Spectroscopic Confirmation of Uranium Bioreduction in a Contaminated Subsurface System

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Introduction

The fate and transport of uranium in the environment is governed by its oxidation state. Uranium in groundwater commonly exists in one of two oxidation states. In aerated environments, uranium is generally found in the hexavalent form and is quite soluble. U(VI) solubility is increased several orders of magnitude by complexation with carbonate, a common groundwater ligand, rendering U(VI) highly mobile in groundwater systems [1,2]. Conversely, reduced U(IV) species are only sparingly soluble and thus immobile within soils and sediments [1,2]. Under anaerobic conditions, a diverse set of microorganisms are known to reduce U(VI) to U(IV), leading to its precipitationas the sparingly soluble uraninite phase [6-9]. *In situ* bioreduction of U(VI) may therefore offer an attractive means of immobilizing uranium in soil and sediments.

The feasibility of this approach was evaluated using a column packed with U(VI)-contaminated soil obtained from the S-3 waste disposal area at the US DOE BWXT Y-12 site in Oak Ridge, Tennessee. The subsurface at the site is highly contaminated with uranium and other pollutants that were discharged into the S-3 ponds [10]. Near the source, contaminated groundwater has a low pH (3.5), and high concentrations of U(VI) (up to ~60 mg/L), nitrate (40,000 mg/L), and sulfate (1,000 mg/L). Soil within this zone has high levels of precipitated or sorbed U(VI) (up to 800 mg/kg), calcium, aluminum, and phosphate. Although sorption or precipitation of U(VI) has provided a natural mechanism of U(VI) attenuation, groundwater U(VI) levels remain unacceptably high, underscoring the need for an effective means of immobilization. To date, studies of the bioreduction of U(VI) have mostly been performed in homogeneous solutions and have shown that U(VI) can be rapidly reduced to U(IV) species by a diverse set of microorganisms [6-9]. However, relatively few studies have examined the bioreduction of U(VI) in soil or sediments, particularly in such a highly contaminated site [10]. Therefore, the present study was aimed at evaluating the bioreduction of U(VI) in soil columns as part of a larger-scale field investigation of *in-situ* reduction of U(VI) at the Oak Ridge Field Research Center.

Methods and Materials

Soil Column Preparation

A contaminated sediment core (sample FWB 104-00-38) was collected from a depth of 11.6 to 13.1 m from the DOE NABIR field research center (FRC) in Oak Ridge, Tennessee. The moist soil was gently crushed into small aggregates, which were then carefully packed into a glass column (25×150 mm). The column was first equilibrated with a pH 4 solution of 10 mM KCl and 10 mM NaCl. The column was then conditioned for ~20 h by flushing with a solution consisted of 30 mM $NaHCO_3$, 30 mM $KHCO_3$, and 5 mM Na_2SO_4 at pH 7.0. Based on the concentration of U(VI) in the column effluent, less than three percent of the solid phase U(VI) was removed during the preceding steps.

Soil column operation for U(VI) reduction

After flushing and conditioning, the soil column was operated anaerobically with a closed-loop continuous recirculation of effluent through the column. The reservoir was filled with 80 mL of the bicarbonate-nutrient solution consisting of $NaHCO_3$ (30 mM), $KHCO_3$ (30 mM), trimetaphosphate (3.0 mg/L) and NH_4Cl (10 mg as N/L) with a nitrogen headspace. Ethanol was added to the reservoir to an initial concentration of 3 mM, and system recirculation was then started (day 1) at a flow rate of 0.2 mL/min. Samples (2 mL) were withdrawn from the reservoir using a sterilized syringe weekly and replaced by the same volume of oxygen-free bicarbonate solution. Neither ethanol consumption nor U(VI) bioreduction were observed in the soil column between day 1 and day 30 (Fig. 1).

The column was subsequently inoculated with 2 mL of a denitrifying bacterial culture. The column was operated continuously after inoculation and the effluent sampled weekly. Subsamples were analyzed for U(VI), ethanol, and acetate concentrations.

Solution Phase Analysis

Total U(VI), Fe, and Mn were determined using an inductively coupled plasma mass spectrometer (ICP-MS) Anions (including NO_3^-, Cl^-, SO_4^{2-} and PO_4^{3-}) were analyzed with an ion chromatograph equipped with an IonPac AS-14 analytical column and an AG-14 guard column (Dionex DX-120, Sunnyvale, CA). Ethanol, acetate, and methane were analyzed with a HP6890 gas chromatograph equipped with a FID detector.

Solid Phase Analysis

To validatebioreduction of U(VI), X-ray absorption near edge structure (XANES) spectroscopy was used to determine the oxidation state of uranium after completion of the experiment. The column was broken apart, and sediment samples removed from the bottom, middle, and top sections of the column. Samples were dried in an anaerobic glovebox, mounted on a Teflon plate, and sealed with Kapton polymide film to prevent oxidation while minimizing X-ray absorption. XANES spectra were collected on beamline 13-BM-C (GSE-CARS) at the Advanced Photon Source (APS). The APS ring was operated at 7 GeV with a current of 100 mA, and energy selection was accomplished with a water-cooled Si(111) monochromator. Higher-order harmonics were eliminated by detuning the monochromator ~40%. Fluorescence spectra were recorded by monitoring the U $L_{III\alpha}$ fluorescence with a 16-element Ge semiconductor detector. Incident and transmitted intensities were measured with in-line ionization chambers. The energy range studied was -200 to +500 eV about the $L_{III\alpha}$-edge of U (17.166 keV). All spectra were collected at ambient temperature

and pressure, and between 2 and 4 individual spectra were averaged for each sample. Spectra were analyzed using IFEFFIT and WinXAS software. Fluorescence spectra were normalized, background subtracted and the atomic absorption normalized to unity. First derivative XANES spectra were smoothed with 17.6% Savitsky-Golay smoothing. The relative amount of U(IV) in each sample was determined by fitting a series of Gaussian functions to the smoothed derivative spectra using PeakFit v4 (AISN Software Inc). The ratio of the amplitudes of the Gaussian functions centered at the U(IV) and U(VI) first derivative inflection points (17.173 and 17.176 keV, respectively) was related to U(IV)/(VI) proportions using five standards having U(VI) percentages ranging from 10 to 90%. The uncertainty of the fitting routine is ±10%.

Results and Discussion

Bioreduction of U(VI)

During the bicarbonate conditioning, the column effluent pH increased from 4.0 to 6.7. The column was subsequently operated as a closed-recirculation system using ethanol as the electron donor for microbial uranium reduction. During the first month of operation, the effluent pH decreased from 6.7 stabilizing 6.6 for more than 20 days. During this period, the was no evidence of ethanol consumption or U(VI) reduction (Fig. 1). This suggests that the indigenous microbial population capable of ethanol consumption and/or uranium reduction were small or not active.

On day 30 the column was inoculated with a low level of biomass obtained from a pilot-scale denitrifying fluidized bed bioreactor. Immediately after inoculation, ethanol degradation commenced with complete consumption by day 45 (data not shown).

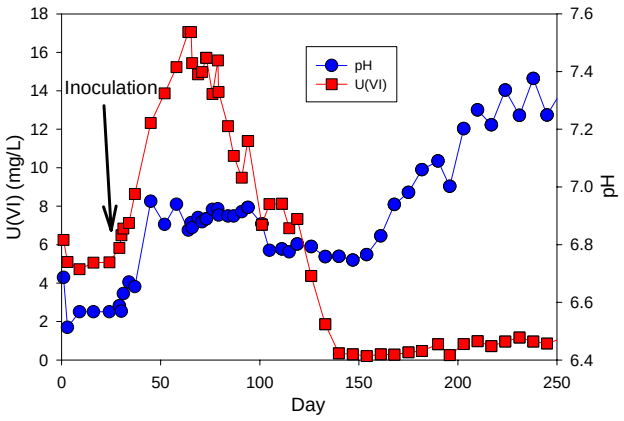

Fig. 1. The pH and U(VI) profiles in the column effluent during the course of biostimulation.

During the initial biostimulation period (day 30 – 70), the U(VI) concentration in the pore water increased from 5.3 to ~12.3 mg/L, peaking on day 64 at 17 mg/L when the pH was 6.9 (Fig. 1). This initial increase in U(VI) concentrations was the result of the bicarbonate production and increase in pH from microbial activity. U(VI) sorption within these sediments is strongly dependent on pH and bicarbonate concentrations [11]. If the rate of U(VI) bioreduction was slower than the U(VI) desorption/dissolution rate, the aqueous U(VI) concentration will increase continuously. With continued electron donor addition, the bioreduction rate should eventually exceed the desorption/dissolution rate resulting in a decrease in aqueous U(VI).

Indeed, on day 85 the U(VI) concentrations decrease from ~17 to 12.2 mg/L after introduction of 4 mM ethanol. Ethanol was generally completely consumed within one week. During this period the pore water U(VI) concentration continuously decreased, reaching 0.3 mg/L on day 140 (Fig. 1). The slight increase in U(VI) concentration after day 220 was due to an concomitant increase in pH.

Validation of U(VI) Bioreduction by XANES Spectroscopy

The presence of bioreduced U(IV) species in the soil column was confirmed by XANES spectroscopic analysis following dissection of the column at the completion of the biostimulation experiment. The soil column was taken apart and sectioned into three parts (bottom, middle, and top) in an anaerobic chamber. U(IV) was found in all column sections, particularly in the middle of the soil column, where approximately 47% of total uranium was present as U(IV) species (Fig. 2). Lower percentages of U(IV) were found in the bottom and top sections (~10% and 20%, respectively) of the soil column (Fig. 2). These results provide direct evidence that U(VI) in the contaminated sediment was being biologically reduced and immobilized.

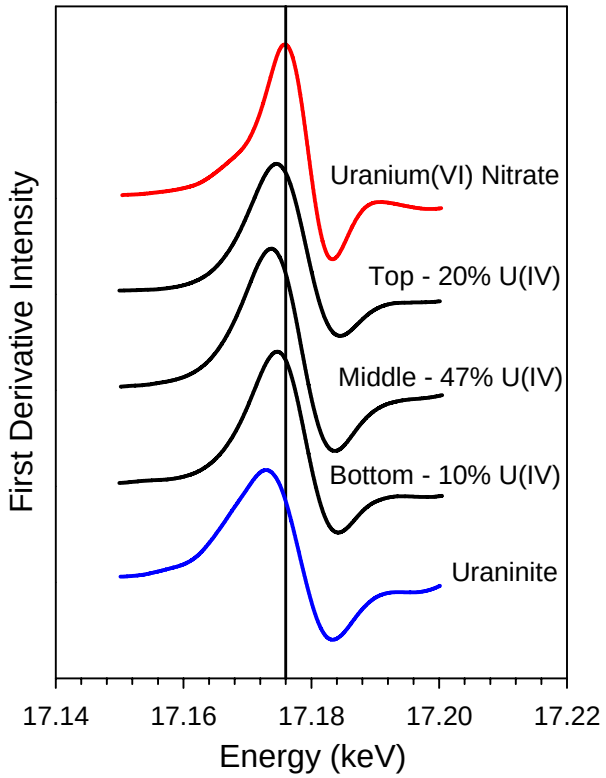

Fig. 2. First derivative X-ray absorption near edge structure (XANES) spectra of uranium in soil samples taken from the top, middle and bottom sections of the soil column. Vertical line indicates U(VI) derivative maxima (17.176 keV)

The fact that residual U(VI) remained in the soil column after more than 3 months of extended bioreduction period (after observing a low effluent U(VI) concentration at day 140) could be attributed to several factors, including: (i) a large portion of U(VI) is sorbed inside aggregates and not accessible; (ii) methanogenesis likely decreased the rate of U(VI) bioreduction; and (iii) some U(IV) species might have been reoxidized to U(VI).

Potential reoxidation is likely because the column remained static for approximately one month prior to destructive sampling and analysis by XANES spectroscopy. Oxygen may have slowly diffused into the soil column through the bottom and top end plugs (made of Teflon) causing partial reoxidation of U(IV). The fact that the top and bottom of the soil column showed the lowest U(IV) percentages supports this assumption. Reoxidation of U(IV) species is rapid in the presence of O_2. Previous studies have shown that the reoxidation of U(IV) can occur on the order of a few hours to days upon contacting air [2]. Other oxidants such as MnO_2 or nitrate and nitrite can also inhibit bioreduction of U(VI) or cause the reoxidation of reduced U(IV) although we have no evidence that such oxidants played a role in this study [4].

Implications

This study clearly demonstrates that oxidized forms of U(VI) (either sorbed or precipitated) in contaminated soil/sediment can be reduced to relatively insoluble U(IV) by the recirculation of groundwater amended electron donors, provided appropriate microbial populations are present and active. The results of this study are consistent with previous findings that microbial reduction of U(VI) to U(IV) may offer an effective remediation strategy to immobilize uranium in soil and groundwater, and they support the proposed sequence of field operations planned for the NABIR field research site. During bioreduction, other reduced minerals, such as iron sulfides, are produced. These minerals help to maintain a low redox condition in soil and serve as a reservoir of reducing power capable of scavenging oxygen and other oxidants that may enter the system. However, our results also highlight potential challenges to the field application of this technology; future studies must address the stability of bioreduced U(VI), prevention of U(IV) reoxidation and potential competitive bioreactions, such as methanogenesis.

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