2006 ERSD Annual Report

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***In situ* Microbial Community Control of the Stability of Bio-Reduced Uranium**

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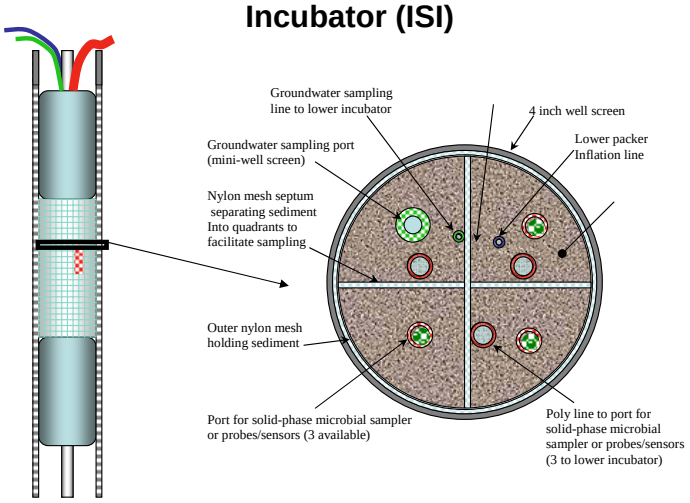
Research Objectives: In aerobic aquifers typical of many Department of Energy (DOE) legacy waste sites, uranium is present in the oxidized U(VI) form which is soluble and thus mobile compared to U(IV). Previous work at the Old Rifle Uranium Mill Tailings Remedial Action (UMTRA) site demonstrated that biostimulation by acetate injection promoted growth of *Geobacteraceae* and stimulated the microbial reduction of U(VI) to less soluble U(IV) (1, 4). Despite the potential for oxidative dissolution of bio-reduced U(IV), field experiments at the Old Rifle site show that although the rate of U(VI) reduction decreases following the on-set of sulfate reduction, U(VI) reduction continues even following the cessation of acetate injection (1, 4). However, U(VI) reduction is reversible and the basis for the observed maintenance of U(VI) reduction post-stimulation is a critical but as yet unresolved issue for the application of biostimulation as a treatment technology. The continued U(VI) reduction and the maintenance of reduced U(IV) may result from many factors including U(VI) reduction by sulfate reducing bacteria (SRB), generation of H_2S or $FeS_{0.9}$ which serves as an oxygen sink, or the preferential sorption of U(VI) by microbial cells or biopolymers. The overall goal of the project is to develop an understanding of the mechanisms for the maintenance of bio-reduced uranium in an aerobic aquifer under field conditions following the cessation of electron donor addition.

Research Progress and Implications: This report summarizes work after 2 years of a 3 year project. Laboratory scale microcosms allow precise control over system variables, but extrapolation of results to the field scale may be difficult. Therefore, in-well sediment incubators (ISIs – Figure 1) were developed to permit field deployment of laboratory-generated native sediment microbial communities to investigate the role of microbial community composition in the maintenance of bio-reduced U(IV). The use of ISIs in conjunction with on-going field activities allows the ready field deployment of multiple permutations microbial communities under different field conditions. This approach makes it possible to assess actual *in situ* conditions during the experiment and to directly observe reoxidation (or bioreduction) end points after the field experiment is completed without drilling. Finally, the production of in-well sediment incubators is

Figure 1: Conceptual design and top view (section) of In-Well Sediment Incubator (ISI)



relatively inexpensive and could ultimately become an alternative to field-scale electron donor amendment experiments as a means of assessing site response to bioremediation and long-term stability of both biostimulated and naturally bioattenuated sites.

Two initial ISI deployments have been conducted. The first ISI experiment consisted of deployment of an ISI containing both Rifle Aquifer Background Sediment (RABS) and laboratory bio-reduced RABS in a background well (B-02) to ascertain changes in microbial community composition and geochemistry/mineralogy in a microaerobic environment. The second experiment consisted of deployment of an ISI containing RABS in a previously simulated downgradient well (M-22). Following each deployment, the ISI was retrieved from the well and sediment samples were sent to the Center for Biomarker Analysis (CBA) at UT for phospholipid fatty acid (PLFA), denaturing gradient gel electrophoresis (DGGE), and quantitative polymerase chain reaction (qPCR) analysis. Sediment samples were split with PNNL for geochemical and mineralogical analyses.

B-02 ISI Deployment Following deployment of RABS and reduced RABS in the background well B-02 (DO=0.44mg L^{-1}), sediment samples from the ISI were obtained for PLFA- and DNA-based analyses. Monounsaturated PLFAs, the main PLFA of *Proteobacteria* including *Geobacteraceae* (2), were the dominant PLFAs in the laboratory reduced RABS (~60%) prior to ISI deployment in B-02 suggesting laboratory stimulation of reducing conditions (Figure 2). Furthermore, *Geobacter* spp. 16S rRNA gene copy numbers increased following laboratory electron donor addition to the background sediment (Figure 3). Nucleotide sequences of the selected bands in the DGGE profiles suggested enrichment of *Firmicutes* (mainly *Clostridiaceae* and *Bacillaceae*) following electron donor addition in the laboratory. An apparent increase in *Firmicutes* has also been observed at one location (M-08) at the site following electron donor injection (4). However, the low proportion of terminally branched saturated PLFAs in the reduced RABS suggests that *Firmicutes* were not the dominant members of the microbial community. In reduced sediment following ISI deployment in B-02, PLFA composition results showed loss of monounsaturated PLFA suggesting a

Figure 2: PLFA Composition

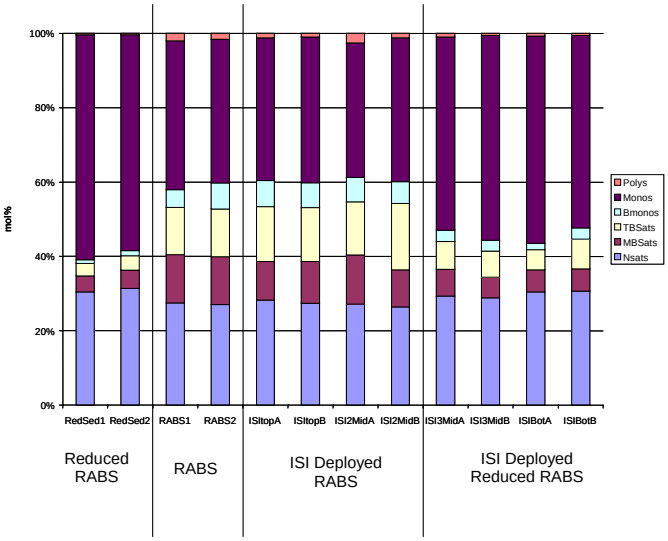
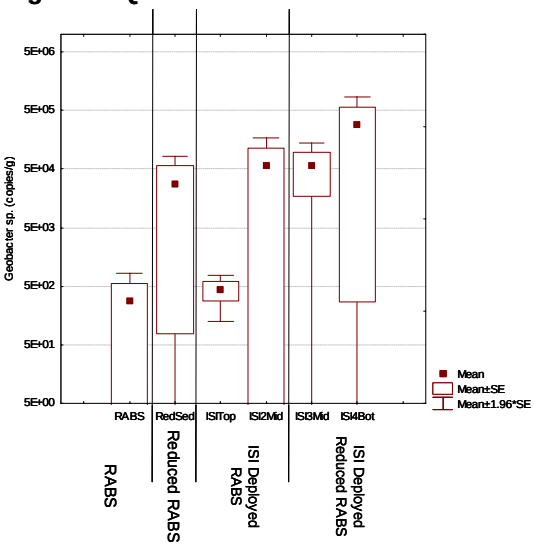


Figure 3: Quantification of *Geobacteraceae*

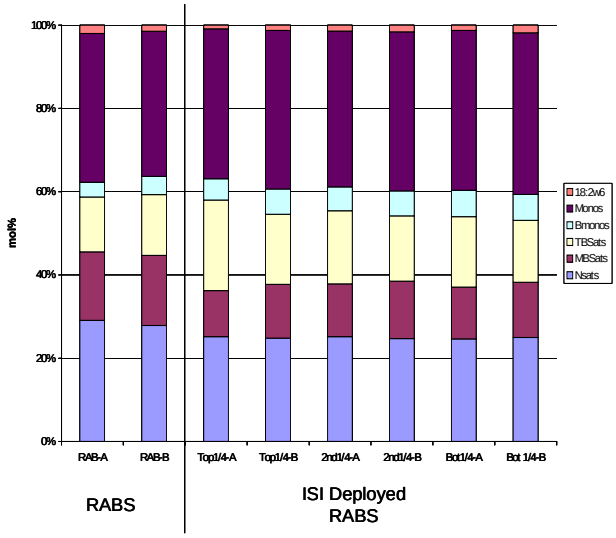


decrease in *Proteobacteria* as would be expected following incubation in a microaerobic well.

Despite the loss of monounsaturated PLFA following incubation in B-02, *Geobacter* spp. 16S rRNA gene copy numbers from field deployed reduced RABS remained comparable to those detected in the reduced RABS prior to in-well incubation (Figure 3).

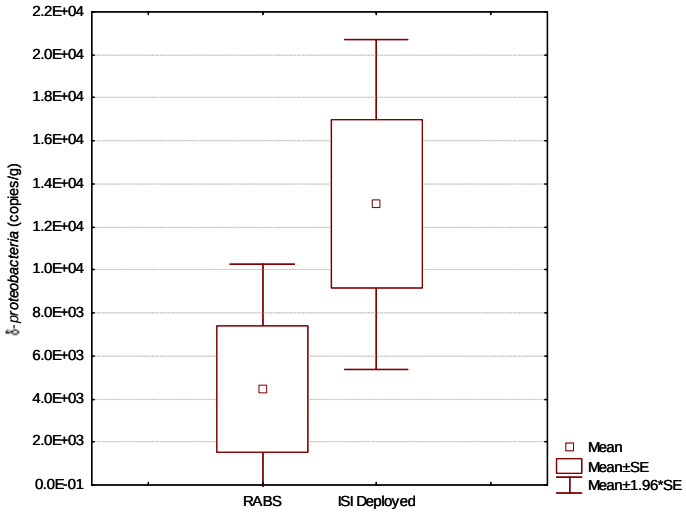
M-22 ISI Deployment: RABS was loaded into the ISI and deployed in previously stimulated well M-22 (2005) prior to the 2006 acetate injection experiments. PLFA results showed an increase in monounsaturated PLFA following a three month incubation in M-22 suggesting enrichment of *Proteobacteria* (Figure 4). Likewise, 16S rRNA gene copy numbers of δ-*proteobacteria*, often used as an indicator of iron and sulfate reducing bacteria (3) increased by nearly an order of magnitude following incubation in M-22 (Figure 5). *Geobacteraceae* populations also appeared to increase, however, the overall 16S rRNA gene copy numbers from *Geobacter* spp. were less than 10^3 copies g^{-1} in the RABS both prior to and following deployment. Overall the ISI results are consistent with the observed environmental conditions and previous field results. Based on the initial deployment results, ISIs will permit expedient interrogation of the microbial community response to environmental conditions and will be a valuable tool for examining microbial community control of bio-reduced uranium in the field.

Figure 4: PLFA Composition



Planned Activities: Additional ISIs are being constructed and will be available for deployment in November 2006. As mentioned previously, the mechanism of continued U(VI) reduction and the maintenance of bio-reduced U(IV) is a pivotal issue in the use of biostimulation as a treatment technology and remains unclear. U(VI) reduction by sulfate reducing bacteria (SRB), generation of H_2S or $FeS_{0.9}$, or the preferential sorption of U(VI) by microbial cells or biopolymers are all potential factors relating to the observed field results. ISIs containing reduced sediments

Figure 5: δ-*proteobacteria* Quantification



dominated by either iron reducing bacteria or sulfate reducing bacteria will be deployed in downgradient wells M-19 and M-24, respectively. A dual deployment of ISIs containing iron reducing and sulfate reducing populations will also be deployed in background well B-02. Comparisons of changes in the microbial community composition and uranium concentrations will be used to ascertain the relative importance of iron and sulfate reducing bacteria in the maintenance of bio-reduced uranium.

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Pacific Northwest National Laboratory's Role in ERSD Project "***In situ* Microbial Community Control of the Stability of Bio-Reduced Uranium**":

Pacific Northwest National Laboratory (PNNL) coordinates and supports field activities for this project. PNNL also performs collection and analysis of field geochemical parameters. PNNL arranges site access to the UMTRA sites (mainly the Old Rifle Site) and provided prototype ISI design and construction. PNNL staff participate in data analysis and interpretation of results.

Philip E. Long and James P. McKinley are the the Principal Investigators at Pacific Northwest National Laboratory (Project number 47284).