ERSP FY2006 Annual Progress Report
An Integrated Assessment of Geochemical and Community Structure Determinants of Metal Reduction Rates in Subsurface Sediments

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Research Objective

Our current research represents a joint effort between Oak Ridge National Laboratory (ORNL), the University of Tennessee (UT), and Florida State University (FSU). ORNL serves as the lead institution with Dr. A.V. Palumbo responsible for project coordination, integration, and deliverables. This project is in its second year. The overall goal of our project is to provide an improved understanding of the relationships between microbial community structure, geochemistry, and metal reduction rates.

This work is being conducted with subsurface materials collected from the Oak Ridge Field Research Center (ORFRC) established by the DOE-ERSP program in Oak Ridge, Tennessee. The primary objective of year 1 under Task 1 was the development and optimization of microcosm experiments designed to manipulate uranium reduction activity. Under Task 2 in years 1 and 2, we proposed to further define experimental conditions for the perturbation of microbial community structure by manipulating electron donor, electron shuttle, and nutrient concentrations. Tasks 1 and 2 are now complete and more than five microcosm experiments have been conducted. Results from these experiments have been submitted as abstracts to American Geophysical Union and American Society for Microbiology Meetings. The combination of experiments will be processed into peer-reviewed manuscripts. Summary results are provided below. A list of papers and other products delivered follows the summary of our results.

Research Progress and Implications

In order to provide a further understanding of the coupled microbiological and geochemical processes limiting U(VI) immobilization, we determined the rates of nitrate and uranium reduction and the changes in microbial community composition when different electron donors were used in microcosm experiments. The microcosm experiments used ORFRC subsurface sediments and groundwater under close to in situ conditions. Results are presented below and in companion reports from FSU and UT.

Microcosms were constructed with site materials and seven carbon substrates. Sediments were homogenized under anaerobic conditions in the FRC glove box prior to use in the microcosms. Experiment three used a full factorial design with pH and substrate. Four pH values used were 5.5, 6.0, 6.5, and 7.0. The carbon substrates included methanol (40 mM), ethanol (20 mM), glucose (10 mM), acetate (30 mM), lactate (20 mM), pyruvate (24 mM), glycerol (17 mM), and a control with no added electron donor. Carbon substrate concentrations were adjusted to give equivalent electron donor potential. Triplicate microcosms were run for each treatment. Each microcosm contained 20 g of sediment and 80 ml of groundwater. The pH was adjusted using sodium bicarbonate. Analytical measurements were made weekly on each microcosm. The HACH method was used to measured nitrate spectrophotometrically. A Chemchek KPA (kinetic phosphorescence analyzer) was used to measure the uranium in diluted samples. Microbial community composition was determined for the final time point using the phospholipid fatty acid methyl ester (PLFA) and quinone methods.

Typical results are similar to those seen in Experiment 3 where nitrate reduction was rapid and that differences among substrates were small (Figure 1a). U reduction lagged nitrate reduction (Figure 1b) and sulfate reduction generally lagged both (data not shown).

The methanol showed a lag time while other substrates did not and the controls did not exhibit nitrate reduction. There was minimal to no effect of pH in the experiment.

Uranium reduction showed initial increases which could be related to kinetic effects on equilibrium in slurries, reoxidation due to nitrate, or leakage of air into microcosms (Figure 1b). However, this has been observed repeatedly even when the microcosms were incubated in an anaerobic glove bag. Thus the most likely explanation is either an equilibrium effect which would be an artifact of setting up the experiments and changing the solid to liquid ratio or a real effect of nitrate reduction on U sorption or speciation. No reduction of uranium was observed in the control or methanol treatments. Uranium in the pyruvate treatment started very high and continued to increase (data not shown). Thus, there must be additional interactions occurring with pyruvate. We did see evidence of transformation of substrates (see FSU report) where, for example, ethanol was utilized and acetate produced and persisted past the point of U reduction.

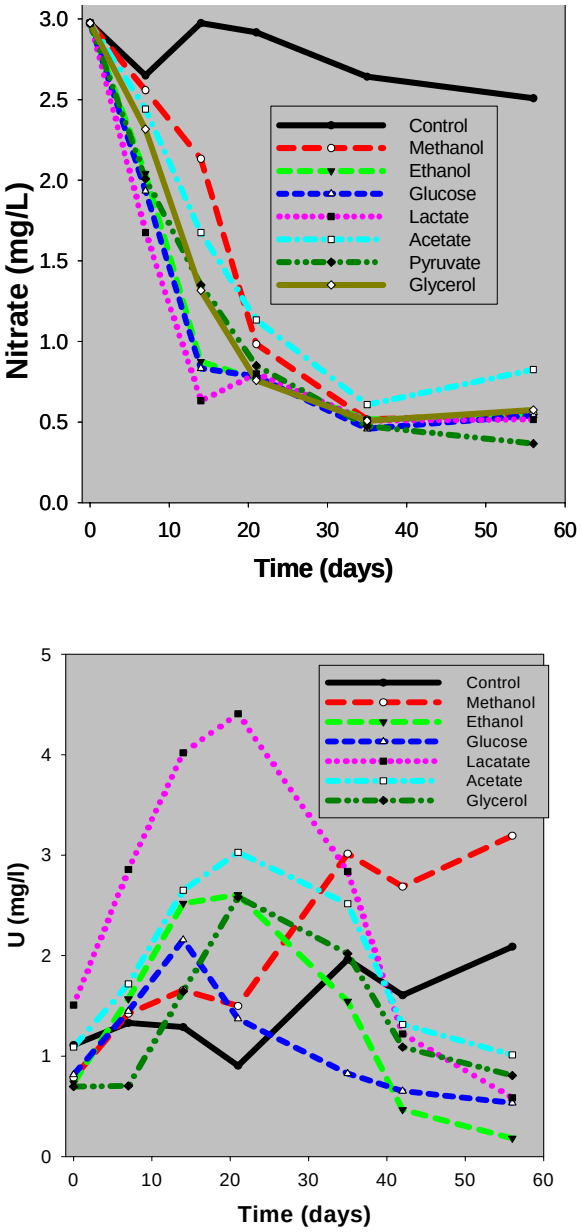

Figure 1. Nitrate (1a - top) and uranium (1b - bottom) reduction in microcosms.

In subsequent experiments we have found some evidence of possible microbial heterogeneity effects on substrate utilization for U reduction. We had found consistently high rates of nitrate and uranium reduction (e.g., Figure 2) with some electron donors (e.g., glucose and ethanol). However, while methanol stimulation consistently promoted nitrate and sulfate reduction, in only one of 5 independent experiments with different sediment samples was U reduction stimulated by methanol addition. Four of the five sediment samples for these experiments were taken within 3 meters of each other in a zone with consistent geochemical characteristics. Among these 4 samples was the only sample in which U reduction was observed with the methanol addition. After this first observation of methanol stimulation of U reduction, we confirmed the result by repeating the

methanol addition on additional archived sediment material from the same sample. Thus, there appear to be sample-scale heterogeneities in the community structure with a relatively uncommon community able to reduce U when stimulated with methanol. We are currently sampling and screening a broader array of sediments from the site to determine the prevalence of this community type.

XANES analysis confirmed the reduction of U in the microcosms and indicated differences in the degree of reduction in different treatments (Figure 3). There was evidence for U reduction in samples from an ethanol treatment (13%), a glucose treatment (43%) and the single methanol treatment (93%) in which we observed loss of U from solution (Figure 2). The monitoring data above (Figures 1 and 2) show U in solution and it does not necessarily reflect the total amount of U in the combined sediment U system. Thus, the XANES data obtained with the cooperation of ANL confirm the reduction of U with methanol as the electron donor. The XANES data also appear to indicate that the loss of U from solution may only reflect a portion of the U in the solid phase. The portion in the solid phase that is reduced appeared to differ greatly among the treatments. U reduction with the methanol treatment appears to be uncommon but substantial when it does occur.

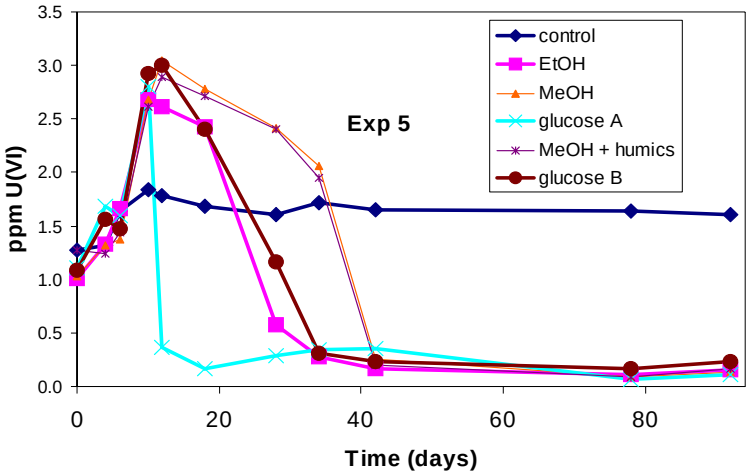

Figure 2. Uranium in solution in Experiment 5.

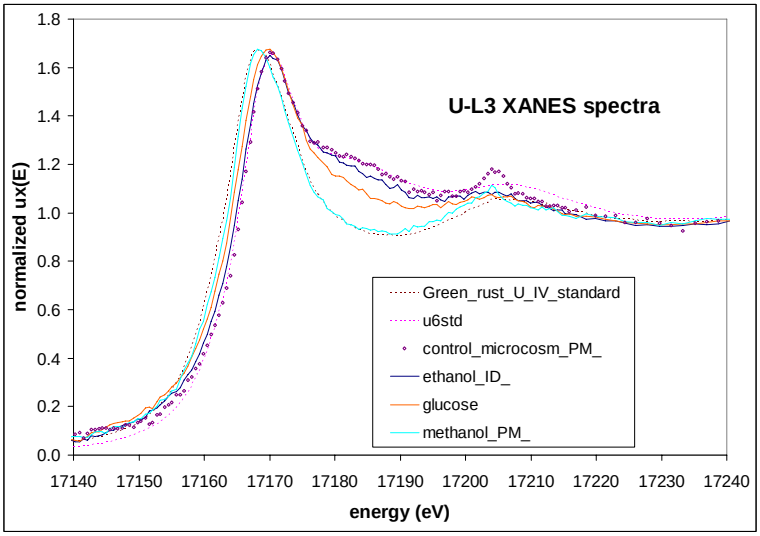

Figure 3. XANES analysis done in collaboration with Kelly, Kemner, and Ravel [(Adv. Photon Source at ANL) working with A. Madden (ORNL).

Characterization of the microbial community from the initial five experiments using a variety of methods (PLFA [see UT report], T-RFLP, PCR) indicated that there were substantial differences in the community structure related to the type of electron donor added

(e.g., see UT report). For example, the community structure observed in the methanol treatment (FSU data) in which U reduction was observed (e.g., Figure 4) was substantially different than the U reducing glucose and acetate treatments. In those treatments, the relative abundance of the *Betaproteobacteria* was less while the *Alphaproteobacteria* and *Actinobacteria* were more abundant (data not shown). The project is providing significant insight into optimization of uranium reduction by manipulation of communities and of the importance of microbial community structure in uranium bioremediation outcomes.

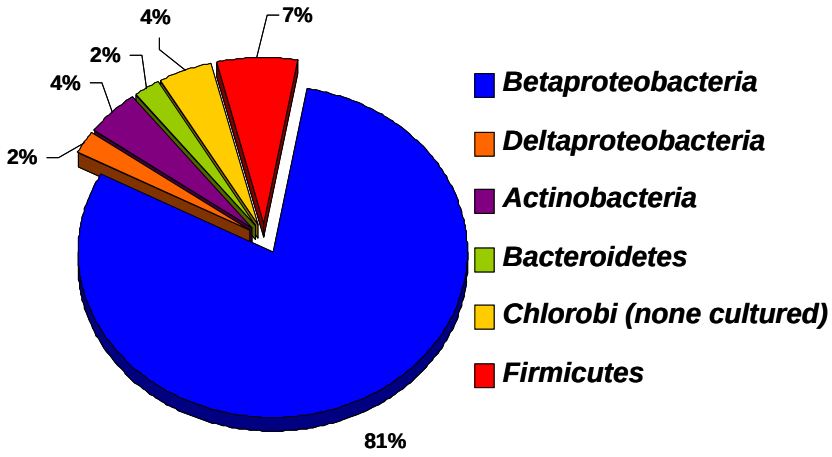

Figure 4. Community structure in methanol treatment in which U reduction was observed (Experiment 5).

Planned Activities

Additional analysis of the community structure is ongoing (e.g. functional gene arrays, T-RFLP, clone libraries) and comparisons of community structure in treatments that don't reduce U with those that do will be emphasized. These community composition measurements will be related to uranium reduction rates by non-linear data analysis techniques to understand the specific differences in the communities stimulated by methanol that result in the observed differences in the ability to reduce U.

Additional studies will take place with glucose, ethanol, and methanol with humics (no changes in rate in experiments to date) and different C/P ratios. Finally, we are sampling many more locations to assess the possible effects of microbial heterogeneity on the outcomes of these biostimulation experiments (e.g., the varying results with methanol).

A summary of the microcosm experiments indicates generally consistent findings but also some heterogeneity in response to methanol. All substrates promoted nitrate reduction and sulfate reduction. Methanol did not promote uranium reduction (or likely iron reduction) in most samples, but glucose and ethanol promoted rapid uranium reduction in all samples. Changes in PLFA and quinone community profiles were seen with methanol and glucose. Thus, there appear to be limitations on community structure related to substrate (methanol) that can lead to an inability to reduce U. Also, there is often enough metabolic diversity to accommodate many different electron donors (e.g., glucose, ethanol, glycerol, acetate) but perhaps not at all locations (e.g., for methanol).

Contributions to the project are made by C. Brandt, C. Schadt, M. McNeilly, L. Fagan. J. Tarver and A. Palumbo at ORNL, S. Pfiffner and S. (Bottomly) Difurio at UT, and D. Akob, H. Mills, and J. Kostka at FSU.

Information Access

Akob, D.M., H.J. Mills, T.M. Gihring, L. Kerkhof, and J.E. Kostka. 2006. Structure and function of metal- and nitrate-reducing microbial communities in subsurface sediments contaminated with radionuclides. Annual Meeting of the American Society for Microbiology, Orlando, FL, May.

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