydroxide (△), with biogenic lepidocrocite (◇), and with biogenic goethite (□). Total U(VI) was considered the sum of soluble U(VI) and solids-associated U(VI). Letters in this figure designate corresponding XRD spectra in Figure 7. Bars represent 1 standard deviation.

and magnetite (47) have been found to be produced by the enzymatic activity of aerobic and/or anaerobic Fe(II)-oxidizing bacteria. It is not clear if biogenic Fe(III) mineralogy is specific to the organism producing the Fe(III) mineral. While we observed the formation of goethite by one bacterial culture and lepidocrocite by another, it is important to note that biogenic goethite was formed from soluble Fe(II) by resting cells while biogenic lepidocrocite was formed from FeS in growth medium. Therefore, whether biogenic Fe(III) mineralogy is bacterial-species-dependent is difficult to assess. It is likely that biogenic Fe(III) mineralogy is dependent on the oxidation rate, prevailing geophysical and geochemical conditions, Fe(II) substrate, and characteristics of bacterial cell surfaces (22, 66, 69–75). In similar studies with *Dechlorosoma suillum* biogenic magnetite and green rust were produced during growth on Fe(II) with nitrate as the terminal electron acceptor, but resting cells of this organism produced ferrihydrite under nitrate-reducing conditions (47, 49). In this case, we predict on the basis of thermodynamic considerations and experimental results that ferrihydrite could effectively oxidize U(IV), while green rust and magnetite would not (76, this work). The reaction between U(IV) and Fe(III) may also be limited by the physical proximity of the solid phases, which could exert significant control on in situ rates of nitrate-dependent U(IV) oxidation. For instance, Frederickson et al. (18) have noted that accumulation of U(IV) in the periplasm of U(VI)-reducing bacterial cells may protect U(IV) from reoxidation. Further work to elucidate controls on microbiological Fe(II)-oxidizing activity and biogenic Fe(III) mineralogy may allow us to better predict the stability of U(IV) in aquifers in which uranium has been reductively immobilized.

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