

ER63973-1025340-0011052

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**Title:** Reduction and Reoxidation of Soils During & After Uranium Bioremediation; Implications for Long-Term Uraninite Stability & Bioremediation Scheme Implementation

The goal of this research was to study the reoxidation of biologically precipitated U(IV). Several experiments were performed and are summarized below. These experiments include: (1) a long-term (~200 days) U(VI) reduction experiment under low sulfate conditions in order to study in detail changes in iron phases and biomass and determine how they affect/buffer reoxidation; (2) a short term (~70 days) experiment where we tracked the uranium profile via XANES prior to reoxidation and during reoxidation in order to determine the U speciation; (3) a short term experiment where we compare the oxidation of U(IV) by oxygen and nitrate in the absence of FeS; and (4) a short term experiment where we compare the oxidation of U(IV) by oxygen and nitrate in the presence of FeS precipitates.

**1. Long Term Reduction Experiment.** The biological reduction and precipitation of uranium in groundwater has shown potential to prevent uranium migration from contaminated sites. Although previous research has shown that uranium bioremediation is maximized during iron reducing conditions, little research has been performed to understand how long iron/uranium reducing conditions can be maintained. Similarly, questions remain about the stability of the bioreduced uranium and that of the uranium-reducing microbial population after iron/uranium biostimulation conditions are terminated and an oxidant (i.e. oxygen) is introduced into the previously reduced zone. To gain further insights into these processes, columns, packed with sediment containing iron as Fe-oxides (mainly Al-goethite) and silicate Fe (Fe-containing clays), were operated in the laboratory under field-relevant flow conditions to measure the long-term (> 200 d) removal efficiency of uranium from a simulated groundwater during biostimulation with acetate under low sulfate conditions. The biostimulation experiments were then followed by reoxidation of the reduced sediments with oxygen. During biostimulation, Fe(III) reduction occurred simultaneously with U(VI) reduction. Both Fe-oxides and silicate Fe(III) were partly reduced, and silicate Fe(III) reduction was detected only during the first half of the biostimulation phase while Fe-oxide reduction occurred throughout the whole biostimulation period. Mössbauer measurements indicated that the biogenic Fe(II) precipitate resulting from Fe-oxide reduction was neither siderite nor  $\text{FeS}_{0.09}$  (mackinawite). U(VI) reduction efficiency increased throughout the bioreduction period, while the Fe(III) reduction gradually decreased with time. Effluent Fe(II) concentrations decreased linearly by 30% over the final 100 days of biostimulation, indicating that bioreducible Fe(III) in the sediment was not exhausted at the termination of the experiment. Even though Fe(III) reduction did not change substantially with time, microorganisms not typically associated with Fe(III) and U(VI) reduction (including methanogens) became a significant fraction of the total microbial

population during long-term biostimulation, meaning that most acetate was utilized for other biological processes than Fe(III) and U(VI) reduction.

Selected columns were reoxidized after 209 days by discontinuing acetate addition and purging the influent media with a gas containing 20% oxygen. Uranium reoxidation occurred rapidly with 61% of the precipitated uranium resolubilized and transported out of the column after 21 days and virtually all of the uranium being removed by day 122. During the first 21 days of reoxidation, the Fe(III) and U(VI) reducing microbial population, as measured by quantitative PCR, remained at pre-oxidation levels (even though the gene transcripts that represent the methanogen population decreased by 99%) indicating that short-term disruptions in biostimulation (equipment failure, etc.) would not negatively affect the uranium reducing microbial population.

**2. XANES speciation experiment.** The biological reduction of uranium from soluble U(VI) to insoluble U(IV) has shown potential to prevent uranium migration in groundwater. To gain insights into the extent of uranium reduction that can occur during biostimulation and to what degree U(IV) reoxidation will occur under field relevant conditions after biostimulation is terminated, X-ray Absorption Near Edge Structure (XANES) spectroscopy was used to monitor i) uranium speciation *in situ* in a flowing column while active reduction was occurring and ii) *in situ* post biostimulation uranium stability and speciation when exposed to incoming oxic water. Results show that after 70 days of bioreduction in a high (30mM) bicarbonate solution the majority (> 90%) of the uranium in the column was immobilized as U(IV). After acetate addition was terminated and oxic water entered the column, *in situ* real-time XANES analysis showed that U(IV) reoxidation to U(VI) (and subsequent remobilization) occurred rapidly (on the order of minutes) within the reach of the oxygen front and the spatial and temporal XANES spectra captured during reoxidation allowed for real-time uranium reoxidation rates to be calculated.

**3. Oxidation of U(IV) by oxygen and nitrate in the absence of FeS.** Flow-through sediment column experiments examined the reoxidation of microbially reduced uranium with either oxygen or nitrate supplied as the oxidant. The uranium was reduced and immobilized via long-term (70 days) acetate biostimulation resulting in 62-92% removal efficiency of the 20  $\mu$ M influent uranium concentration. Uranium reduction occurred simultaneously with iron reduction as the dominant electron accepting process. The columns were reoxidized by discontinuing the supply of acetate and either replacing the anaerobic gas used to purge the influent media with a gas mixture containing 20% oxygen (resulting in a dissolved oxygen concentration of 0.27 mM) or adding 1.6 mM nitrate to the influent media. Both oxygen and nitrate resolubilized the majority (88% and 97%, respectively) of the uranium precipitated during bioreduction within 54 days. Though oxygen is more thermodynamically favorable an oxidant than nitrate, nitrate-dependant uranium oxidation occurred significantly faster than oxygen-dependant uranium oxidation at the beginning of our experiment due, in part, to oxygen reacting more strongly with other reduced compounds. Nitrate breakthrough at the effluent of the column occurred within 12 hours, which was significantly earlier than when oxygen was detected at the effluent (26 days). Though, over time, the majority of uranium was

reoxidized by either oxidant, these results indicate that the type of oxidant and its reactivity with other reduced compounds will influence the fate of reduced uranium during a short-term oxidation event that may occur during a uranium bioremediation scenario.

#### **4. Oxidation of U(IV) by oxygen and nitrate in the presence of FeS precipitates.**

Sediment column experiments were performed to quantify the reoxidation of previously bioreduced uranium. Reoxidation was achieved by providing oxygen or nitrate to the influent media. Significant sulfate reduction occurred during the biostimulation, producing iron sulfide precipitates that were more effective at protecting U(IV) from reoxidation with dissolved oxygen than with nitrate. A constant supply over a 50 day period of 0.25 mM and 1.6 mM nitrate resulted in uranium resolubilization of 11% and 60%, respectively, while less than 1% uranium was resolubilized in the column supplied 0.27 mM dissolved oxygen and none in the column supplied 0.03 mM dissolved oxygen during the same time period. Over time, oxidation increased pore water channeling, which was more pronounced during reoxidation with nitrate. Increased channeling with time of oxidation could affect the transport of an oxidant through the previously reduced zone, and hence the reoxidation dynamics of the reduced species.

### **Publications**

#### **(i) Journals**

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Komlos, J., B. Mishra, A. Lanzirotti, S.C.B. Myneni, and P.R. Jaffé, "Real Time Speciation of Uranium during Active Bioremediation and U(IV) Reoxidation," *J. of Environmental Engineering*, ASCE, Vol. 134, No. 2., 2008, pp. 78-86.

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Komlos, J., A. Peacock, R.K. Kukkadapu, and P.R. Jaffé, "Long-Term Dynamics of Uranium Reduction/Reoxidation under Low Sulfate Conditions," *Geochim Cosmochim Acta*, 72, 2008, pp. 3603-3615.

Komlos, J., H.S. Moon, and P.R. Jaffé, “Effect of Sulfate on the Simultaneous Bioreduction of Iron and Uranium,” *Journal of Environmental Quality*, Vol. 37, 2008, pp. 2058-2062.

**(ii) Conference Presentations (except PI meetings)**

Komlos, J., P.R. Jaffe, K.R. Kukkadapu, and J.M. Zachara. Quantification of Iron Reduction in Microbially Reduced Sediments. American Geophysical Union Fall Meeting, San Francisco, December 2005, abstract No. B23B-1052.

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