

In-Situ Evidence for Uranium Immobilization and Remobilization

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The in-situ microbial reduction and immobilization of uranium was assessed as a means of preventing the migration of this element in the terrestrial subsurface. Uranium immobilization (putatively identified as reduction) and microbial respiratory activities were evaluated in the presence of exogenous electron donors and acceptors with field push–pull tests using wells installed in an anoxic aquifer contaminated with landfill leachate. Uranium(VI) amended at 1.5 μM was reduced to less than 1 nM in groundwater in less than 8 d during all field experiments. Amendments of 0.5 mM sulfate or 5 mM nitrate slowed U(VI) immobilization and allowed for the recovery of 10% and 54% of the injected element, respectively, as compared to 4% in the unamended treatment. Laboratory incubations confirmed the field tests and showed that the majority of the U(VI) immobilized was due to microbial reduction. In these tests, nitrate treatment (7.5 mM) inhibited U(VI) reduction, and nitrite was transiently produced. Further push–pull tests were performed in which either 1 or 5 mM nitrate was added with 1.0 μM U(VI) to sediments that already contained immobilized uranium. After an initial loss of the amendments, the concentration of soluble U(VI) increased and eventually exceeded the injected concentration, indicating that previously immobilized uranium was remobilized as nitrate was reduced. Laboratory experiments using heat-inactivated sediment slurries suggested that the intermediates of dissimilatory nitrate reduction (denitrification or dissimilatory nitrate reduction to ammonia), nitrite, nitrous oxide, and nitric oxide were all capable of oxidizing and mobilizing U(IV). These findings indicate that in-situ subsurface U(VI) immobilization can be expected to take place under anaerobic conditions, but the permanence of the approach can be impaired by dissimilatory nitrate reduction intermediates that can mobilize previously reduced uranium.

Introduction

When uranium is recovered from ore, H_2SO_4 and/or HNO_3 are frequently used as extractants (1). The waste from this process often contains high levels of soluble oxidized uranium (U(VI)) along with sulfate and/or nitrate, which are mobile

in the environment and can contaminate groundwater. A proposed means of preventing the migration of U(VI) is the in-situ immobilization of soluble U(VI) (as UO_2^{2+} , usually complexed with carbonate in natural systems) via microbial reduction to insoluble U(IV) (as UO_2) (2, 3). Several microorganisms are known to mediate this process, including the Fe(III)-reducing *Geobacter* sp. and *Shewanella* sp. (2), the Fe(III)- and sulfate-reducing *Desulfotomaculum* sp. (4), the sulfate-reducing *Desulfovibrio* sp. (5, 6), and the fermentative anaerobic *Clostridium* sp. (7). Work to assess the influence of alternate terminal electron acceptors on U(VI) reduction has focused mostly on Fe(III) and sulfate since the organisms that reduce these substances are expected to be primarily responsible for U(VI) reduction in contaminated aquifers. Spear et al. (8) reported that U(VI) reduction rates were greater in the presence of sulfate than without in both a pure culture of *Desulfovibrio desulfuricans* and a *D. vulgaris*–*Clostridium* sp. co-culture. Ferrihydrite, but not goethite, was reported to inhibit U(VI) reduction by the Fe(III)-reducing *Shewanella alga* (BrY) (9). Several organisms are capable of nitrate and U(VI) reduction, and experiments performed by Ganesh et al. (10) suggested that U(VI) reduction by *D. desulfuricans* is slightly inhibited by the presence of 190 mM nitrate but not by nitrate concentrations lower than 95 mM.

On the basis of the potential for H_2 oxidation coupled to alternate terminal electron acceptors under standard conditions, the sequence of processes to be expected is denitrification ($\Delta E_o' = -1160$ mV), uranium reduction ($\Delta E_o' = -820$ mV), and sulfate reduction ($\Delta E_o' = -190$ mV) (as calculated from ref 11). This sequence of reductive processes was demonstrated in electron donor-amended groundwater and in a sediment core column from a U(VI)-contaminated site in Tuba City, AZ (12, 13). Sediment slurry incubations from a uranium mill tailings disposal site in Shiprock, NM, revealed that uranium reduction will not commence until nitrate is completely depleted (14). Subsequently, U(VI), Fe(III), and sulfate reduction occur, with both Fe(III) and U(VI) reduction stimulated by glucose or acetate addition (14).

We determined the impact of electron donor and terminal electron acceptor amendments on the fate of U(VI) in anaerobic sediments from a landfill leachate impacted aquifer in Norman, OK. This aquifer had not been previously contaminated with U(VI). Field experiments and laboratory incubations reveal the potential for in-situ U(VI) immobilization via concerted microbiological and geochemical processes. We also present evidence that nitrate-reducing activity leads to the mobilization of previously immobilized uranium and may be due to oxidation of U(IV) by dissimilatory nitrate reduction intermediates.

Materials and Methods

Single Well Push–Pull Tests. Single well push–pull tests were performed as described by Istok et al. (15). Each test consists of three phases: extraction of groundwater and amendment with test chemicals, injection of amended groundwater, and in-situ incubation with periodic extraction. Drive point wells were installed in an aquifer impacted by leachate from the Norman Municipal Landfill to a screen depth of 3.5 m. Sediments and groundwater from this site have been previously characterized microbiologically and geochemically (16). Groundwater (50 L) was pumped from the wells with a peristaltic pump into carboys and amended with electron donor or electron acceptor (below) while being constantly sparged with 80:20 $\text{N}_2:\text{CO}_2$. Norman landfill leachate has a pH of 6.9–7.2 when sparged with 80:20 $\text{N}_2:\text{CO}_2$. The groundwater was then injected back into the aquifer.

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TABLE 1. Summary of Amendments to Groundwater for Push–Pull Tests^a

test	NaNO ₃ (mM)	Na ₂ SO ₄ (mM)	NaCH ₃ COO (mM)	NaCH ₃ CHOHCOO (mM)	NaCOO (mM)
Round 1					
1					
2			11		
3				8	
4					17
5		0.5			
6	5	0.5			
Round 2					
7					
8	5				
9	1				

^a All tests were amended with U(VI) and bromide as described in the Materials and Methods section.

Samples were taken during injection after every 10 L (of the 50 L total). All analytes were quantified as described below to see if any U(VI), sulfate, or nitrate loss occurred during injection. No transformation of amendments occurred during the injection phase of the tests (data not shown).

During the incubation phase, groundwater (2 L) was periodically removed from the wells and filtered (0.2 µm) into sterile tubes, stored at 4 °C, and subsequently analyzed for U(VI), bromide, nitrate, sulfate, and electron donor concentrations. These concentrations were normalized to account for dilution by the surrounding groundwater. This was accomplished by first calculating the ratio of the bromide concentration at each time point to the injected bromide concentration. This value was used to normalize the measured amendment concentration.

Two rounds of tests were performed. Six different manipulations were performed in the first round where all wells were amended with 1.5 µM UO₂(NO₃)₂, 1.5 mM KBr, and different amendments of electron donor or acceptor as summarized in Table 1. Approximately 2.5 months after completion of the first round of tests, a second round of tests was performed in wells that contained immobilized uranium from the previous round of tests. These tests were performed nearly identically to the previous round with 1.0 µM U(VI), 1.5 mM bromide, and nitrate as summarized in Table 1. Approximately 1 month after the conclusion of the second round of tests, a core was collected approximately 0.5 m from each well. At the screened depth interval, 20 cm of core was divided into approximately 3-cm segments, and triplicate 1-g subsamples were taken from each segment and sequentially extracted as described below to determine soluble U(VI), solids-associated U(VI), and U(IV).

Laboratory Incubations. Sediments were collected from a shallow, alluvial, landfill leachate-impacted aquifer in Norman, OK, using a hand auger. The depth from which the sediments were collected was slightly below the water table (approximately 2 m) in the anoxic zone, as indicated by blackening of the sediments. The sediments were transferred to sterile jars and transported back to the laboratory where they were flushed with N₂ and refrigerated prior to use. Sediments (5 g; 25% water) were placed in 25-mL serum bottles and stoppered while inside an anoxic glovebox. The bottles were then removed from the glovebox and flushed with 80:20 N₂:CO₂. Autoclaved (20 min) slurries served as negative controls. Amendments (0.1 mL) were made from sterile stock solutions to reach the following concentrations: uranyl acetate (2.5 µM), Na₂SO₄ (1 mM), or NaNO₃ (0.6 or 7.5 mM). NaCl was added to bottles, if needed, to achieve a final Na⁺ concentration of 7.5 mM. After addition of groundwater (1.05 mL) and amendments (0.2 mL total), the final liquid

volume of the incubations was 2.5 mL. All sediment incubations were performed in duplicate; incubated at room temperature in the dark; and periodically sacrificed for analysis of nitrate, nitrite, and sulfate as well as soluble U(VI), solids-associated U(VI), and U(IV) as described below.

U(IV) Oxidation. For U(IV) oxidation experiments, autoclaved sediment slurries were prepared as described above and amended with uranyl acetate (U(VI)) or uraninite (UO₂) (U(IV)) (Alfa Products, Danvers, MA) to an initial concentration of 75 nmol/g of sediment slurry. Fine-grained, clayey aquifer material was used, as opposed to the sandier sediments used for the sediment slurry incubations. Samples were allowed to equilibrate for 1 week, representative bottles were sacrificed, and uranium was sequentially extracted as described below to determine any changes in the uranium pool (i.e., reduction or adsorption) over the week-long equilibration period. After equilibration, NaNO₃ (5 µmol/g of sediment), NaNO₂ (5 µmol/g of sediment), NO (0.4 µmol/g of sediment), N₂O (5 µmol/g of sediment), or N₂ (5 mL to the 20 mL of 80:20 N₂:CO₂ headspace) were added to the sediment slurries. For the incubations in which gas was added, NaCl (5 µmol/g of sediment) was added to produce an ionic strength equivalent to the nitrate- and nitrite-amended bottles. Subsamples were removed periodically from the soluble fraction and analyzed for U(VI) as described below. At the conclusion of the experiments, any remaining insoluble uranium was characterized by the sequential extraction procedure described below.

Analytical Techniques. Uranium speciation was determined by sequential extraction of soluble U(VI), solids-associated U(VI), and U(IV) in samples from nonsterile and heat-inactivated laboratory sediment incubations and cores collected at the conclusion of the push–pull tests. This was accomplished using a variation of methods described by Phillips et al. (17) and Fredrickson et al. (18), which has been shown to effectively remove solids-associated U(VI) from neutral pH sediments with a bicarbonate solution. Sediments were separated from porewater by centrifugation (6000g for 10 min), and the supernatant containing the soluble U(VI) fraction was removed. To solubilize any solids-associated U(VI), a volume of anoxic sodium bicarbonate (100 mM; pH 8.3) equivalent to the supernatant removed (0.5 mL) was added to the remaining pellet (1 g of sediment) under anoxic conditions. After being incubated overnight in the sodium bicarbonate solution, samples were centrifuged again, the supernatant was removed, and nitric acid was added under aerobic conditions in a volume equivalent to that of supernatant removed to oxidize and solubilize remaining uranium. Phillips et al. (17) reported that bicarbonate extracted 20–94% of the uranium extracted by nitric acid. The mean extraction efficiency for the first two extractions (soluble and bicarbonate extraction) was at least 67% for heat-inactivated incubations, assuming that no reduction took place. Therefore, the maximum U(VI) potentially measured as U(IV) was 33% of the total uranium quantitated. Furthermore, when commercial UO₂ was added to sterile incubations (U(IV) oxidation experiments described above), the vast majority (approximately 96%) of uranium could only be recovered in the nitric acid-extractable fraction, and 62–100% of the UO₂ added to the bottles was recovered. On the basis of these results, we were thus able to conclude that the nitric acid-extractable fraction was mainly U(IV). This extraction method allowed us to quantify the uranium in three pools: the soluble fraction, the solids-associated fraction (i.e., adsorbed), and the reduced fraction. While spectroscopic evidence for U(VI) reduction was not obtained in these experiments, uranium that could only be recovered in the nitric acid-extractable fraction was putatively identified as reduced and is referred to as reduced in the Results and Discussion section. U(VI) concentration in all samples was

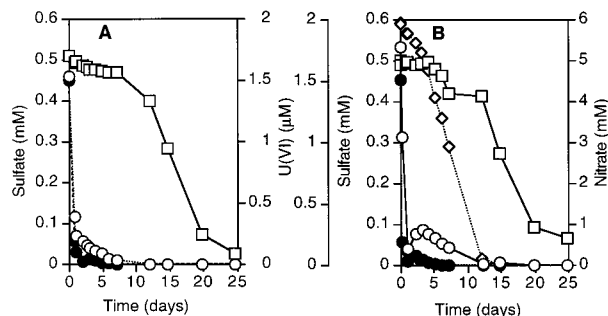


FIGURE 1. U(VI) immobilization in the presence of (A) 0.5 mM sulfate or (B) 5 mM nitrate and 0.5 mM sulfate, as assessed in-situ by push-pull tests. In both graphs, (●) represents U(VI) concentration in the terminal electron acceptor-unamended well, (○) represents U(VI) concentration in the amended wells, and (□) represents sulfate. Nitrate concentration is represented by ◇. All concentrations were adjusted for dilution by dividing measured concentrations by the C/C_0 of bromide as described in the Materials and Methods.

determined by kinetic phosphorescence analysis (KPA) (KPA-11; ChemChek Instruments, Richland, WA) (19). Bromide, sulfate, and nitrate were quantified by ion chromatography (Dionex DX 500 fitted with an AS-4A column and conductivity detector; Dionex Corporation, Sunnyvale, CA). Electron donors (formate, acetate, and lactate) were quantified by ion chromatography using the same system fitted with an AS-11 column.

Results and Discussion

Push-Pull Tests: Effect of Electron Donors and Acceptors on U(VI) Reduction. Single well push-pull tests were used to assess the impact of electron donors and alternate terminal electron acceptors on U(VI) reduction. Acetate, lactate, and formate were all degraded relative to bromide over approximately a 2-week period during these tests (data not shown). However, there was no detectable influence of electron donors on U(VI) immobilization. Complete U(VI) immobilization in the absence of exogenous electron donor or acceptor occurred within 10 d (Figure 1), and a substantial amount ($\geq 44\%$) of immobilization occurred within a few hours of injection, due either to adsorption of U(VI) on sediments or to a rapid rate of reduction. When sulfate was injected as an alternate terminal electron acceptor with U(VI), a slight inhibition of the rate of U(VI) immobilization was evident (Figure 1A). In the presence of nitrate and sulfate, more extensive inhibition of U(VI) reduction was observed especially as nitrate reduction proceeded (Figure 1B). Although the leachate-impacted sediments in this aquifer are generally considered to represent a sulfate-reducing or methanogenic environment (16, 20), the presence of rapid nitrate-reducing activity was far from surprising. Many anaerobic environments exhibit extensive nitrate reduction even though they do not receive regular inputs of nitrate (21–23), and several sulfate-reducing bacteria are capable of dissimilatory nitrate reduction to ammonia (24). Immobilization of U(VI) was not complete until nitrate was removed (~ 15 d). As is typical of anaerobic environments (25–29), significant sulfate reduction was not evident until the nitrate was depleted to low levels (Figure 1B). The percent recovery of U(VI) was determined by dividing the amount removed from the well by the mass injected in 50 L of groundwater. This value was then normalized by the corresponding percent recovery of bromide to account for dilution of U(VI) by the surrounding groundwater. While the percent recovery of U(VI) in the sulfate amended well was only slightly higher than that of the untreated well, nearly half of the injected U(VI) was recovered from the nitrate-amended well (Table 2).

TABLE 2. Percent Recoveries for Wells Treated with U(VI) and Nitrate or Sulfate^a

treatment	% recovery U(VI)/ % recovery Br ⁻
U(VI) only	4
U(VI) and 0.5 mM sulfate	10
U(VI), 0.5 mM sulfate, and 5 mM nitrate	54
U(VI) and 0.5 mM nitrate ^b	40
U(VI) and 5 mM nitrate ^c	80

^a All percentages are adjusted by dividing by the percent recovery of bromide to account for dilution as described in the Results and Discussion. ^b Well had been treated with 1.5 μM U(VI) and 17 mM sodium formate in the previous round of tests. ^c Well had been treated with 1.5 μM U(VI), 0.5 mM sodium sulfate, and 5 mM sodium nitrate in the previous round of tests.

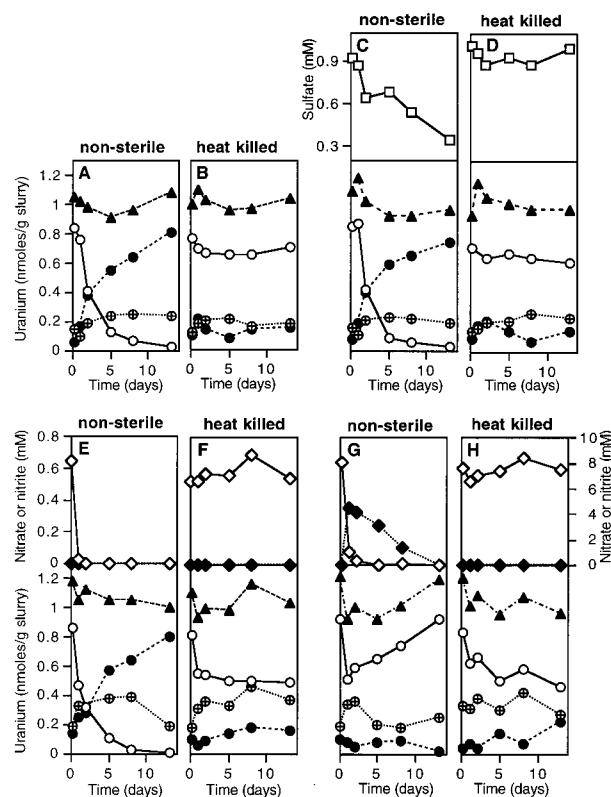


FIGURE 2. U(VI) immobilization in nonsterile and heat-killed sediment incubations with (A) U(VI) and no alternate terminal electron acceptor and (B) heat-killed control; (C) U(VI) with 1 mM sulfate and (D) heat killed control; (E) U(VI) with 0.6 mM nitrate and (F) heat-killed control; (G) U(VI) with 7.5 mM nitrate and (H) heat-killed control. Bottom panels represent uranium species found in sequential extractions: (○) soluble U(VI), (●) solids-associated U(VI), (●) U(IV), and (▲) total U. Top panels represent alternate terminal electron acceptors; either (□) sulfate, (◇) nitrate, or (◆) nitrite. All uranium concentrations are expressed as nmol/g of sediment slurry. Each gram of sediment slurry contained 0.6 g dry sediment and 0.4 mL of water.

Laboratory Incubations. Laboratory U(VI) immobilization profiles in sediment incubations were similar to the in-situ tests. Uranium was reduced in the sediments, with near complete immobilization of uranium observed after 13 d (Figure 2A,B). Upon addition of U(VI) to the sediment slurries, a near immediate (approximately 1 h) immobilization of U(VI) was observed in both live incubations and in heat-inactivated incubations. As solids-associated U(VI) increased at the start of the incubations, the initial decrease in soluble U(VI) is partly due to sorption. This was also evident initially in the heat-inactivated sediments, possibly as a result of surface-

catalyzed U reduction by Fe(II) or on iron sulfides. The sum of all uranium pools has been plotted in Figure 2 to demonstrate that no pools of uranium are unaccounted for. Abiotic immobilization of uranium (via adsorption or reduction) has been well-documented, occurring on iron sulfides and iron oxyhydroxides by both adsorption and reduction (12, 30–36) and by adsorption onto clay minerals (37, 38). However, biological activity was required for complete immobilization of U(VI) in our experiments. It has also been suggested that Fe(II) produced by iron-reducing bacteria can reduce U(VI) and other radionuclides on Fe(III) minerals (17, 39). Whether the biological reduction we observed in nonsterile incubation is a result of direct enzymatic reduction or is from the evolution of reducing bacterial endproducts is unknown. Finneran et al. (14) have suggested that Fe(II) and sulfide do not significantly contribute to U(VI) reduction in Shiprock, NM, sediments. Furthermore, both sulfate and microbially reducible Fe(III) were not abundant (100 μ M and 200 nmol/g of sediment, respectively) in the sediment samples used for these experiments, while Fe(II) levels were high in both sterile and live sediments and minimal abiotic reduction occurred in the heat-killed incubations. Uranium and sulfate reduction occurred concomitantly in sediment incubations, but sulfate appeared to neither stimulate nor inhibit U(VI) reduction relative to the unamended incubations (Figure 2A–D). This suggests that U(VI) reduction did not occur as a result of sulfide generated by sulfate reduction, as the addition of sulfate would then have stimulated U(VI) reduction. As noted above, slight “inhibition” of U(VI) immobilization by sulfate was observed in the push–pull tests, but this was not observed in the laboratory incubations. On the basis of the sediment incubations, it appears that the apparent “inhibition” of U(VI) reduction by sulfate that occurred in the push–pull tests may be due to slightly different sorptive characteristics of the sediments surrounding the push–pull test wells.

When 0.6 mM nitrate was added to sediment slurries, no inhibition of U(VI) reduction occurred (Figure 2E,F). Nitrate was completely reduced within 2 d, and no accumulation of nitrite was evident. In this case, nitrate was present in relatively low concentration, reduced quite rapidly, and had no detectable effect on U(VI) reduction. However, in sediment incubations with U(VI) and 7.5 mM nitrate, an initial immobilization of U(VI) was observed, but remobilization occurred as nitrate was reduced and nitrite and likely other denitrification intermediates accumulated (Figure 2G,H).

Abdoulas et al. (40) performed column experiments suggesting that terminal electron acceptors were consumed in sequence. Nitrate reduction was followed by U(VI) reduction and finally sulfate reduction. Complete reduction of nitrate occurred in an acetate and trimetaphosphate-amended column after 7.5 d, with the transient accumulation of nitrite. U(VI) loss began after nitrate had been consumed and while nitrite reduction occurred. There was no evidence for the remobilization of U(VI) in these experiments. While our experiments also show that U(VI) reduction will not commence until nitrate is consumed, we present both field and laboratory evidence (below) that remobilization/oxidation of U(IV) occurs only after nitrate reduction has commenced.

Oxidation and Remobilization of Uranium under Nitrate-Reducing Conditions. The inhibition of U(VI) reduction by nitrate in sediment incubations and the first round of push–pull tests could be interpreted simply as a result of nitrate controlling the redox potential rather than remobilization of previously reduced uranium. An increase in the nitrous oxide to N_2 ratio can result from the addition of nitrate to environmental samples (41–43), indicating that denitrification is occurring along with the transient accumulation of denitrification intermediates. To test the hypothesis that

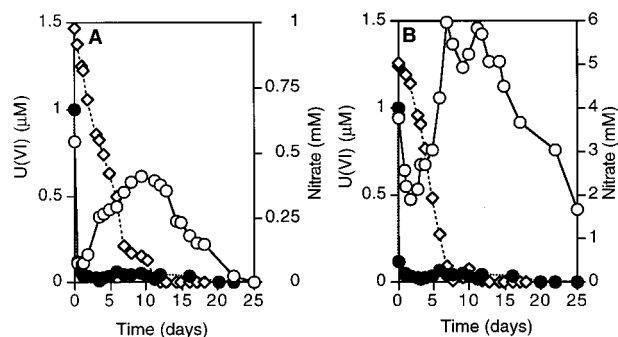


FIGURE 3. U(VI) immobilization and remobilization as (A) 1 and (B) 5 mM nitrate is reduced. Nitrate concentration is represented by $\diamond$, U(VI) concentration in nitrate amended wells is represented by $\circ$, and U(VI) concentration in the unamended well is represented by $\bullet$. All concentrations were adjusted for dilution by dividing measured concentrations by the C/C_0 of bromide as described in the Materials and Methods.

U(IV) was oxidized and remobilized by dissimilatory nitrate reduction intermediates, a second set of push–pull tests was performed in the wells from the previous experiments in which uranium was co-injected with two different nitrate concentrations in separate wells (1 and 5 mM). The sediments surrounding these wells already contained reduced uranium based on both our calculated recoveries and the apparent reduction observed in the laboratory experiments. A control well was tested with U(VI) only and exhibited a similar pattern of immobilization to the first round of tests (Figure 3A,B). However, when 1 or 5 mM nitrate accompanied U(VI), initial immobilization occurred over the first 1–2 d. Remobilization subsequently occurred during the period of active nitrate reduction. In the well with 5 mM nitrate, the dilution-adjusted U(VI) actually reached a higher concentration than that of the injected U(VI) (Figure 3B); percent recoveries of U(VI) in both wells were quite high (Table 2).

After completion of push–pull tests, 0.5-m cores were collected from two wells, and uranium was sequentially extracted. The majority of the uranium extracted from the cores was reduced (Table 3), suggesting that the immobilization observed in the push–pull tests was a result of reduction and precipitation. The cores were collected from the well used to conduct tests 1 and then 7 (see Table 1) and the well used to conduct tests 4 and then 9 (see Table 1). Interestingly, less total uranium was present in the core collected adjacent to a well that received an input of nitrate (during test 9). This would be expected as this well had been treated with nitrate, and the resulting remobilized uranium would either be extracted or be transported away from the well vicinity by regional groundwater flow, as opposed to the unamended well where U(VI) was immobilized in close proximity to the well. This observation, coupled with the slight lag in uranium remobilization until nitrate reduction had commenced, suggested that highly reactive nitrate reduction intermediates might be oxidizing previously reduced uranium.

To test the hypothesis that U(IV) was oxidized by intermediates as nitrate reduction proceeded, U(VI) and U(IV) were added independently to autoclaved sediments. The sediments used in these tests contained higher amounts of clay and fine-grained material to achieve more U(VI) adsorption and allow us to differentiate between remobilization via U(IV) oxidation or via liberation from solid phases to which U(VI) adsorbed. Within 30 min, 51% of the U(VI) had adsorbed to these sediments (as opposed to 17% in sandy sediments used for the nonsterile incubations to assess biological activity). After a 7-d equilibration period, 68% of the U(VI) was reduced, and 24% was solids-associated. More abiotic U(VI) reduction was observed in the heat-killed

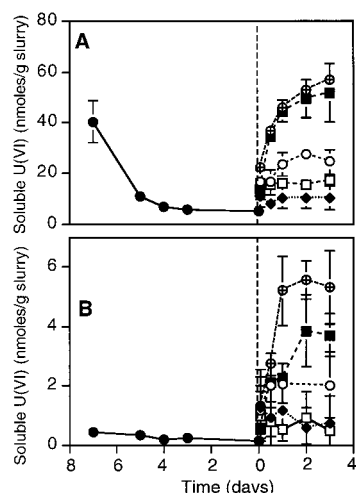


FIGURE 4. Oxidation and remobilization of uranium in the presence of denitrification intermediates. U(VI) was added to heat killed sediments (A) and allowed to equilibrate for 7 d (●), during which time 68% of the U(VI) was reduced and 24% was adsorbed to sediments. (B) U(IV) was added in parallel sediment incubations. The dashed line represents the point at which intermediates were added, and soluble U(VI) was monitored in the presence of (□) nitrate, (■) nitrite, (⊕) nitric oxide, (○) nitrous oxide, and (◆) nitrogen gas. The dashed line at 0 d represents the point at which nitrogen oxides were added. Error bars represent standard deviation of duplicate incubations.

sediments in these experiments than the previous set, which were used to assess biological reduction. This was likely a result of different mineralogical characteristics in the sediments and may be attributed to a combination of the abiotic pathways of U(VI) reduction noted above. Sediments surrounding the wells have been shown to contain high levels of iron sulfides (20), which could be involved in U(VI) reduction. Marsh et al. (44) also observed abiotic Cr(VI) reduction in dark clayey sediments from the same aquifer, but abiotic Cr(VI) reduction did not occur in light-colored, sandy sediments. When dissimilatory nitrate reduction intermediates (NO_2^- , N_2O , NO) were injected into these bottles, rapid uranium oxidation/remobilization occurred, providing evidence that the remobilization observed in laboratory experiments and push-pull tests was due to oxidation of U(IV) and reduced adsorbent minerals (i.e., iron sulfides) by denitrification intermediates (Figure 4). Nitrite and nitric oxide lead to the most extensive U(IV) oxidation/remobilization. When nitrite and nitric oxide were added to bottles initially treated with U(VI), all of the U(IV) present was oxidized, with any U(VI) not in solution being in the solids-associated fraction (20% solids-associated, 80% soluble). In incubations initially treated with U(IV), approximately 18% of the U(IV) was oxidized by nitrite and nitric oxide. Preliminary light-microscopic examinations of biogenic UO_2 and commercial UO_2 (Alfa Products, Danvers, MA) revealed that commercial UO_2 is considerably larger (approximately 5–7 μm diameter) than that recovered from resting cell incubations (less than 1 μm in diameter) of *D. desulfuricans*, performed as described by Lovley and Phillips (6). On the basis of particle diameter, it is reasonable to predict that biogenic UO_2 would present a greater surface area than commercial UO_2 and might be more easily oxidized.

The Gibbs free energies were calculated for some reactions in which U(IV) is oxidized with the reduction of inorganic nitrogen compounds involved in denitrification. These calculations reveal that U(IV) oxidation by nitrate and dinitrogen are less favorable than U(IV) oxidation by nitrite, nitric oxide, and nitrous oxide (Table 4). The presence of sulfides in the test sediments may also have accentuated the

TABLE 3. Sequential Extraction of Oxidized and Reduced Uranium Species at Conclusion of Push-Pull Tests

	well A (μmol of U/ kg of sediment) ^a	well D (μmol of U/ kg of sediment) ^b
U(IV) (nitric acid-extractable fraction)	784	460
solids-associated U(VI) (bicarbonate-extractable fraction)	61	36
soluble U(VI) (water-extractable fraction)	48	32

^a Well had been treated with 1.5 μM U(VI) for two rounds of tests.

^b Well had been previously treated with 1.5 μM U(VI) and 17 mM formate, followed by 1.5 mM U(VI) and 1 mM nitrate.

TABLE 4. Gibbs Free Energies of Reactions Coupling the Oxidation of Amorphous Uraninite ($\text{UO}_2(\text{am})$) to the Reduction of Various Compounds Involved in Denitrification^a

Reaction	ΔG_R° (kJ/mol)
$\text{UO}_2 + 0.33 \text{N}_2 + 2.67 \text{H}^+ \rightarrow \text{UO}_2^{2+} + 0.33 \text{NH}_4^+$	+80
$\text{UO}_2 + \text{N}_2\text{O} + 2 \text{H}^+ \rightarrow \text{UO}_2^{2+} + \text{N}_2 + \text{H}_2\text{O}$	−235
$\text{UO}_2 + 2\text{NO} + 2\text{H}^+ \rightarrow \text{UO}_2^{2+} + \text{N}_2\text{O} + \text{H}_2\text{O}$	−200
$\text{UO}_2 + \text{NO} + 2\text{H}^+ \rightarrow \text{UO}_2^{2+} + 0.5 \text{N}_2 + \text{H}_2\text{O}$	−218
$\text{UO}_2 + 0.4 \text{NO} + 2.2 \text{H}^+ \rightarrow \text{UO}_2^{2+} + 0.4 \text{NH}_4^+ + 0.4 \text{H}_2\text{O}$	−47
$\text{UO}_2 + 0.33 \text{NO}_2^- + 2.67 \text{H}^+ \rightarrow \text{UO}_2^{2+} + 0.33 \text{NH}_4^+ + 0.67 \text{H}_2\text{O}$	−159
$\text{UO}_2 + 0.67 \text{NO}_2^- + 2.67 \text{H}^+ \rightarrow \text{UO}_2^{2+} + 0.33 \text{N}_2 + 1.33 \text{H}_2\text{O}$	−40
$\text{UO}_2 + \text{NO}_3^- + 2\text{H}^+ \rightarrow \text{UO}_2^{2+} + \text{NO}_2^- + \text{H}_2\text{O}$	−57
$\text{UO}_2 + 0.25 \text{NO}_3^- + 2.5 \text{H}^+ \rightarrow \text{UO}_2^{2+} + 0.25 \text{NH}_4^+ + 0.75 \text{H}_2\text{O}$	−44
$\text{UO}_2 + 0.4 \text{NO}_3^- + 2.4 \text{H}^+ \rightarrow \text{UO}_2^{2+} + 0.2 \text{N}_2 + 1.2 \text{H}_2\text{O}$	−118

^a Values calculated from Dean (11).

effects of nitric oxide and nitrous oxide. Nitrous oxide and nitric oxide reductases are inhibited by sulfide (45), and the respective intermediates have been shown to accumulate in transition zones from nitrate reduction to sulfate reduction in coastal marine sediments (46).

Our results suggest that U(VI) can be reduced through the concerted action of microbiological and geochemical processes. However, dissimilatory nitrate reduction could lead to conditions in which U(IV) is oxidized. We do not know if the oxidation observed in the nonsterile incubations and push-pull tests is due exclusively to abiotic oxidation by denitrification intermediates or if there is a role for lithotrophic sulfide, Fe(II), or U(IV) oxidation coupled to nitrate reduction. The simplest explanation for our observations is that uranium remobilization during nitrate reduction is an abiotic process, occurring as dissimilatory nitrate reduction intermediates accumulate. Fe(II) is oxidized by nitrite and nitric oxide in the same manner (47). If Fe(II) is oxidized by denitrification intermediates, it is possible that the Fe(III) produced may oxidize U(IV) (48). Several denitrifying organisms have been shown to couple Fe(II) oxidation to nitrate reduction either lithotrophically or mixotrophically at neutral pH (49–52). Interestingly, the organisms isolated by Straub et al. (49) were able to oxidize insoluble iron(II) phosphates and carbonates, suggesting that insoluble reduced metal minerals such as UO_2 can act as electron donors for lithotrophic organisms. An instance of lithotrophic U(IV) oxidation by *Thiobacillus ferrooxidans* under aerobic and acidic conditions has been reported (53). Therefore, lithotrophic U(IV) oxidation coupled to nitrate reduction is conceivable (Table 4) but not documented under neutral conditions.

This work provides an explanation for persistent U(VI) solubility in contaminated sites in which nitrate is present. Evolution of the dissimilatory nitrate reduction intermediates apparently creates a highly oxidizing environment, leading

to the oxidation of U(IV), reversing the reducing conditions required for uranium immobilization. If electron donor is injected into a U(VI)-impacted aquifer as part of a remediation strategy, nitrogen could be cycled in the sediments, maintaining conditions favorable for U(IV) oxidation. Therefore, it will be necessary to stimulate the removal of nitrogen from systems via denitrification before the immobilization of U(VI) can commence.

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