Biological Reduction of Uranium in Oak Ridge Source Zone Sediment

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Introduction

The former S-3 Waste Disposal Ponds located at Oak Ridge National Laboratory (ORNL) were constructed in 1951 and consisted of four unlined surface impoundments. They received liquid nitric acid and uranium-bearing waste from the Y-12 plant via a pipeline at a rate of 10 million gallons per year until 1983. The ponds were neutralized and biodenitrified in 1984 and subsequently capped in 1988. These waste disposal activities created a mixed plume of contamination in the underlying unconsolidated residuum (primarily saprolite and fill) and shale bedrock. This plume is quite acidic (pH of 3) and contains, on average, several hundred milligrams of uranium per liter, tens of grams of nitrate per liter, and several molar aluminum [1]. The plume moves primarily along the saprolite's strike, with surface discharge to Bear Creek and several of its tributaries.

One promising approach to subsurface immobilization of uranium is *in situ* transformation of soluble U(VI) to insoluble U(IV) by the stimulation of naturally occurring metal-reducing microbial communities [2]. These microbial communities are generally capable of using nitrate and certain metals, including uranium, as terminal electron acceptors. This *in situ* remediation technique is being tested at Area 3 of the U.S. Department of Energy (DOE) Natural and Accelerated Bioremediation Research (NABIR) Field Research Center (FRC) at ORNL. The extreme conditions of the contaminant plume (low pH, high total dissolved solids [TSD]) make *ex situ* water conditioning (denitrification, pH adjustment, etc.) necessary prior to stimulation of microbial activity and *in situ* precipitation of uranium. Here we investigate what conditions are required for uranium reduction, what types of microbial communities might be responsible for *in situ* uranium reduction, and the time scale of uranium bioreduction under optimal conditions.

Methods and Materials

We examined the microbial reduction of uranium in the presence of sediments from ORNL in a series of batch experiments. Batch experiments were prepared in batch tubes under anaerobic conditions by using 4 to 5 g of sediment from a radionuclide-contaminated site at ORNL. Then 10 mL of synthetic groundwater media, denitrified with a fluidized-bed reactor, were then to each tube. Various organic carbon sources were tested to determine which combination most effectively reduced U(VI) to U(IV) under conditions similar to those found in contaminated subsurface areas at ORNL. Control experiments were sterilized by autoclaving. Vials were maintained in the dark and inverted several times a day over the course of each experiment.

In experimental series #1, 5 g of three different types of soil from the ORNL subsurface were used. Soil type "red" was from an oxidized zone and contained 256 mg/kg U and 0.07 μmol/g nitrate. Soil type "green" contained 155 mg/kg U and 20.9 μmol/g nitrate, and was from a high-flow zone. Soil type "black" was high in Mn and Fe, with 730 mg/kg U and 95.4 μmol/g nitrate. One third of the batch tubes lacked an electron donor and were not inoculated with metal-reducing bacteria. Another third of the tubes contained 500 g electron donor (ethanol/lactate mix). The final third contained 500 g electron donor and were inoculated with a metal-reducing bacterial enrichment from ORNL. Three replicates of each type were sacrificed after 30 days and homogenized for more complete analysis.

The sediment used in experimental series #2 and #3 was obtained from the depth of bioremediation below Area 3 at the FRC. In experimental series #2, various combinations of 5 mL of effluent from a denitrifying fluidized-bed reactor (FBR) (eff), concentrated biomass from the FBR (bio), and 2 g/L of ethanol (ED) were added to 4 g of sediment and 50 mg/L of uranium. Three replicates of each combination were sacrificed after 65 days and homogenized for more complete chemical, mineralogical, and microbiological analysis.

Each tube for experimental series #3 contained 4 g of soil, 61 mg/L U, and 22 mM of ethanol and was inoculated with 5 mL of effluent from the denitrifying FBR. Control samples identical to the viable samples were sterilized. Sets of three control and viable tubes were sacrificed on days 1, 14, 27, and 63.

Aqueous samples were taken from batch systems and analyzed for soluble U(VI) by using a kinetic phosphorescence analyzer (KPA).

The oxidation state of solid-phase uranium and iron was determined by using x-ray absorption near-edge structure (XANES) spectroscopy. X-ray absorption spectroscopy was performed at beamline station 13-BM-C at the APS. XANES analysis was conducted by using WinXAS and SixPACK [3]. Spectra were background subtracted by using a low-order polynomial and normalized by setting the total cross-sectional

absorption to unity. The first derivative of each spectra was obtained by using a Savitky-Golay algorithm. Fe and U oxidation states were determined by comparing the main-edge peak position in the first derivative of the samples to the main-edge peak position of Fe and U standards.

Results

Experimental Series #1

Uranium reduction was not observed in any of the control cultures (data not shown). Little or no uranium reduction was observed in cultures containing red sediment (Fig. 1), probably as a result of the presence of large amounts of oxidized solids. Slight uranium reduction was observed in green and black cultures, but the presence of high levels of nitrate inhibited more extensive reduction (Fig. 1), since the electron donor amendment was only 19 mg COD per culture, while nitrate demand was 4.18 g COD and 19.1 g COD, respectively, for cultures containing green and black sediment types.

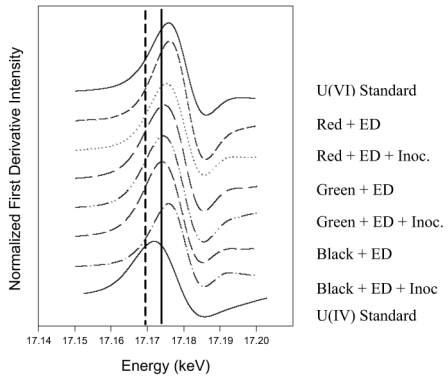

FIG. 1. First-derivative U L_{III} XANES spectra of samples from experimental series #1. Solid vertical line indicates U(VI) edge position (17.176 keV), and dashed vertical line indicates U(IV) edge position (17.173 keV).

Experimental Series #2

Soluble uranium decreased rapidly in sediment-free systems (Fig. 2). In these systems, reduced uranium was associated with the bacterial cells in all preparations (Fig. 3).

In systems containing contaminated sediment in the absence of viable cells (FBR effluent or biomass), sediment-associated uranium remained oxidized (Fig. 4). Additionally, the soluble uranium concentration in the abiotic systems stabilized at roughly one quarter of the initial uranium concentration (Fig. 5).

When viable cells (effluent and/or biomass) were added to the system, the soluble uranium concentration decreased to less than 5 mg/L (Fig. 5). The solid-

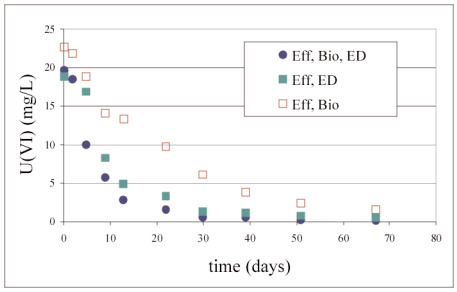

FIG. 2. Soluble uranium concentration as a function of time in systems inoculated with denitrifying bacteria and no sediment. Effluent from FBR = Eff, electron donor = ED, and biomass from FBR = Bio.

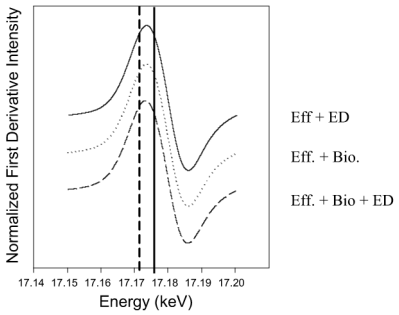

FIG. 3. First-derivative U L_{III} XANES spectra of biomass exposed to U(VI) at day 60. Solid vertical line indicates U(VI) edge position (17.176 keV), and dashed vertical line indicates U(IV) edge position (17.173 keV).

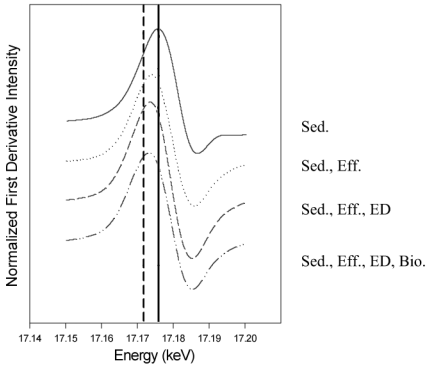

FIG. 4. First-derivative U L_{III} XANES spectra of sediment exposed to U(VI) at day 60. Solid vertical line indicates U(VI) edge position (17.176 keV), and dashed vertical line indicates U(IV) edge position (17.173 keV).

associated uranium in these inoculated systems was largely reduced (Fig. 4) regardless of the type of amendment. Iron reduction was not observed in any of the preparations.

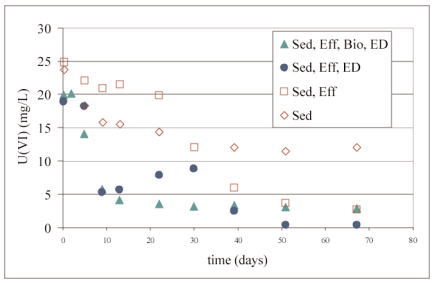

FIG. 5. Soluble uranium in inoculated systems containing sediment.

Experimental Series #3

Neither reduced iron (data not shown) nor reduced uranium (Fig. 6) was observed in control cultures. Solid-associated uranium was largely reduced after only one day of exposure to viable bacterial cells (Fig. 6), while iron reduction was not observed during the course of the experiment.

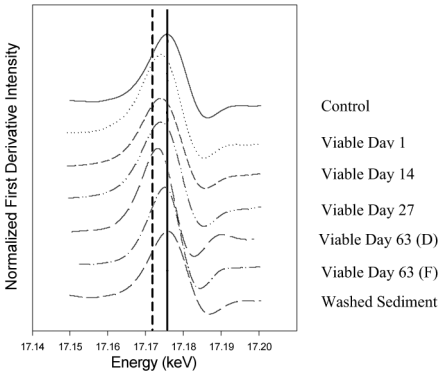

FIG. 6. First-derivative U L_{III} XANES spectra of contaminated sediment over time. Solid vertical line indicates U(VI) edge position (17.176 keV), and dashed vertical line indicates U(IV) edge position (17.173 keV). (D) refers to cultures in which soluble uranium continually decreases, while (F) refers to those in which soluble U leveled off.

Average aqueous uranium concentrations continued to decline noticeably until day 30 (Fig. 7). At this point, the soluble uranium concentrations leveled off in some cultures and continued to decline in others, resulting in the large error bars in Fig. 7. The sediment-associated uranium in both of these cases remained largely reduced (Fig. 6).

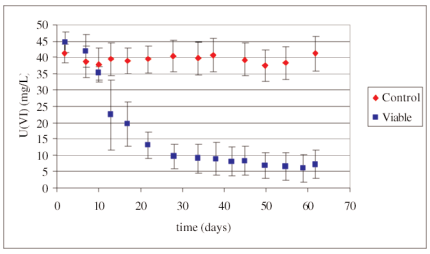

FIG. 7. Average soluble U(VI) concentration in all sediment cultures. Data points represent the average of at least 12 data points, and the error bars represent one standard deviation.

Conclusions

Uranium was reduced in the presence of contaminated sediments by a metal-reducing enrichment from the FRC as well as by bacteria from a denitrifying FBR similar to the one being used at Area 3 to treat water. These two types of bacterial communities are expected dominate the subsurface microbial community during bioremediation operations. The presence of high levels of nitrate inhibited uranium reduction, justifying the *ex situ* denitrification of the groundwater prior to stimulation of subsurface uranium reduction.

The initial reduction of soil-associated uranium occurred quickly, and reduction of soluble uranium continued for more than 60 days. Once uranium reduction ceased, resolubilization of the sequestered uranium did not occur. The stimulation of metal-reducing bacteria in the Oak Ridge subsurface, along with the addition of denitrifying bacteria and effluent from a FBR, should result in the reduction and sequestration of uranium contamination from S-3 waste disposal ponds.

Acknowledgments

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