Mesoscale Biotransformations of Uranium: Identifying Sites and Strategies where Reductive Immobilization is Practical (Project Number 1024940)

PI: Tetsu K. Tokunaga

Contributors: Jiamin Wan, Terry C. Hazen, Mary K. Firestone, Eoin Brodie, Yongman Kim, Rebecca Daly

Research Objectives:

Bioreduction of U in contaminated sediments is an attractive strategy because of its low cost, and because of short-term studies supporting its feasibility. However, any in-situ immobilization approach for U will require assurance of either permanent fixation, or of very low release rates into the biosphere. Our previous long-term (2 years) laboratory experiments have shown that organic carbon (OC) based U(VI) bioreduction to UO_2 can be transient even under sustained reducing (methanogenic) conditions. The biogeochemical processes underlying this finding urgently need to be understood. The current research is designed to identify mechanisms responsible for anaerobic U oxidation, and identify conditions that will support long-term stability of bioreduced U. We are investigating: (1) effects of OC concentration and supply rate on remobilization of bioreduced U, (2) the roles of Fe- and Mn-oxides as potential U oxidants in sediments, and (3) the role of microorganisms in U reoxidation, and (4) influences of pH on U(IV)/U(VI) redox equilibrium.

Research Progress and Implications:

A summary of progress made during the first 2 years of this project (begun FY 2005) is provided here.

Effects of OC concentration and supply rate on remobilization of bioreduced U.

Part of our current work examines effects of varying influent OC concentrations on U mobility under reducing conditions. Through a long-term laboratory column experiment using FRC Area 2 soils, under continuous infusion of OC (lactate, at an OC concentration of 32 mM), our earlier study showed that U was reduced during the first 100 days, then reoxidized (Wan et al., 2005). These soil columns were subsequently infused with solutions having different OC concentrations (0 to 100 mM), beginning on Day 510. Rapid changes in effluent U concentrations occurred in response to these changes in OC supply. Both the 0 and 6 mM OC treatments yielded decreased U concentrations (contrary to conventional expectation), and the 100 mM OC treatment caused even higher levels of U in effluents (also contrary to conventional expectation). The system continuously supplied with 32 mM OC sustained a nearly steady outflow U concentration of about 1 μM. These new results support our hypothesis that carbonate enrichment (from microbial oxidation of OC) promotes U(IV) oxidation because of the stability of U(VI) carbonate complexes, and also show that U-soil systems can be highly sensitive to OC supply. The terminal electron acceptor (TEA) driving U reoxidation is hypothesized to be a residual reactive Fe(III) fraction which becomes progressively depleted under sustained reduction. The decline in effluent U(VI) concentrations after about 700 days is indicative of TEA depletion.

Roles of Fe- and Mn-oxides as potential U oxidants in sediments.

While our work on OC concentrations is demonstrating the importance of U(VI) carbonate complexes in elevating aqueous U(VI) concentrations in bioreduced sediments, we are

conducting additional new experiments for the purpose of identifying TEAs that drive U(IV) oxidation under reducing conditions. Our previous study led to the hypothesis that amorphous and poorly crystalline Fe(III) oxides were the most likely TEAs responsible for U reoxidation in these reducing sediments (Wan et al., 2005). In order to test this hypothesis, our new column experiments were designed to allow simultaneous oxidation state measurements on U, Fe, and Mn by micro- X-ray absorption near-edge structure (μ-XANES) spectroscopy. Under continuous supply of OC solutions (different concentrations of lactate and acetate), we showed that Mn reduction to Mn(II) was complete at relatively early times, such that Mn(III,IV) are unlikely TEAs for U(IV) reoxidation. In contrast, reduction of Fe(III) is relatively slow. Results to date (312 days) continue to support the presence of a residual reactive Fe(III) fraction that can require years of sustained reduction to deplete.

Roles of microorganisms in U reoxidation.
Soil samples taken from representative columns during the net uranium reduction phase were used as inoculum for enrichment and subsequent isolation of microbes that demonstrated changes in activity between net reduction and oxidation phases of the previous column study. Enrichment media were designed to correspond to column conditions. Organic carbon was supplied in the form of acetate or lactate in quantities proportional to the column conditions the soil inoculum was taken from. Enrichments were designed for acetoclastic methanogens, Verrucomicrobia, and iron, sulfate, and nitrate reducers. Isolates obtained thus far include diverse genera in the α- and β- Proteobacteria, and the majority of isolates have previously been demonstrated to be dominant members in prior FRC bioremediation studies when analyzed by culture-independent methods. Isolates are currently being tested for the ability to reduce iron and uranium. Future enrichments will be inoculated with soil from the net reoxidation phase and will be tailored to enrich for microbes active during reoxidation as determined by 16S rRNA PhyloChip analysis.

Influences of pH on U(IV)/U(VI) redox equilibrium.
A set of columns packed with the U-contaminated sediments from FRC Area 2 were subjected to infusion with 30 mM OC (lactate) solutions at pH 6, 7, and 8.3 in order to test pH-dependence of U bioreduction. The effluents from these columns show relatively little variations in effluent pH because of buffering from the sediment, hence relatively minor differences in effluent U concentrations.

Planned Activities:
Near-future activities on subsets of sediment columns include sequential extractions of U and Fe at different stages in redox, further μ-XANES spectroscopy, X-ray fluorescence analyses for characterizing elemental redistribution, and geochemical modeling. Microbial community composition in the columns will be examined to determine if different column treatments significantly affect community structure and to determine if there is a relationship between community structure and uranium mobility. DNA extracted from representative columns during the net uranium reduction phase is in the process of being analyzed by 16S rDNA PhyloChip microarrays to determine which microorganisms are present in the communities. Future work includes direct analysis of 16S rRNA on the PhyloChip to determine changes in microbial

activity between the net uranium reduction and net uranium oxidation phases as well as analysis by functional gene arrays.

Information Access:

Publications.

Zheng, Z., T. K. Tokunaga, and J. Wan. 2003. Influence of calcium carbonate on U(VI) sorption to soils. Environ. Sci. Technol. 37, 5603-5608.

Tokunaga, T.K., J. Wan, J. Pena, S.R. Sutton, and M. Newville. 2004. Hexavalent uranium diffusion in soils from concentrated acidic and alkaline solutions. Environ. Sci. Technol. 38, 3056-3062.

Wan, J., T.K. Tokunaga, E. Brodie, Z. Wang, Z. Zheng, D. Herman, T.C. Hazen, M.K. Firestone, and S.R. Sutton. 2005. Reoxidation of bioreduced uranium under reducing conditions. Environ. Sci. Technol. 39, 6162-6169.

Tokunaga, T.K., J. Wan, J. Pena, E. Brodie, M. Firestone, T.C. Hazen, S.R. Sutton, A. Lanzirotti, and M. Newville. 2005. Uranium reduction in sediments under diffusion-limited transport of organic carbon. Environ. Sci. Technol. 39, 7077-7083.

Brodie, E.L., T.Z. DeSantis, D.C. Joyner, S.M. Baek, J.T. Larsen, G.L. Andersen, T.C. Hazen, P,M. Richardson, D.J. Herman, T.K. Tokunaga, J.M. Wan, and M.K. Firestone. 2006. Application of a high-density oligonucleotide microarray approach to study bacterial population dynamics during uranium reduction and reoxidation. Appl. Environ. Microbiol. 72, 6288-6298.

DeSantis, T.Z., Brodie, E.L., Moberg, J.P., Zubieta, I.X., Piceno, Y.M. and G.L..Andersen. High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. Microb. Ecol. (in press).

Presentations.

Tokunaga, T. K., J. Wan, J. Pena, S. Sutton, and M. Newville. Uranium diffusion in soils. Remediation of Chorinated and Recalcitrant Compounds, The 4th International Conference. Monterey, CA, May 24-27, 2004.

Tokunaga, T.K., J. Wan, J. Pena, E. Brodie, M.K. Firestone, T.C. Hazen, S.R. Sutton, A. Lanzirotti, M. Newville. Uranium bioreduction dynamics in low permeability sediments. 10th International Symposium on Microbial Ecology. Cancun, Mexico, August 22-27, 2004.

Brodie, E. L., T. Z. DeSantis, J. P. Moberg, I. X. Zubieta, Y. M. Piceño, T. C. Hazen, and G. L. Andersen. A High Density Microarray for Rapid Profiling of 16S rDNA and 16S rRNA of Prokaryotic Communities. International Symposium on Subsurface Microbiology. Jackson Hole, WY, August 2005.

Brodie, E.L., Hazen, T.C. and H. Tabak. Advances in Bioremediation of Soils and Sediments Polluted with Metals and Radionuclides: 2. Field Research on Bioremediation of Metals and

Radionuclides. International Conference on Chlorinated and Recalcitrant Compound Remediation. Monterey, CA, May 2006.

Brodie, E.L. and T.C. Hazen. Integration of the Omics, Bioinformatics, and Biogeochemistry: The New Frontier for Environmental Biotechnology. International Conference on Chlorinated and Recalcitrant Compound Remediation. Monterey, CA, May 2006.