Field-scale evaluation of biological uranium reduction and reoxidation in the near-source zone at the NABIR Field Research Center in Oak Ridge, TN
ERKP382

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Objectives: The primary objective of the project is to advance the understanding and predictive capability of coupled hydrological, geochemical, and microbiological processes that control the *in situ* transport and bioremediation radionuclides and co-contaminants at multiple scales ranging from the molecular to the . Specific objectives include:

(1) Investigate the feasibility of in situ bioremediation of uranium in a highly contaminated region within the subsurface of Area 3 of the DoE ERSP FRC
(2) Using a variety of tracer strategies, develop and model a system that establishes hydraulic control of the target region for biostimulation
(3) Perform long term in situ biostimulation studies that create a microbial communities capable of reducing residual nitrate to N_2 and mobile U(VI) to sparingly soluble U(IV)
(4) Use a variety of solid and solution phase interrogation techniques to quantify the extent of in situ reduction and immobilization of U(VI).
(5) Investigate a variety of geochemical factors that influence the stability and possible reoxidation of reduced uranium.

Mission Relevance: The following research will have a significant impact on the *Oak Ridge Reservation Groundwater Strategy* document (DOE 2004) that describes a watershed-based strategy for making decisions about groundwater remediation on the Oak Ridge Reservation (ORR). The document was prepared by the DOE-Oak Ridge Office of Environmental Management (EM) and its remediation contractor Bechtel Jacobs Company LLC (BJC), in consultation with the state and federal regulators. The ORR *Groundwater Strategy* document emphasizes the need for timely and focused research investigations on natural hydrogeologic systems to help evaluate the technical feasibility and cost-effectiveness of various remediation strategies. Proposed first steps include "field investigations of the natural attenuation processes at the ORR, followed by numerical modeling sensitivity studies to identify critical parameters for further evaluation". Our research provides new experimental and numerical knowledge and information in previously unexplored areas of *in situ* rates and mechanisms and long-term effectiveness of bioremedial strategies for immobilizing contaminants in the subsurface. We have demonstrated through field experiments that the addition of electron donor to the subsurface stimulates metal and sulfate reducing bacteria that can reduce U(VI) to sparingly soluble U(IV) in both solution and on the solid phase. Bioreduction rates are relatively rapid and groundwater U concentrations can maintained below US EPA maximum contaminant limit (MCL) for drinking water and groundwater, and

solid phase U(IV) remains stable under anaerobic conditions. Furthermore, our research has quantified what geochemical and hydrological conditions are necessary to maintain the stability and impede the reoxidation of solid phase U(IV). This fundamental information will have maximum impact on ORR groundwater remediation decisions by increasing the scientific understanding of subsurface processes and by providing the characterization and numerical modeling tools needed to predict contaminant fate and transport under a variety of bioremediation scenarios.

Research Progress: This report summarizes research after 5 y of a 5 y project. Initially within this section, research endeavors and major discoveries are briefly highlighted for years 1 through 5. This is followed by a more detailed description of research activities and major accomplishments specific to year 5 (FY 06).

Project Progress Review:

1. Site characterization (Jan. 2001 to Aug. 2003)

   (1) Characterized hydrogeology, geochemistry and microbiology of the field site (Gu et al., 2004; Watson et al., 2004; Fields et al., 2005).

   (2) Feasibility studies performed to assess the propensity of uranium reduction and immobilization at the field site.

   (3) Column and microcosm investigations of bioreduction/immobilization of uranium with field based solid phase material and site groundwater (Gu et al., 2005; Wu et al., 2005; Nyman et al., 2005).

2. Design and installation of in situ and above ground remedial systems (Jan. 2002 to Aug. 2003)

   (1) Development of a nested well system (double dipole injection/withdraw system) for hydraulic control of groundwater flow through coupled groundwater tracer investigations and numerical modeling (Luo and Kitanidis, 2004; Fienen et al., 2004; 2005; 2006; Luo et al., 2005 a,b; 2006 a,b) .

   (2) Designed, installed, and implemented a sophisticated computer-based above-ground remediation system to remove high concentrations of PCE, Al, Ca, Mg, Ni, and nitrate from groundwater (Wu et al., 2006a; Hwang et al., 2005).

3. Field investigations (August 23, 2003 to present)

   (1) Flushed site with pH adjusted tracer water to remove bulk nitrate and Al and to increase pH to 6.0 (day 1-136) (Chen et al., 2006).

   (2) Biostimulation was initiated to induce in-situ denitrification in an effort to remove residual nitrate (day 137-184) (Wu et al., 2006b).

   (3) Biostimulation continued to induce in-situ U(VI) reduction/immobilization (day 185-712) (Wu et al., 2006b).

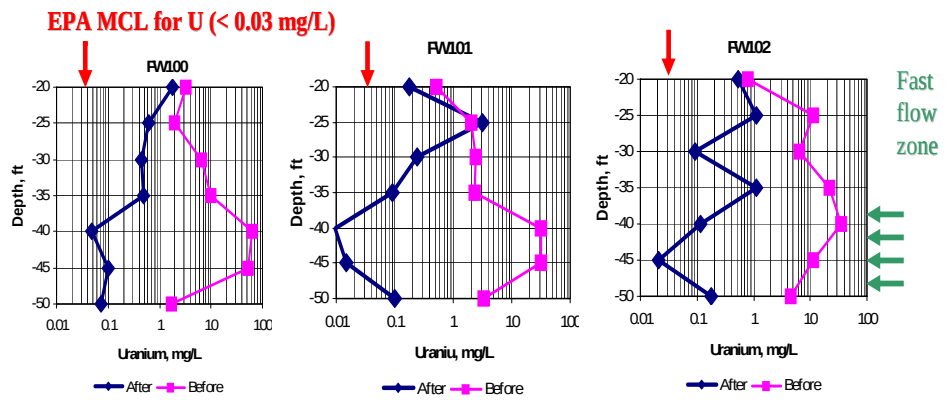
   (4) Stability studies (day 713-present) were initiated to evaluate geochemical and hydrologic factors that influence the stability of immobilized uranium (day 713-present) (Wu et al., 2007; Luo et al., 2007).

   (5) Microbial community analysis of the treatment area using clone libraries, MPN, and microarrays was conducted during the biostimulation and stability investigation in order to understand bacteria related to bioremediation and population dynamics (Fields et al., 2006).

   (6) Characterization of uranium speciation and chemical environment in sediments from the field site was performed before and after biostimulation using high resolution spectroscopy such as X-ray Absorption Near Edge Structure (XANES) & Extended X-ray Absorpation Fine Structure (EXAFS) to confirm bioreduction and immobilization of uranium (Ginder-Vogel et al., 2006; 2007; Kelly et al, 2007).

Major Achievements and Discoveries

•Low U concentrations, below US EPA maximum contaminant limit (MCL) for drinking water and groundwater (< 0.03 mg/L), were achieved by stimulated in situ bioreduction.



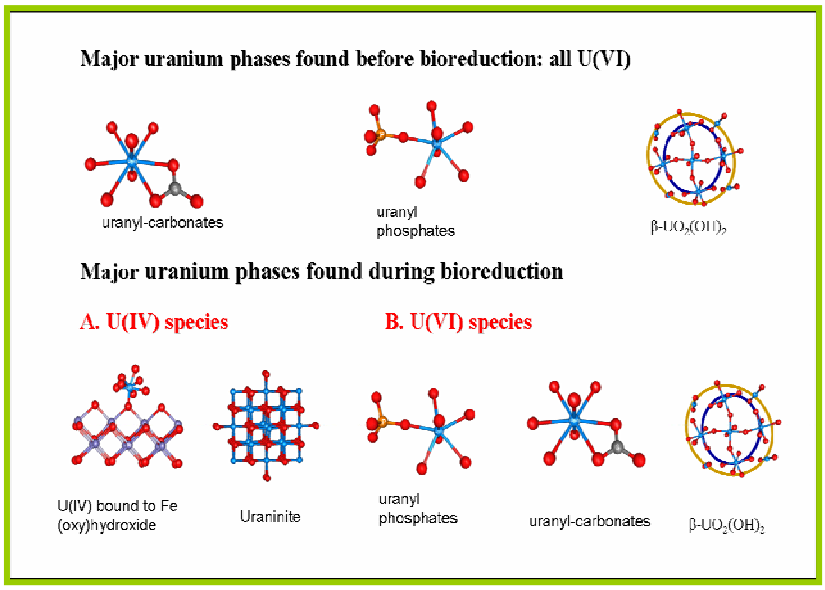
U concentrations in several monitoring wells before and after three years of bioremediation

(2) In situ bioreduced/immobilized U has been stable under anaerobic conditions for 1-2 years. No anaerobic re-oxidation was observed.

(3) Dissolved oxygen and/or nitrate intrusion did reoxidize and remobilize bioreduced U(IV) since the reduced subsurface zones have a decreased capacity to protect immobilized U(IV).

(4) Both field and laboratory investigations confirmed that metal-reducing Geobacter spp., and sulfate reducing Desulfovibrio spp. were stimulated by additions of the electron donor ethanol and were most likely significant contributors to the bioreduction of U(VI). Microarray analysis indicated the functional genes related to sulfate reducing bacteria were significant during biostimulation.

(5) XANES and EXAFS analysis confirmed significant changes in U speciation and chemical environment following biostimulation and the presence of up to 60-to 80% bioreduced U(IV) within bioreduced sediments.



•Hydraulic control and removal oxidants (DO and nitrate) appear to be essential for the bioreduction and immobilization of U(VI) contaminated site.

•23 multi-disciplinary, multi-institutional peer-reviewed manuscripts have been published thus far with 5 pending. An expensive list of abstracts and presentations. That is one publication for every $165 K of funding.

Detailed FY06 Progress

We have continued pilot-scale tests in Area 3 for in situ bioremediation of uranium at the contaminated site during this period. We have emphasized research that seeks to improve our understanding and predictive capability of the stability of reduced U under a variety of geochemical and hydrological conditions. The major achievement during this period is:

(a) Low U concentration below US EPA maximum contaminant limit (MCL) for drinking water (< 30 µg L^{-1} or 0.126 µM) were achieved by in situ bioreduction. We demonstrated that in-situ bioreduction of U (VI) decreased the levels of dissolved uranium in groundwater from 50-60 mg/L to <0.03 mg/L levels in groundwater. These concentrations fell below the US EPA maximum contaminant limit (MCL) for drinking water (< 30 µg L^{-1} or 0.126 µM) during ethanol injection period. At present, these low concentrations can be maintained under anaerobic conditions.

(b) Bioreduced/immobilized U is stable under anaerobic conditions. Field tests showed that the low U levels are stable under anaerobic conditions in the absence of added ethanol for at least 40 days. To assess longer-term changes in uranium within the reduced sediment, field samples of reduced sediments from the site were incubated anaerobically with low levels of HCO_3^- (<2 mM) and without electron donor. U(VI) concentrations gradually decreased then stabilized at low concentrations (<0.1 µM) for more than one year. Higher U(VI) concentrations and methane production occurred when samples were amended with higher levels of HCO_3^- (15 mM) and Ca^{2+} indicating U speciation changes and reduced reduction rates. Overall, the results indicate that low concentrations of aqueous uranium can be achieved and maintained under anaerobic conditions in the presence of sufficiently low levels of bicarbonate (<1-3 mM) and Ca^{2+} (0.75-1.0 mM).

(c) Dissolved oxygen intrusion into the field site was found to reoxidize bioreduced U(IV). Introduction of oxygenated water containing 4.0-5.5 mg L^{-1} DO into the reduced area caused re-oxidation and remobilization of bioreduced U(IV). However, the rate of U re-oxidation was dependent on groundwater residence time to the various sampling locations. During a 60-day DO injection period, spatially variable changes in aqueous U(VI) levels occurred, with concentrations increasing rapidly from <0.13 to 2.0 µM at MLS wells located in preferential flow paths versus those that were not. Resumption of ethanol addition after dissolved oxygen exposure restored iron reduction, sulfate reduction, and U(VI) reduction within 36 hours at all monitoring locations suggesting viable metal reducing organisms were still present at the field site.

(d) Hydraulic control appeared to be a key factor in maintaining the stability of reduced U. Tests were conducted to quantify the impact of U reduction within the inner loop without outer loop hydrological protection of the test area. Outside groundwater was allowed to penetrate into the reduced site which contained high concentrations of nitrate (>2 mM). Results suggested that rapid increases in U were observed in some monitoring well within 3-4 days of nitrate intrusion.

(e) Additional XANES analysis confirmed the presence of bioreduced U(IV) after two years of ethanol addition. XANES analyses indicated that the reduced product U(IV) made up 60 to 80% of the total uranium in sediment samples from the monitoring wells.

(f) Additional bacterial community analysis of both the groundwater and solid phase indicated that bacteria known to reduce uranium were being stimulated in the subsurface including Desulfovibrio spp. and Geobacter spp., In sediment samples, Geothrix spp. were also present and were the predominant Fe(III)-reducing bacteria.

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Also see: http://www.stanford.edu/group/evpilot/oakridge.htm
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