

July 28, 2004

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Appl. Environ. Microbiol. 2004, 70(12):7558. DOI:
10.1128/AEM.70.12.7558-7560.2004.

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Resistance of Solid-Phase U(VI) to Microbial Reduction during In Situ Bioremediation of Uranium-Contaminated Groundwater

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Received 21 June 2004/Accepted 28 July 2004

Speciation of solid-phase uranium in uranium-contaminated subsurface sediments undergoing uranium bioremediation demonstrated that although microbial reduction of soluble U(VI) readily immobilized uranium as U(IV), a substantial portion of the U(VI) in the aquifer was strongly associated with the sediments and was not microbially reducible. These results have important implications for in situ uranium bioremediation strategies.

The large volumes of uranium-contaminated groundwater at many uranium-contaminated sites precludes pump and treat remediation strategies. In situ immobilization of uranium via microbial reduction of soluble U(VI) to insoluble U(IV) is a potential strategy for preventing further migration of uranium with the groundwater (11, 12). Addition of acetate to a uranium-contaminated aquifer effectively stimulated the growth of dissimilatory metal-reducing microorganisms in the family *Geobacteraceae* and the removal of U(VI) from the groundwater (1) in accordance with previous predictions from laboratory incubations of uranium-contaminated sediments (4, 5, 7). Continuing field analysis has suggested that once uranium is precipitated from groundwater, it is immobilized for substantial periods of time after the acetate additions are stopped (unpublished data).

Speciation of uranium in subsurface sediments from a bioremediation site. In order to further evaluate the immobilization of uranium during in situ bioremediation, sediment cores were collected from the previously described uranium bioremediation site in Rifle, Colo. (1), from depths of 4.0 to 5.2 m by a diamond-rotosonic method (Boart Longyear, Environmental Drilling Division, Little Falls, Minn.), 38 days into a second summer of acetate injection. Core samples were sectioned and transferred in the field to an anaerobic (N_2 -filled) portable glove bag, and subsamples were placed in anaerobic pressure tubes and packed on ice for transport back to the laboratory. In the laboratory, sediments were extracted (1 g of sediment per 5 ml of extractant) under anaerobic conditions in a glove bag (83:10:7 N_2 - CO_2 - H_2) with 100 mM sodium bicarbonate (15) for 48 h. Preliminary studies with subsurface sediments from the study site demonstrated that this procedure was sufficient to extract over 90% of the U(VI) that could be extracted with 1 M nitric acid. U(VI) in the extract was measured with a kinetic phosphorescence analyzer (4, 15). The sediment extractions were then aerated for 24 h to oxidize U(IV) to U(VI) (6), and U(VI) was again measured. U(IV)

was calculated as the increase in U(VI) in the extract following aeration.

Sediments from locations in which U(VI) had been effectively reduced in the groundwater contained U(IV), but most of the uranium associated with the sediments was in the form of U(VI) (Fig. 1). There were also substantial quantities of U(VI) in sediments upgradient from the acetate injection zone. This fact indicated that U(VI) adsorption was not associated with the in situ uranium bioremediation. When a given sediment volume is considered, U(VI) associated with the sediments was ca. eightfold more abundant than U(VI) in the groundwater.

Sediment incubations. In order to evaluate the apparent persistence of U(VI) in the acetate-injection zone under strictly anaerobic conditions, sediments (115 g) from outside the acetate injection zone at the Rifle study site were incubated with associated uranium-contaminated groundwater (30 ml) under anaerobic conditions in sealed serum bottles as previously described (4, 5). These sediments, which are naturally partially reduced, as evidenced by the presence of Fe(II), contained U(IV) and well as U(VI) (Fig. 2A). If no amendments were made to the sediments, there was an increase in soluble U(VI) and a decline in U(VI) associated with the sediments, presumably due to reequilibration of the sediment-bound uranium with the added groundwater. At apparent equilibrium, the soluble and sorbed U(VI) fractions contributed nearly equally to the total U(VI) pool. Addition of acetate (2 mM) to simulate in situ uranium bioremediation stimulated microbial metal reduction, as was evident from an accumulation of Fe(II) as well as a loss of U(VI) from the groundwater and an accumulation of U(IV) in the sediments (Fig. 2B). However, there was no loss of U(VI) from the sediments, suggesting that the U(VI) associated with the sediments was resistant to microbial reduction.

In order to further evaluate this phenomenon, a second study was conducted in which the concentration of U(VI) was artificially increased with the addition of an additional 20 μ M U(VI) (Fig. 3A). Circa 40% of the added U(VI) was lost from solution and adsorbed onto the sediments, further demonstrating that sorption of uranium can be an important sink for uranium in the subsurface. The addition of acetate (5 mM) to stimulate dissimilatory metal reduction enhanced the adsorp-

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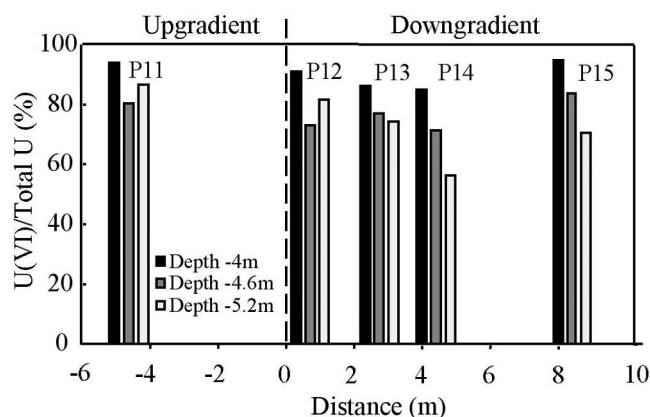


FIG. 1. Percentages of U(VI) out of the total U of sediment cores collected at three different depths in a uranium-bioremediation site located in Rifle, Colo. The dashed line indicates the position of the acetate injection gallery. P11, upgradient sediment core; P12 to P15, downgradient cores from the point of acetate injection into the subsurface. The results are the means of results of five replicates, and the standard deviations were below 10% of the means.

tion of U(VI) relative to that with controls and resulted in increased removal of U(VI) from the groundwater, which was accompanied by an increase in U(IV) in the sediments (Fig. 3B). As Fe(III) was depleted and sulfate reduction became the predominant process, the loss of soluble U(VI) and the production of reduced U(IV) both stopped. This result is consistent with previous observations from field experiments which have suggested that Fe(III) reducers, but not sulfate reducers,

are effective in reducing soluble U(VI) to insoluble U(IV) in contaminated aquifer sediments in the presence of acetate (1).

Implications. U(VI) adsorption onto iron oxides and hydroxides (2, 3, 8, 10, 16, 17) and clays (9, 13, 14) in sediments is well known. However, previous studies of in situ uranium bioremediation have primarily focused on the reduction of dissolved U(VI) to insoluble U(IV) because immobilization of contaminant uranium is of paramount importance. The results presented here suggest that although dissimilatory metal-reducing microorganisms can effectively reduce soluble U(VI), U(VI) associated with the solid phase is not microbially reducible. The inability of microorganisms to reduce U(VI) adsorbed to sediments is not a limitation during the acetate injection phase of in situ bioremediation because this U(VI) is already immobile. However, it does have an impact on strategies for eventually extracting the immobilized uranium. For example, it has previously been proposed that once uranium has been immobilized in a discrete zone via U(VI) reduction, it may be resolubilized and extracted by reoxidizing precipitated U(IV) to soluble U(VI) (5). The finding that a high proportion of the immobilized uranium is likely to be in the form of U(VI) suggests that the oxidation of U(IV) should be supplemented with procedures for extracting U(VI), such as the use of bicarbonate (15), a common extraction technique for in situ uranium mining. In a similar manner, the injection of bicarbonate upgradient of a zone of acetate injection may solubilize U(VI) from sediments, which may be followed by reductive precipitation within the acetate injection zone to remove uranium which otherwise may later serve as a source of dissolved U(VI) in the groundwater via desorption. Further investigations into the form of the U(VI) associated with sed-

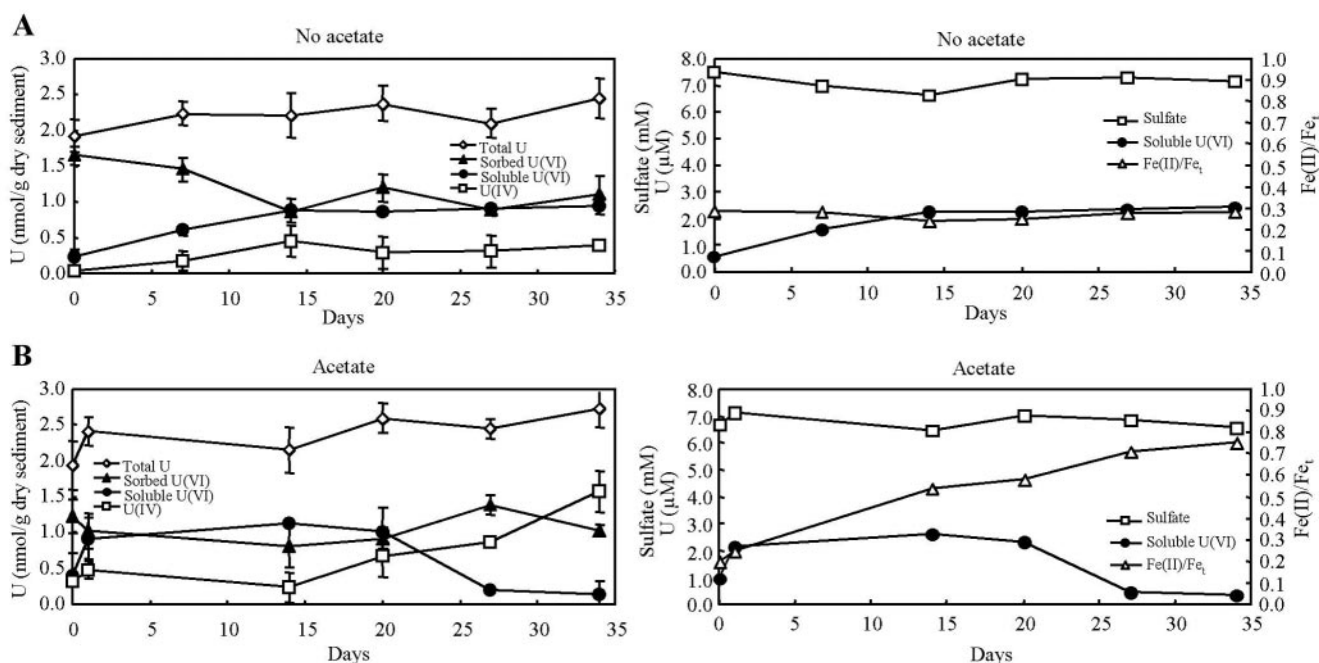


FIG. 2. Sediment and soluble uranium, sulfate, and ratio of Fe(II) to total acid-extractable iron (Fe_t) in contaminated aquifer sediments (115 g) with uranium-contaminated groundwater (30 ml) incubated with (B) or without (A) added acetate (2 mM). The results are the means of results of triplicate sediment incubations.

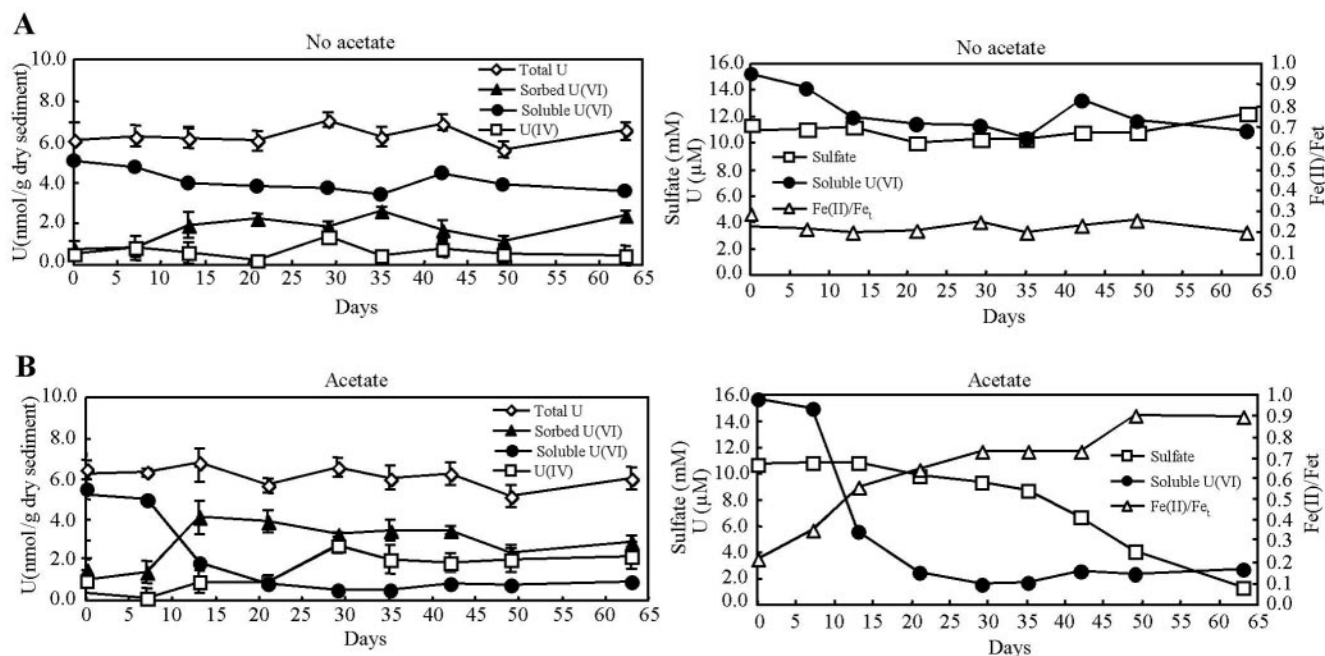


FIG. 3. Sediment and soluble uranium, sulfate, and ratio of Fe(II) to total acid-extractable iron (Fe_t) in contaminated aquifer sediments (115 g) with uranium-contaminated groundwater (30 ml) amended with an additional 20 μ M U(VI) and incubated with (B) or without (A) added acetate (5 mM). The results are the means of results of quintuplet extractions made on duplicate sediment incubations.

iments and the mechanisms preventing the microbial reduction of this U(VI) are warranted, as such studies may provide insights into strategies for promoting the microbial reduction of this important pool of U(VI) in uranium-contaminated aquifers.

This research was supported by the Office of Science (BER), U.S. Department of Energy, grant DE-FG02-04ER63719. I.O.-B. was the recipient of a postdoctoral fellowship from the Secretaría de Estado de Educación y Universidades (Madrid, Spain), cofunded by the European Social Fund.

We thank Philip E. Long (Pacific Northwest National Laboratory), Aaron Peacock (Microbial Insights), and Richard Dayvault (S. M. Stoller Corporation) for core sample collection. We also thank Donald R. Metzler (U.S. Department of Energy) for coordinating activities conducted at the Old Rifle UMTRA site.

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