

**4<sup>th</sup> Annual DOE-ERSP PI Meeting:  
Abstracts**

**April 20–23, 2009**

**National Conference Center (NCC)  
Lansdowne, Virginia**

**Environmental Remediation Sciences Program (ERSP)**

**This work was supported by the Office of Science, Biological and Environmental  
Research, Environmental Remediation Sciences Program (ERSP),  
U.S. Department of Energy under Contract No. DE-AC02-05CH11231.**



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## 2009 Annual ERSP Principal Investigators Meeting

Welcome to the 2009 Annual Environmental Remediation Sciences Program (ERSP) Principal Investigators (PI) Meeting, and thank you for setting aside time in your busy schedules to participate in this important annual meeting. The ERSP seeks to advance fundamental science to understand, predict, and mitigate the impacts of environmental contamination from past nuclear weapons production and provide a scientific basis for the long-term stewardship of nuclear waste disposal. These ambitious goals cannot be achieved by any one project alone. Therefore, ERSP funds a combination of research programs at the DOE national laboratories, individual projects at universities and federal agencies, and large long(er)-term field site research. Integration of these activities to advance the ERSP goals is a constant challenge, but made significantly simpler by bringing together all funded ERSP researchers once a year to discuss the very latest research results. It is at these meetings where new ideas and/or scientific advancements in support of ERSP goals can be discussed and openly debated among all PIs in the program. The ERSP thrives, in part, on the new ideas, concepts, scientific connections, and collaborations generated as a result of these meetings.

The annual PI Meeting is very much a working meeting with three major goals: (1) to provide opportunities for scientific interaction among the ERSP scientists, a critical element for the program; (2) to provide the ERSP program staff with an opportunity to evaluate the progress of each program and project; and (3) to showcase the ERSP to interested parties within DOE and within other federal agencies. In addition to program managers from within OBER, there will be representatives from other offices within DOE and other federal agencies in attendance at the meeting.

This year, we have another full agenda. The meeting begins on Monday afternoon with an update on activities at the Oak Ridge IFRC and an evening poster session. Tuesday begins with morning plenary talks from individual ERSP projects, followed by two concurrent breakout sessions during the free time after lunch. The afternoon plenary session will continue with an update on activities at the Hanford 300 Area IFRC, followed by an evening poster session after dinner. Wednesday will follow a similar format, with morning plenary session talks, free time over lunch, and an afternoon plenary session on activities at the Rifle IFRC and an evening poster session. Plenary talks will continue Thursday morning, and the PI meeting formally adjourns at noon on Thursday. Immediately after the meeting, ERSP program managers will be conducting a mid-term SciDAC review and a review of proposals submitted in response to the latest call to the DOE national laboratories for a program in Transuranics contamination research.

While the meeting agenda is necessarily full, we have attempted to preserve free time in the afternoons for research groups and individuals to meet privately. We know that many groups use this time at the PI Meeting to meet face to face with project members, and we do not wish to discourage this. We have also provided a large block of time for the evening poster presentations. This year, each SFA program will be presenting posters as a group and will be assigned to one of the three evening poster sessions. Individual university/federal agency projects (in addition to the IFRC projects) will also be assigned to one of the poster sessions. Each ERSP-funded project will be represented by a poster and at least one of the investigators. The ERSP program managers encourage all PIs to attend the poster session, whether you are presenting your project or not, to view results obtained from others in the program.

On behalf of the ERSP program managers and staff, I thank you for attending this annual meeting. The past year has been quite productive, and we look forward to reviewing the progress you have made and discussing future plans for your research.

Best Regards,

R. Todd Anderson  
ERSP Program Manager



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# DOE-ERSP Annual PI Meeting

National Conference Center, Lansdowne, VA

## Preliminary Agenda

**Monday, April 20, 2009**

**Afternoon Plenary Session**

3:00 p.m.     **Opening Comments and ERSP Programmatic Overview**  
*Todd Anderson, ERSP Program Manager*

3:30 p.m.     **ERSP Strategic Planning Process**  
*David Lesmes, ERSP Program Manager*

### *Integrated Field-Scale Subsurface Research Challenge (IFC) Presentations*

4:00 p.m.     **Oak Ridge (IFRC)**  
*Phil Jardine, ORNL Lead PI and research team members*

6:00 p.m.     **DINNER**

7:00 p.m.     **POSTER SESSION 1**

**Tuesday, April 21, 2009**

**Morning Plenary Session**

### *Mercury Biogeochemistry*

8:30 a.m.     **Overview of Mercury Contamination at the Oak Ridge Y-12 site**  
*Scott Brooks, ORNL*

9:00 a.m.     **Reduction of Mercury in Saturated Subsurface Sediments and its Potential  
to Mobilize Mercury in its Elemental Form**  
*Tamar Barkay, Rutgers University*

**9:30 a.m.**     ***Mercury Interactions with Natural Organic Matter***  
*Kathy Nagy, University of Illinois/Chicago*

10:00 a.m.    **BREAK**

### *Radionuclide Transport and Immobilization Mechanisms*



## Wednesday, April 22, 2009

### Morning Plenary Session

#### *Measurement and Monitoring Techniques*

8:30 a.m.     **Development of Highly Sensitive Micro-Biosensors for in-situ Monitoring of Mercury (II) and Chromium (VI) Contaminants through Genetic-evolution and Computer modeling of Metal-binding Proteins**  
*William Goddard, California Institute of Technology*

9:00 a.m.     **Contaminant Flux Measurement in the Environment**  
*Kirk Hatfield, University of Florida*

9:30 a.m.     **Electrode Techniques for Monitoring Microbial Activity in the Environment**  
*Ken Williams, LBNL*

10:00 a.m.    **BREAK**

#### *Microbiological Processes Influencing Contaminant Mobility*

10:30 a.m.    **Role of Microbial Synergies in Immobilization of Metals**  
*Slava Epstein, Northeastern University*

11:00 a.m.    **Optimization and Directed, Natural Evolution of Biologically Mediated Chromate Reduction in Subsurface Soil Microcosms**  
*Dorothea Thompson, Purdue University*

11:30 a.m.    **TBA**

**12:00 noon – 3:00 p.m.     LUNCH AND FREE TIME**

### Afternoon Plenary Session

3:00 p.m.     **Update on Groundwater Contamination Issues across the DOE Complex**  
*Terry Hazen, LBNL*

#### *Integrated Field-Scale Subsurface Research Challenge (IFC) Presentations*

3:30 p.m.     **Old Rifle Site IFRC**  
*Philip Long, PNNL Lead PI and research team members*

5:30 p.m.     **DINNER**

6:30 p.m.     **POSTER SESSION 3**

**Thursday, April 23, 2009**

**Morning Plenary Session**

***Molecular Scale Biogeochemistry***

- 8:30 a.m.     **Molecular Scale Mechanisms of Biogeochemical Electron Transfer Underlying U/Tc Valence Transformation and Stability at Hanford**  
*Kevin Rosso, PNNL*
- 9:00 a.m.     **Reactivity of Biogenic Uraninite in Ground Water: Stability Controls and Corrosion**  
*John Bargar, SLAC*
- 9:30 a.m.     **TBA**
- 10:00 a.m.    **BREAK**

***Subsurface Transport Simulation***

- 10:30 a.m.    **Reactive Transport Modeling of Microbially-Mediated Processes at the Old Rifle and Hanford 100H Sites**  
*Carl Steefel, LBNL*
- 11:00 a.m.    **Multiscale-Multiphase-Multicomponent Subsurface Reactive Flows Using Advanced Computing**  
*Peter Lichtner, LANL*
- 11:30 a.m.    **Hybrid Numerical Methods of Multiscale Simulations of Biochemical Processes**  
*Tim Scheibe, PNNL*
- 12:00 p.m.    **Final Comments and Adjourn**  
*Todd Anderson, ERSP Program Manager*



# Abstracts



## ERSP University-Led Research



# Geochemical and Physical Aquifer Property Heterogeneity: A Multiscale Sedimentologic Approach to Reactive Solute Transport

*Session: ERSP-Led University Research*

Richelle M. Allen-King, (PI), Guohui Wang, Kelly Lilienthal—University at Buffalo (SUNY), Buffalo, NY; Gary Weissmann—University of New Mexico, Albuquerque, NM; Christopher Murray, Tim Scheibe, Bruce Bjornstad, George Last—PNNL, Richland, WA

This project is testing the hypothesis that sedimentary lithofacies determine the geochemical and physical hydrologic properties that control reactive solute transport. The representative geochemical and physical aquifer properties selected for quantification in the proposed project are the properties that control carbon tetrachloride (CT) transport at the Hanford Site (Ringold Formation): hydraulic conductivity and reactivity (sorption distribution coefficient and transformation rate constant). We are combining observations at outcrop analog sites (to measure lithofacies dimensions and statistical relations) with measurements from archived and fresh core samples (for geochemical experiments and to provide additional constraint to the stratigraphic model) to estimate the spatial distributions of properties that control reactive solute transport in the subsurface.

Through analysis of the boreholes and outcrop, we determined that (1) the Ringold E unit (the primary saturated zone unit contaminated by the CT plume) consists primarily of gravel to cobble deposited by large multichannel streams, and (2) this unit is lithologically relatively homogeneous with minor sand-body inclusions that appear to be bar-top facies. To further assess the influence of lithologically constrained K heterogeneity on groundwater flow and reactive transport, we created high-resolution lithofacies maps from lidar scans of Ringold outcrops for use in 2D groundwater models. The two high-resolution terrestrial lidar scans (collected in the previous year) provide an accurate 3D survey of lithologic unit geometry, along with intensity of laser reflection. The two outcrop scans are oriented approximately perpendicular to each other to allow us to assess the influence of facies orientation on groundwater flow and reactive solute transport.

We have used a surrogate compound approach to determine that (1) the CT sorption distribution coefficient ( $K_d$ ) is low in the Ringold formation, and (2) reasonable  $K_d$  estimates can be obtained from the measured organic carbon content ( $f_{oc}$ ) of the sediments using the literature or reference value for  $K_{oc}$  independent of CT concentration. This finding allows us to "rule out" sorption as a significant mass-storage mechanism for CT in the Ringold E and to recognize that CT retardation is minimal. Measurements on the Ringold mud are ongoing.

A protocol for CT transformation experiments that preserves sample integrity to the maximum extent practical has been developed, and CT transformation experiments with the fresh samples from a range of depths in the Ringold formation have been initiated. Transformation was not detectable above background control loss in initial experiments with a Ringold sand and gravel sample. This is consistent with the observation of low to nondetectable sorbed Fe(II) in samples from the same unit. Variable transformation, sorbed Fe(II) concentration, and CF suggest variable transformation potential with the Ringold mud sample, which exhibits variable redox color indicators.

We have also begun work on reactive transport simulations that will be used to predict the impacts of the combined heterogeneity of the physical and geochemical aquifer attributes on reactive transport.

## Investigating Geophysical Signatures of Microbial Nanowires

*Session: ERSP University-Led Research*

Estella A Atekwana (PI), David Corter, Gamal Z Abdel Aal—Oklahoma State University, Stillwater, OK; Dwayne Elias, University of Missouri-Columbia

The primary goal of this research is to establish a fundamental and mechanistic understanding of the relationship between microbial activities involved in electron transfer, including nanowires, and the geophysical/geoelectrical responses of subsurface environments. More specifically, our objective is to conduct laboratory studies to accomplish the following:

1. Correlate the spatial and temporal bacterial nanowire distribution with geoelectrical responses, including induced polarization (IP) and self-potential (SP) measurements.
2. Demonstrate that in electron-acceptor limiting conditions, microbial nanowires will be produced and discernable differences in geoelectrical signatures will exist.

In a previous set of experiments using saturated sand columns and the metal-reducing bacterium *Shewanella oneidensis* MR-1, we suggested that electrically conductive extracellular appendages enhanced the measured electrical potentials over time. However, the source mechanism of the enhanced measured electrical potential is not fully understood. In the present set of experiments, we investigated the effect of electron-acceptor limitation on bacterial electron transport. A flow-through column experiment under anaerobic conditions was conducted to investigate the effect of sulfate-limitation on electron transport using the bacterium *Desulfovibrio vulgaris*. Two sets of experimental columns were utilized; one simulating electron-acceptor “excess” conditions (containing 4 mM lactate and 3 mM sulfate) and the second simulating electron-acceptor “limiting” conditions (5 mM lactate and 1.5 mM sulfate). Both columns contained 0.0015 mM of Fe(II) as FeCl<sub>2</sub> to minimize precipitation of iron sulfide. Low frequency electrical measurements (0.1–1000 Hz) and self-potential (SP) measurements were made on the columns over a period of 36 days. Fluid and soil samples were extracted from the sand columns to investigate the changes in the geochemical and biological properties over time. We observed that a difference in the ratio of electron donor to electron acceptor caused a significant change in the phase (up to 8 mrad) and imaginary conductivity component (up to  $3.5 \times 10^{-4}$  S/m) of the sulfate limited columns compared to the sulfate excess columns. However, both columns showed significant changes in the SP values (up to 500 mV for the sulfate-limiting column and 600 mV for the sulfate excess column). The changes in the SP were likely related to chemical reactions occurring at the electrodes. A steady decrease in pH from 7.5 to 6.9 suggests that the cells were viable for the length of the experiment. Geochemical analyses including organic acid concentrations and scanning electron microscope imaging are currently under way to help explain the differences in the low frequency electrical measurements.

## Reduction of Hg(II) to Hg(0) by Magnetite

*Session: ERSP University-Led Research*

Nathan Yee, Heather A. Wiatrowski, Soumya Das, Chu-Ching Lin, Madhavi Parikh—Rutgers University, New Brunswick, NJ; Ravi Kukkadapu, Pacific Northwest National Laboratory, Richland, WA; Tamar Barkay, Rutgers University (lead PI)

Mercury is a component of mixed waste that has contaminated vast areas of soil and groundwater as a result of nuclear weapons production. While the complexation of mercuric Hg (Hg[II]) with soil organic matter and mineral surfaces can retard mercury migration, episodes of groundwater contamination are known. In some cases, mercury mobilization in groundwater results in potable water supersaturated with elemental Hg (Hg[0]). Coupled biotic/abiotic processes that reduce Hg(II) to Hg(0) in saturated subsurface sediments may contribute to this problem.

Previously, we demonstrated that three dissimilatory metal reducing bacteria (DMRB), *Shewanella oneidensis* MR-1, *Geobacter sulfurreducens* PCA, and *Geobacter metallireducens* GS-15 reduce Hg(II) to Hg(0), while utilizing ferrihydrite as a terminal electron acceptor. One possibility of enhanced mercuric Hg reduction under iron-reducing conditions is Hg(II) reduction by biogenic ferrous iron. The goal of this project is to investigate the coupled biotic/abiotic pathways of Hg(II) reduction by DMRB. The research objectives are: (1) to quantify Hg(II) reduction by solid-phase Fe(II) minerals; and (2) to elucidate the abiotic and biotic pathways of Hg(II) reduction in complex enrichment cultures that are derived from saturated subsurface sediments.

Here we report the discovery that magnetite (Fe<sub>3</sub>O<sub>4</sub>) can reduce Hg(II) to Hg(0). Kinetic and spectroscopic experiments were performed to elucidate reaction rates and mechanisms. The experimental data demonstrated that reaction of Hg(II) with magnetite results in the loss of Hg(II) and the formation of volatile elemental Hg (Hg[0]). Kinetic experiments showed that Hg(II) reduction occurred within minutes, with reaction rates increasing with increasing magnetite suspension density (0.05 to 0.2 g/L) and solution pH (4.8 to 6.7), and decreasing with increasing chloride concentration (10<sup>-6</sup> to 10<sup>-2</sup> mol/L). Mössbauer spectroscopic analysis of reacted magnetite samples revealed a decrease in Fe(II) content, corresponding the oxidation of Fe(II) to Fe(III) in the magnetite structure. These results suggest that Hg(II) reaction with solid-phase Fe(II) is a kinetically favorable pathway for Hg(II) reduction in magnetite-bearing environmental systems.

## Microscale Metabolic, Redox and Abiotic Reactions in Hanford 300 Area Subsurface Sediments

Session: ERSP University-Led Research

Haluk Beyenal, Washington State University, Pullman, WA (lead PI), Jim Fredrickson, Pacific Northwest National Laboratory (PNNL), Richland, WA, Jeffrey S. McLean, J.Craig Venter Institute, Paul D. Majors, PNNL

The biotransformation of aqueous uranium species associated with the solid phase can occur in established communities of cells growing on mineral surfaces. In previous studies, when the natural populations of microbes associated with subsurface sediments were stimulated with organic electron donors in laboratory microcosms, the number of active cells increased rapidly. Development of bacterial communities on mineral surfaces can result in highly metabolically active cells contained within a matrix consisting of extracellular polymeric substances (EPS). In previous ERSP research, biogenic  $\text{UO}_2$  nanoparticles were shown to associate with the extracellular matrix material, supporting the importance of microbial processes and the potential role of EPS in nanoparticle collection and stabilization. Hence, microbial processes and abiotic redox reactions that operate at the microscale are critical to understanding factors controlling the macroscopic fate and transport of contaminants in the subsurface.

The primary goal of our research program is to understand how microscale conditions control uranium mobility and how biotic and abiotic reactions interact to influence contaminant behavior. Our research focuses on the Hanford 300 Area subsurface environment and uses a combination of novel microscale approaches and modeling to understand these relationships. Core samples from 300 Area IFRC site were used to prepare eight column reactors. We then isolated facultative bacteria that could reduce soluble uranium (VI). Currently, we are in the process of identifying these isolates through 16s rRNA analysis. Laboratory NMR microscopy studies were performed to monitor spatial and temporal metabolite concentrations and material-transport rates within biofilms. We developed a new system that is optically accessible for real-time nondestructive imaging during biofilm growth on controlled electrodes simulating redox states of solid-phase electron acceptors. New microelectrodes measuring uranyl concentration ( $[\text{UO}_2^{2+}]$ ), as well as pH and dissolved oxygen have been developed and constructed to measure local chemistry in the laboratory and within 300 Area subsurface biofilms.

Live NMR metabolism experiments demonstrated that the apparent diffusion rate for water decreases linearly with biofilm depth. These values were used in a reaction-diffusion model describing metabolite profiles, and the results were compared with the experimentally measured steady-state metabolite profiles (lactate, acetate, fumarate, and succinate). In parallel studies with the optically accessible controlled electrode chamber, we demonstrated that there is quantifiable competition between a soluble electron acceptor (fumarate) and insoluble acceptor (electrode) in a *Shewanella* biofilm of average thickness (80  $\mu\text{m}$ ). Experiments are under way to correlate the uranium reduction and oxidation rates with the presence of biofilms whose overall activity is modulated by the availability of soluble and insoluble alternative electron acceptors. Microelectrodes, controlled electrochemical devices, and NMR in combination provide capabilities for measuring metabolism, chemistry, and uranium solubility at the microscale in Hanford 300 Area mixed species biofilms, as well as in pure culture biofilms. These data will be used in developing a model to ultimately understand and predict the fate of uranium at the microscale, and then be linked to macroscale properties and processes.



## Release of Aged Contaminants from Weathered Sediments: Effects of Sorbate Speciation on Scaling of Reactive Transport

*Session: ERSP University-Led Research*

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Our work aims for a predictive-mechanistic understanding of the coupling between mineral weathering and contaminant (Cs, Sr, I) fate in caustic waste-impacted sediments across space, time, and geochemical gradients observed at the Hanford Site. Our research approach centers on a series of flow-through saturated and unsaturated column studies conducted on Hanford Formation sediments reacted with synthetic tank waste leachate (STWL) for up to 1 yr in the presence and absence of CO<sub>2</sub>. STWL-reacted sediments are then subjected to flow-through column studies using simulated background pore water (BPW) solution to assess subsequent contaminant release. We are using a multifaceted approach (XAS, XRD, DRIFT, NMR, TEM/EDS, wet chemistry) to investigate molecular-scale mechanisms that give rise to macroscale response. In all treatments, added Sr, Cs, and I were incorporated into the solid phase with 3 to 12 times more uptake of Sr than Cs, and much smaller uptake of I. Sediment CEC and specific surface area decreased with STWL reaction time, consistent with dissolution of clay minerals, but a finer granulometry resulted as well. XRD, DRIFT and microscopic characterizations indicated the neoprecipitation of Ca-rich Na-zeolites for the highest contaminant concentrations (HIGH: 10<sup>-3</sup> m Cs and Sr, 10<sup>-5</sup> m I) and NO<sub>3</sub>-feldspathoids (sodalite/cancrinite) for the low concentrations (LOW: 10<sup>-5</sup> m Cs and Sr, 10<sup>-7</sup> m I) with detectable amounts of Sr and Cs in the neophases for HIGH. Iodine uptake could not be localized in the solid phase. Intensities of neophase XRD reflections increased with reaction time. No major effect of the pCO<sub>2</sub> was observed. Homogenous nucleation experiments conducted under similar conditions revealed the precipitation of similar neophases with a mineral transformation from zeolite to feldspathoids occurring between 30 and 365 days in both HIGH and LOW. Therefore, assuming similar neophases in sediments and given the presence of zeolite in HIGH after 1 year, mineral transformation (i.e., ripening) appears to be inhibited for HIGH sediment treatments. When these reacted sediments were leached with simulated BPW (pH=7) in flow-through saturated and unsaturated columns, major cation release and pH stabilized at PV > 300, with data fit to a pseudo steady-state condition comprising rapid ion exchange and slower dissolution/precipitation reactions. Long-term fractional release of Sr and Cs in all treatments falls within a narrow range of 0.0001–0.0004 PV<sup>-1</sup>. Conversely, rapid I desorption was observed, with the total sorbed pool diminished by ca. 50% in within 50 PV. XRD of solids after 600 PV indicated persistence of NO<sub>3</sub>-feldspathoids in the LOW and incongruent zeolite dissolution in the HIGH. Numerical modeling of the long-term release of Cs and Sr is consistent with feldspathoid dissolution control in the LOW, whereas the presence of zeolite in the HIGH makes the modeling more complex. When considered in light of our prior studies on specimen clays and homogeneous nucleation processes, results indicate that initial contaminant concentration and sediment composition exert a strong impact on the solid phase transformations and contaminant fate/mobility in both STWL and BPW scenarios.

## The Genome Sequences of the Fe(II) Oxidizers *Diaphorobacter* sp. strain TPSY and *Pseudogulbenkiania* sp. strain 2002

Session: ERSP University-Led Research

Kathy G. Byrne-Bailey, Antinea H Chair, John D. Coates—U. of California, Berkeley, CA (lead PI)

Nitrate-dependent Fe(II)-oxidizing bacteria (NFOxB) produce a broad range of insoluble iron forms as endproducts, including amorphous hydrous Fe(III)-oxides and mixed-valence iron minerals such as green rust, maghemite, and magnetite. These iron minerals regulate soluble metal concentrations, including those of uranium, chromium, and cobalt, in natural waters through adsorption and co-precipitation. Although, it has been over a decade since nitrate-dependent Fe(II) oxidation was first identified, there is still relatively little known about the microorganisms responsible, and nothing is known regarding the genetic or biochemical mechanisms involved. During enumeration studies from a variety of sediments, including the Oak Ridge FRC, we isolated and characterized two novel nitrate-dependent Fe(II)-oxidizing Betaproteobacteria, *Diaphorobacter* sp. strain TPSY and *Pseudogulbenkiania* sp. 2002. *Diaphorobacter/Acidovorax* species, of the *Comamonaceae* order, are ubiquitous in soil and aqueous environments, and are often found as potential symbionts and pathogens in eukaryotic hosts. *Pseudogulbenkiania* spp. of the order *Neisseriales* are typically eukaryotic pathogens. Both strains grew by nitrate-dependent Fe(II) oxidation mixotrophically with acetate as the carbon source, while in addition, strain 2002 also grew lithoautotrophically with Fe(II), CO<sub>2</sub>, and nitrate. Both strains also oxidized insoluble U(IV) to soluble U(VI) coupled to nitrate reduction. The genomes of strains 2002 and TPSY were 4.3 Kb (draft) and 3.8 Kb (completed) respectively, with 3927 and 3479 predicted protein coding sequences. At the 16S rRNA level strain TPSY had 99.8% similarity to *Acidovorax* sp. strain JS42, whereas the closest relative to strain 2002 was *Pseudogulbenkiania subflava* (99.3% similarity). Previously, we demonstrated that c-type cytochromes are involved in nitrate-dependent Fe(II) oxidation. Both strains TPSY and 2002 had approximately 40 cytochrome domain-containing proteins. Strain 2002 appears to have a more extensive suite of cytochromes, including a b<sub>5</sub> and a Ni-Fe hydrogenase b-type cytochrome (not observed in strain TPSY), which may have a role in its ability to oxidize U(IV). A number of genes encoding candidate proteins with similarity to PioABC and MtrABC were present in both TPSY and 2002. These proteins, particularly MtrABC, are centrally involved in Fe(III) respiration by *Shewanella* spp. Interestingly, neither strain 2002 nor TPSY are capable of Fe(III) reduction, suggesting that the MtrABC protein may be involved in Fe(II) oxidation in a reverse pathway. Further investigations are needed, both at the molecular and biochemical level, to determine the role of these candidates in nitrate-dependent Fe(II) oxidation in strains TPSY and 2002, as well as to investigate whether the mechanisms involved differ between these two strains.

## Microbial Activity and Precipitation at Solution-Solution Mixing Zones in Porous Media

*Session: ERSP University-Led Research*

Frederick Colwell, Oregon State University, Corvallis, OR (lead PI); Robin Gerlach, Montana State University, Bozeman, MO; George Redden, Idaho National Laboratory, Idaho Falls, ID; Dorthe Wildenschild, Brian Wood—Oregon State University

Use of biological and chemical processes that degrade or immobilize contaminants in subsurface environments is a cornerstone of remediation technology. The enhancement of biological and chemical processes *in situ* involves transport, displacement, distribution, and mixing of one or more reactive agents. Biological and chemical reactions require diffusive transport of solutes to reaction sites at molecular scale; and accordingly, the success of processes at the meter scale and larger is dictated by the success of phenomena occurring at the micron scale. However, current understanding of scaling effects on the mixing and delivery of nutrients in biogeochemically dynamic porous media is limited, despite the limitations this imposes on the efficiency and effectiveness of the remediation challenges at hand.

We therefore propose to experimentally characterize and computationally describe the growth, evolution, and distribution of microbial activity and mineral formation, as well as changes in transport processes in porous media that receive two or more reactive amendments. The model system chosen for this project is based on a method for immobilizing  $^{90}\text{Sr}$ , which involves stimulating microbial urea hydrolysis with ensuing mineral precipitation ( $\text{CaCO}_3$ ), and co-precipitation of Sr. Studies at different laboratory scales will be used to visualize and quantitatively describe the spatial relationships between amendment transport and consumption that stimulate the production of biomass and mineral phases that subsequently modify the permeability and heterogeneity of porous media.

*Mixing Dynamics Hypothesis:* Stimulated biological activity and mineral precipitation reactions in porous media will occur where reactive amendments (i.e., DOC, electron acceptors, and impacted inorganic constituents, including  $\text{Ca}^{2+}$ ,  $\text{CO}_3^{2-}$ ,  $\text{Sr}^{2+}$ ) are mixed. Localized reduction in permeability from accumulation of biomass and precipitates in the pore structure will result in diversion of flow, divergent transport rates for amendments (i.e., restructuring of diffusive and advective components), and spatial differentiation of microbial activity and community structure that leads to decreasing overall and volume-averaged reaction rates.

*Mixing Control Hypothesis:* By controlling spatial distribution and injection schemes for reactive amendments in porous media, *in situ* longitudinal and transverse mixing zones can be controlled to optimize the distribution of intended microbial activity (i.e., calcite precipitation and Sr co-precipitation) and immobilization of metal contaminants when compared with conventional injection schemes.

To test these hypotheses, we will coordinate experimental, modeling and analytical tasks. Biomass growth, activity, and mass deposition in mixing zones will be investigated using two-dimensional micro-model flow cells and flow cells that can be analyzed using synchrotron-based x-ray tomography. Larger-scale flow-cell experiments will be conducted where the spatial distribution of media properties, flow, segregation of biological activity and impact on ancillary constituents (i.e., Sr) will be determined in both homogeneous and heterogeneous media. Model simulations will be conducted using pore-scale simulation methods that address upscale and volume averaging of media properties.

The proposed research will define the key physical, chemical, and biological processes influencing the form and mobility of DOE priority contaminants (e.g.,  $^{60}\text{Co}$ ,  $^{90}\text{Sr}$ , U) in the subsurface. This will (1) aid prediction and control of microbial kinetics and how this translates to field-scale coupled processes, (2) contribute to controlling the juxtaposition of microbial activity, contaminants and amendments, (3) promote new strategies for delivering amendments, and (4) allow new approaches for modifying permeability and flow in porous media. This will translate directly to improving the efficiency of amendment based remediation strategies.

## Mechanism of Bacterial Metal and Radionuclide Reduction by *Shewanella Oneidensis*

Session: ERSP University-Led Research

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*Shewanella oneidensis* respire a variety of terminal electron acceptors under anaerobic conditions, including insoluble Fe(III)-oxides and Tc(VII). *S. oneidensis* is postulated to be in close proximity to, or in contact with, Fe(III) oxide surfaces for efficient electron transfer to Fe(III) oxide surfaces. Previous studies in our laboratories have shown that the presence of cell surface features such as exopolysaccharides influence bacterial adhesion. Exopolysaccharide capsules have been shown to reduce adhesion to Fe(III) oxide surfaces and may limit electron transfer due to physical interruption of cell-Fe(III) oxide surface contact. The goal of this work is to identify novel components involved in *S. oneidensis* adhesion to Fe(III) oxide surfaces, in order to elucidate the mechanism of adhesion. Static adhesion assays have been performed on a battery of in-frame deletion mutants of *S. oneidensis* to determine their ability to adhere to hematite and goethite. Two adhesion-deficient strains have been identified, including those mutants deficient in the Type II (*gspD*) and Type V (*SO3800*) secretion systems. The *Gspd* mutant strain is deficient in its ability to bind to Fe(III) oxides after growth on lactate with oxygen, fumarate, and Fe(III)-citrate as electron acceptors. The *SO3800* mutant strain is deficient in its ability to bind Fe(III) oxide surfaces after growth on lactate coupled with fumarate and Fe(III)-citrate, but adheres to Fe(III) oxides at wild-type levels after growth on lactate aerobically. Mutant strains deficient in the outer-membrane cytochromes *mtrC* and *omcA* displayed wild-type levels of adhesion under all growth conditions. These results indicate that *S. oneidensis* secrete outer membrane proteins that facilitate binding to Fe(III) oxide surfaces, and that the Fe(III)-binding proteins may include proteins other than the outer-membrane cytochromes *mtrC* and *omcA*.

A rapid mutant screening technique has also been developed to identify mutant strains deficient in Fe(III) oxide adhesion. A pool of random mutants is passed repeatedly through hematite suspensions to filter out strains retaining the ability to adhere to hematite. The resulting filtrate is enriched with adhesion-deficient mutants. A bank of adhesion-deficient mutants thus identified is being tested for the ability to adhere to Fe(III) oxides after growth on various electron acceptors. Genes required for adhesion to Fe(III) oxides will be identified by genetic complementation analysis of the adhesion-deficient mutants.

Microbial reduction of soluble Tc(VII), on the other hand, results in formation of highly insoluble Tc(IV) oxides, a Tc immobilization process that forms the basis of alternate remediation strategies. To identify genes required for Tc(VII) reduction by *S. oneidensis*, a rapid mutant screening technique was developed for identification of Tc(VII) reduction-deficient (Tcr) mutants. The Tcr mutant screen was based on the observation that wild-type *S. oneidensis* produced a black Tc(IV) precipitate on its colony surface during Tc(VII) reduction, while putative Tcr mutant colonies remained colorless. Six Tcr mutants were identified via application of chemical mutagenesis and the Tcr mutant screen. Based on their ability to respire an array of 13 alternate electron acceptors with hydrogen, lactate or formate as electron donor, the Tcr mutants were divided into three classes: (1) deficiency in reduction of Tc(VII) with hydrogen or lactate as electron donor, yet retaining the ability to reduce Tc(VII) with formate, (2) deficiency in reduction of Tc(VII), NO<sub>3</sub><sup>-</sup>, Mn(III) or U(VI) with hydrogen as electron donor, yet retaining respiratory capability on all electron acceptors with lactate or formate, and (3) deficiency in reduction of all anaerobic electron acceptors regardless of electron donor. These results suggest that the Tc(VII) reduction pathway of *S. oneidensis* contains separate branches linked to the oxidation of hydrogen, lactate, or formate, and shares structural or regulatory components with pathways for reduction of NO<sub>3</sub><sup>-</sup>, Mn(III) and U(VI). The genes required for Tc(VII) reduction by *S. oneidensis* are currently being identified by genetic complementation analysis of the Tcr mutants.

## The Natural Enrichment of Stable Cesium in Weathered Micaceous Materials and Its Implications for $^{137}\text{Cs}$ Sorption

*Session: ERSP University-Led Research*

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Stable cesium likely has become concentrated in 2:1 phyllosilicates in the subsurface during natural weathering processes. Its presence in exchange sites limits the degree to which these minerals can take up  $^{137}\text{Cs}$ , so knowledge of the amount of exchangeable stable cesium in them is essential. Furthermore, knowledge of how stable cesium has become distributed in various sites in subsurface minerals, during natural processes that have extended over millennia, will provide the basis for a conceptual model of how  $^{137}\text{Cs}$  will behave in the subsurface over its effective lifetime. The hypotheses to be tested are: (1) that stable cesium has become significantly enriched relative to potassium in weathered micaceous materials (2:1 phyllosilicates) within the subsurface at the Savannah River Site (SRS) as a result of natural chemical weathering processes; and (2) that the Cs so present is sufficient to be a major factor determining the ability of the subsurface materials to take up and hold  $^{137}\text{Cs}$ .

The primary objectives in this study are as follows: (1) to determine the mineralogical character of the 2:1 phyllosilicates in various size fractions of subsurface samples from the SRS; (2) to determine the degree to which stable Cs has been enriched by natural weathering processes in cation-exchange sites of the 2:1 phyllosilicates, including fixed Cs as well as Cs in sites where it is immediately accessible for exchange by cations in solution; (3) to determine the character of the microenvironments where stable Cs is now concentrated, insofar as that is possible by measuring species, including strontium isotopes and argon isotopes, released by a set of progressively stronger ion-exchange reactions; (4) to determine the degree to which  $^{137}\text{Cs}$  in contaminated subsurface materials is concentrated in the same microenvironments where stable cesium is concentrated; and (5) to evaluate the potential use of commercially available weathered mica in cleanup of  $^{137}\text{Cs}$ -contaminated waters at Department of Energy sites.

In this study, approximately 15 samples of clayey sand will be collected from SRS cores, including three samples that are contaminated with  $^{137}\text{Cs}$ . After size separations of the uncontaminated samples, the character of the phyllosilicates in the various fractions will be determined by microscopy and x-ray diffraction. The 2:1 phyllosilicates from selected fractions will be concentrated by physical or chemical techniques and characterized further via x-ray diffraction and TEM. The concentrations of K, Rb, and Cs in acid leachates from untreated sample portions and from concentrated phyllosilicates will be determined to indicate the degree of enrichment of cesium relative to the two other elements. Ion exchange between test portions of selected samples and a series of progressively more effective aqueous exchange agents will be used in an attempt to find the amounts of Cs (including  $^{137}\text{Cs}$  from the contaminated samples), Rb, and K at different kinds of sites, including sites where these elements have become fixed. After each step of an exchange sequence, portions of the supernatant solution will be used for determination of pH, amounts of major base cations (flame AAS), and amounts of Al, Cs, Rb,  $^{87}\text{Sr}$ , and  $^{87}\text{Sr}$  (by ICP-MS). For contaminated samples, yet another portion will be used for determination of  $^{137}\text{Cs}$ . For selected samples, ion-exchange reactions will be done in an initially Ar-free reaction vessel to allow the amount and isotopic composition of the Ar released in each step of an ion-exchange process to be determined.

## Accessing Uncultivated Microorganisms Involved in Metal Immobilization

*Session: ERSP University-Led Research*

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The long-term objective of this project is to understand the *in situ* microbial activities affecting metal-contaminant transformation and optimize these activities to achieve sufficient removal of contaminants from the polluted sites. To achieve this, a critical first step is to access and isolate novel microorganisms involved into metal immobilization. Currently, the majority of environmental species remain uncultivated by standard approaches. Therefore, we have developed a new concept for microbial isolation by growing microbial cells in their natural environment inside diffusion chambers. We recently demonstrated that one to several *in situ* incubations lead to domestication of many species that are otherwise uncultivable. In the course of field and lab experiments conducted at the Oak Ridge National Laboratory and Northeastern University, we have obtained approximately 700 cultures of individual species and/or consortia from groundwater of DOE's IFRC sites, which are heavily contaminated with U and other metals. We began screening these cultures on their ability for *in vitro* U and other metal reduction, using inductively coupled plasma mass spectrometry. Our initial results indicate that approximately 75% of the cultures cause precipitation of U(VI) at environmentally relevant concentrations of the pollutant. Currently, we are in the process of completing the screen for all of the 700 cultures obtained, and establishing the identity of species capable of such transformation. Our next goal will be to compare performance of individual species versus consortia of species, which provides valuable insights to address whether synergistic consortia immobilize heavy metals more efficiently than do individual species. Upon completion of this project, we will obtain a collection of novel species capable of *in situ* metal immobilization, and a better understanding of community-level interactions that favor the rates of this important process.

## Microbial Community Trajectories in Response to Accelerated Remediation of Subsurface Metal Contaminants

*Session: ERSP University-Led Research*

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To the degree that remediation of subsurface metal contaminants at DOE sites involves microbial mechanisms of oxidation/reduction or complexation, then that remediation is controlled in large part by the ecology of the microbial community involved. Recognizing and quantifying the relationship between community structure, function, and key environmental factors may yield the quantitative understanding needed to inform future decisions on remediation strategies and trajectories. We have previously found that U bioreduction and maintenance of low aqueous U concentrations is strongly dependent on the organic carbon (OC) supply rate. Our results also showed that OC supply rate had a significant effect on microbial community structure, while the effect of two different OC types was undetectable during the duration of the experiment. The differences between communities attributable to different rates of OC supply diminished through time, despite the fact that different rates of OC supply resulted in different environmental conditions within the columns. We quantified overall temporal changes using a number of biodiversity indices; each emphasizing different aspects of the microbial community structure. We observed significant, directional, temporal trends when comparing taxa abundance curves, taxa richness, evenness, and Simpson's diversity. Together, these data indicate that microbial communities stimulated for bioremediation may follow predictable trajectories in community structure.

Based on our prior work, and operating under the premise that microbial communities can be controlled and predicted as well as the resulting remediation capacity, the objectives of our current project are to: (1) determine if the trajectories of microbial community composition and function following OC amendment can be related to, and predicted by, key environmental determinants; (2) assess the relative importance of the characteristics of the indigenous microbial community, sediment, groundwater, and OC supply rate as the major determinants of microbial community functional response and bioremediation capacity; and (3) provide a fundamental understanding of the microbial community ecology underlying subsurface metal remediation requisite to successful application of accelerated bioremediation and long-term stewardship of DOE sites.

We are testing three sediments and their resident microbial seed communities using a reciprocal transplant experimental design. We are employing small, easily replicated and manipulated flow-through reactors receiving simulated groundwater at two OC supply rates; flow-through reactors will be destructively sampled over a period of 18 months. Microbial community trajectories will be followed using: 16S PhyloChip analysis of community DNA (overall structure) and RNA (active members); GeoChip functional analysis of community DNA (functional potential) and community RNA (active functions); functional metagenomic analyses of DNA and RNA to explore functional capacities not included on extant arrays; and high-resolution deconvolution microscopy and statistical evaluation of microbial spatial organization to document syntrophy and other microbial interactions. Solution chemistry characteristics of flow cell effluents will be used to statistically correlate geochemical and microbial community structural and functional trajectories.

## Long-Term Colloid Mobilization and Colloid-Facilitated Transport in a Semi-Arid Vadose Zone

Session: *ERSP University-Led Research*

Markus Flury (lead PI), Jim Harsh—Washington State University, Pullman, WA; Fred Zhang, Pacific Northwest National Laboratory, Richland, WA; Peter Lichtner, Los Alamos National Laboratory, Los Alamos, NM; Earl Mattson, Idaho National Laboratory, Idaho Falls, ID

Our general goal is to improve the fundamental mechanistic understanding and quantification of long-term colloid mobilization and colloid-facilitated transport of radionuclides in the vadose zone, with special emphasis on the semi-arid Hanford site. Our specific objectives are: (1) to determine the mechanisms of colloid mobilization and colloid-facilitated radionuclide transport in undisturbed Hanford sediments under unsaturated flow, (2) to quantify *in situ* colloid mobilization and colloid-facilitated radionuclide transport from Hanford sediments under field conditions, and (3) to develop a field-scale conceptual and numerical model for colloid mobilization and transport at the Hanford vadose zone, and use that model to predict long-term colloid and colloid-facilitated radionuclide transport.

*Research Plan:* (1) We will conduct *in situ* colloid mobilization experiments with mesoscale, undisturbed sediment columns using natural and forced flow rates and water contents representative of the Hanford site. We will further use a geocentrifuge (a) to separate effects of flow rates and water contents on colloid mobilization, and (b) to speed up the experiments. At the microscale, we will use tensiometry to quantify forces between colloids and the air-water interface. (2) We will study colloid mobilization and transport at the field-scale at Hanford using natural and forced flow rates. Colloids in Hanford pore water will be collected with fiberglass-wick samplers. (3) We will develop a numerical field-scale model to describe colloid and colloid-facilitated contaminant transport through the Hanford vadose zone.

Results: We have taken three, large undisturbed soil cores from the Hanford site from 20 m depth (50 cm diameter, 60 cm height). These mesoscale columns are currently being set up in the laboratory for long-term colloid mobilization experiments. One core has been fully instrumented and is under irrigation at a steady-state flow rate of 20 mm/yr. Once outflow collection begins, we will increase the flow rates sequentially to 40, 80, and 160 mm/year. A second core is being set up for transient flow experiments where we mimic natural rainfall patterns at Hanford. The third core will be used for studying the effects of water table fluctuations on colloid mobilization.

We are currently instrumenting four field lysimeters at the Hanford 300N Lysimeter Facility for colloid mobilization and transport experiments. The lysimeters are being instrumented with neutron-probe access tubes, water content sensors, tensiometers, and fiberglass-wick samplers for collection of water and colloid samples. We will apply Eu colloids to the surface of the lysimeters. We have synthesized 200 nm size  $\text{Eu}_2\text{O}_2\text{CO}_3$  colloids and have characterized their surface and colloid properties. Different-sized polystyrene colloids will be also be applied as tracer colloids. Lysimeters will be subject to different irrigation scenarios. Hanford rainwater has been collected for the purpose of mimicking irrigation solutions.

We have collected seven undisturbed small cores from Hanford formation sediments, which will be used for laboratory bench top and geocentrifuge experiments. Geocentrifuge experiments are scheduled to start in summer 2009. We have begun experiments to quantify the forces on colloidal particles exerted by a moving air-water interface. We have selected representative particles from Hanford sediments and have quantified forces at the air-water interface using tensiometry.

Colloid-facilitated transport of radionuclides is being added to PFLOTRAN, a massively parallel reactive flow and transport code developed under the DOE SciDAC-2 program. The model will include colloid filtration mechanisms, and mechanistic formulations of reversible and irreversible sorption, including ion exchange and surface complexation for variably saturated porous media.



## Developing Highly Sensitive Micro-Biosensors for *In Situ* Monitoring of Mercury and Chromium (VI) Contaminants by Genetically Evolved and Computer-Designed Metal-Binding Proteins

Session: ERSP University-Led Research

Qinhong Wang, Xiangdong Fang, Sheng Wu, Patrick J Shuler, Yongchun Tang, William A Goddard III (lead PI)—Power, Environmental, and Energy Research (PEER) Center, California Institute of Technology, Covina, CA

The Department of Energy's environmental cleanup operations are facing enormous challenges to remediate metal and radionuclide contaminants. In order to gain significant understanding of the mobility and fate of contaminants in the subsurface, one of the key tasks requires the development of novel techniques for detecting and long-term monitoring the contaminants, both before and after remediation efforts. At present, there are lots of sensor-based detection methods, but with different advantages and disadvantages. Whole-cell biosensors, constructed by coupling microbes to transducers that convert cellular responses into detectable signals, are easy to reproduce and have a great potential to reduce the cost.

Our goal is to employ the latest advances in protein engineering, microbiology and photo detection technology to improve the effectiveness of metal-binding protein based biosensor that can be used for accurate detection and monitoring of heavy metal contaminants at DOE sites. Here, based on the metal-lorepressor MerR and red fluorescent protein mCherry, we constructed different whole-cell biosensors for mercury detection (including inorganic mercuric ion and methyl mercury) with different sensitivity with *Escherichia coli* as host strain. These biosensors have a wide range of mercury detection (0.1nM-5,000nM). They also have high selectivity without response to other metal ions, such as  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Mn^{2+}$ ,  $Fe^{3+}$ ,  $Co^{2+}$ ,  $Ni^{2+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$ ,  $Cd^{2+}$ ,  $Pb^{2+}$ , and  $Mo^{3+}$ . By introducing the bio-amplification genetic circuit based on the gene cascade expression system of  $P_{RM}-cI$  from bacteriophage  $\lambda$  and  $P_m-XylS2$  from *Pseudomonas putida*, we have increased detection sensitivity of the whole-cell biosensors by 1~2 fold in our tested conditions. With directed evolution of MerR and subsequent high-throughput screening based on color assay and microplate screening, we further increase detection sensitivity by 5~10-fold at low concentration of mercury (II) of 0.1-10nM.

To fabricate the cheap, portable fluorescence-based detector, our latest effort is to use a commercial off the shelf green laser pointer emitting at 532 nm. The price of such devices has now fallen to \$10/piece, and because it is perfectly collimated with perfect beam quality ( $M^2=1.2$ ) and an ultra narrow linewidth (<0.02 nm) at 532 nm, we save the cost of collimation and focusing lens, as well as excitation filter in the previous setup where an LED is used. This actually brings down the cost of light source submodule in the final system, at the same time improves noise rejection. Our new result was obtained with the 532 nm excitation laser beam orthogonal to the 610 nm passing filter and PMT (Photo Multiplier Tubes) beam path, and we expect to further reduce the noise and improve signal when using inline configuration. Also, we are trying to integrate microfluidic channels with our system so that single cell's fluorescence could be detected, and we hope to demonstrate even higher signal to noise ratio because the fluorescence protein are concentrated inside the bacterial cell.

## Geochemical Conditions Affecting Electrode-based Removal of Uranium

Session: ERSP University-Led Research

Kelvin B. Gregory (lead PI), Juan Peng—Carnegie Mellon University, Pittsburgh, PA

*In situ* remediation is a potential cleanup strategy for sites with subsurface U(VI) contamination. A common suggestion is the addition of bulk electron donor for biotic and abiotic reduction of U(VI), which induces reductive precipitation and immobilization of U as U(IV) in the subsurface. However, because U still resides in the subsurface and re-oxidation may remobilize the contaminant, the risk to humans has not been eliminated. Recently, a new, electrode-based bioremediation approach for *in situ* metal remediation was suggested. In brief, U(VI) may be reduced by indigenous and pure cultures of *Geobacteraceae* when provided electron donor from a cathodic electrode. This method shows significant promise for *in situ* U remediation; however, knowledge gaps in the fundamental principles of metal sequestration at the electrode surface and the environmental factors that affect removal inhibit successful application of electrode technology at the field scale. The objectives of this research address the need for an in-depth understanding of the geochemical factors that affect contaminant removal and shed light on the underlying principles of electrode-based remediation and recovery. The governing hypothesis of this work is that geochemical conditions are a critical factor in the design and implementation of electrode-based remediation.

Batch experiments investigated the removal/recovery of U(VI) at poised, graphite electrode surfaces under various geochemical conditions. Uranium removal at electrode surfaces with an applied external potential of 2 V was strongly influenced by pH. Although at least 90% removal was observed at all pH between 2 and 6, initial removal rates were inversely correlated with H<sup>+</sup> concentration and decreased over time. This detrimental effect of elevated H<sup>+</sup> concentration, however, was counteracted through applying higher potentials between the electrodes. For example, increasing the potential from 2.0 to 2.5 V increased the removal rate of U(VI) by 125%, but potentials above 3.0 V did not have a significant additional effect on the removal rates. In addition to pH, the impact of ionic strength and competing metal cations on U(VI) removal was investigated through the addition of Al(III) and Mg(II) salts at increasing concentrations under acidic conditions (pH = 3.0). At low applied potential (2.5 V), Al(III) up to 540 mg/L and Mg(II) up to 120 mg/L did not impact U(VI) removal rates, nor was Al(III) removed from solution. Interestingly, at higher applied potentials (5V), Al(III) was removed from solution and U(VI) removal rates were significantly decreased in the presence of Al(III) at concentrations less than 135 mg/L.

Findings demonstrate that U(VI) can be removed from water under acidic conditions and in the presence of high concentrations of competing cations, such as those observed at Area III of the FRC. Clearly, applied potential at the electrodes will be an important design criterion for optimized removal rates in the field. Under all conditions tested, at least 80% of the added U(VI) was recoverable from the electrodes, demonstrating that electrode-based remediation offers the advantage of contaminant mass removal not offered by other remedial technologies for heavy metals and actinides. Geochemical investigations are ongoing. Future work will evaluate electrode-based removal processes and optimization using site water, and investigate the microbial community that develops on electrodes deployed in Area III site water.

## Novel Sensor for the *In Situ* Measurement of Uranium Fluxes

*Session: ERSP University-Led Research*

Kirk Hatfield (lead PI), Mark Newman, Michael D. Annable, Jaehyun Cho—University of Florida, Gainesville, FL; James Randville, Valerie Stucker—Colorado School of Mines, Golden, CO; Aaron Peacock, Microbial Insights Inc, Steve Cabaniss, University of New Mexico, Albuquerque; Irina Perminova, Lomonosov Moscow State University

Given the emphasis on field-scale outcomes under ERSP, current hypotheses under evaluation at the Rifle IFRC site, and previous findings of stimulated uranium bioreduction, there is a critical need to develop sensors to quantify uranium fluxes, reaction mass balances, stability, and attenuation rates at the field-scale. Once developed, these sensors could be used with other technologies to investigate apparent relationships between field-scale variations in mineralogy, microbial community biomass/composition, and flux variations for uranium and salient electron donor/acceptors.

The goal of this project is to develop a novel sensor that incorporates field-tested concepts of the passive flux meter to provide direct *in situ* measures of uranium and groundwater fluxes. Measurable uranium fluxes are essentially the product of concentration and groundwater flux or specific discharge. The sensor uses sorbents and tracers to measure uranium flux and specific discharge directly; however, sensor principles and design should apply to fluxes of other radionuclides. Flux measurements will assist DOE in obtaining field-scale quantification of subsurface processes affecting uranium transport (e.g., advection) and transformation (e.g., uranium attenuation) and further advance conceptual and computational models for field scale simulations. Project efforts will expand our current understanding of how field-scale spatial variations in uranium fluxes and those for salient electron donor/acceptors, and groundwater are coupled to spatial variations in measured microbial biomass/community composition, effective field-scale uranium mass balances, attenuation, and stability.

During the first year, laboratory studies focused on sorbent and tracer selection and testing, sensor development and validation under controlled conditions, geochemical modeling, and limited field testing. Several anion-exchange resins tested successfully as sorbents for capturing uranium on the fluxmeter. Seven branched alcohols proved useful as tracers for measuring groundwater flows under laboratory conditions; however, additional work is needed to show tracers are recalcitrant in the field. Geochemical modeling of equilibrium speciation suggests Ca-tricarbonato-uranyl complexes predominate under field conditions, while calculated uranyl ion activities are sensitive to changes in pH, dissolved inorganic carbon, and alkaline earth metals. Further modeling is needed in conjunction with laboratory studies to resolve differences in model predictions. Preliminary field tests at the Rifle IFRC site were also conducted to assess ambient groundwater flow conditions, monitor microbial growth on the sensor during field deployment, and resolve any unforeseen problems evolving from field deployment. Groundwater flows measured November 2008 varied from 2.5–6.5 cm/d (velocity of 9–25 cm/d) or much lower than typically quoted rates, whereas U fluxes ranged from 0.9 to 8.5 mg/m<sup>2</sup>/d. Microbial biomass and community composition measured on fluxmeter sorbents varied between well locations (up/down gradient from biostimulation zones) and sorbent types.

Future field studies will be closely coordinated with existing ERSP projects to complement data collection. For these studies, the uranium flux sensor will be used in conjunction with molecular-biological tools to characterize microbial community and active biomass at synonymous wells. These field efforts will be designed to quantify spatial changes in uranium flux and field-scale rates of uranium attenuation (ambient and stimulated) and uranium stability, and then quantitatively assess how fluxes and effective reaction rates are coupled to spatial variations in mineralogy, the microbial composition, and active biomass. Analyses of data generated will lead to estimates of Monod kinetic parameters that are “effective” in nature and optimal for modeling uranium fate and transport at the field-scale. Proper development of these models is critical for long-term stewardship of contaminated sites in the context of predicting uranium source behavior, remediation performance, and off-site risk.

## Spectroelectrochemical Sensor for Pertechnetate Applicable to Hanford and Other DOE Sites

*Session: ERSP University-Led Research*

William R. Heineman (PI), Carl J. Seliskar—University of Cincinnati, OH; Samuel A. Bryan, Pacific Northwest National Laboratory, Richland, WA

The general aim of our work currently funded by DOE is the design and implementation of a new sensor technology that offers unprecedented levels of specificity needed for analysis of the complex chemical mixtures found at DOE sites nationwide. This project involves a very successful collaboration between scientists at the University of Cincinnati (UC) and several at the Pacific Northwest National Laboratory (PNNL) and the Environmental Molecular Sciences Laboratory (EMSL). The goal of the work is the continued development of a sensor for  $^{99}\text{Tc}$  that is applicable to characterizing and monitoring the vadose zone and associated groundwater. The single focus is pertechnetate,  $\text{TcO}_4^-$ , which is considered to be the dominant species in the vadose zone and groundwater. The sensor will have the capability for on-site monitoring, either by immersion in subsurface water for continuous monitoring or for the immediate analysis of collected samples. The project builds on the substantial progress of a well-established UC-PNNL collaboration which provides the wide range of expertise needed for success: spectroscopy, electrochemistry, device fabrication, thin film technology, synthetic inorganic chemistry, experience with Tc, and facilities for handling radioactive isotopes.

The sensor will consist of an innovative fluorescence-based spectroelectrochemical configuration that we have developed under our previous EMSP grants. The spectroelectrochemical sensor has been demonstrated on a variety of chemical systems including an authentic tank waste sample from Hanford. The following benchmarks have been met:

- Sulfonated polystyrene-block-poly(ethylene-ran-butylene)-block-polystyrene (SSEBS) characterized and demonstrated as an effective preconcentrating polymer film.
- Absorbance-based sensor demonstrated for detection of  $[\text{Re}(\text{dmpe})_3]^+$  (dmpe = dimethylphosphinoethane) where rhenium was used as a nonradioactive analogue for technetium and SSEBS was the preconcentrating film.
- Synthesized lower oxidation-state Tc-complex  $[\text{Tc}(\text{dmpe})_3]^+$  to use as a model compound for developing Tc sensors. It exhibits the important properties of fluorescence at ambient temperatures and reversible electrochemistry.
- Fluorescence spectroelectrochemistry initiated for  $[\text{Tc}(\text{dmpe})_3]^+$  and preconcentration at SSEBS with electrochemical modulation of fluorescence signal demonstrated.
- $\text{TcO}_4^-$  shown to preconcentrate in sensor films containing anion exchange polymers and to undergo electrochemical reduction.
- Ability to selectively modulate fluorescence of specific analyte in mixtures preconcentrated in SSEBS film demonstrated with model systems  $\text{Ru}(\text{bpy})_3^{2+}$ ,  $\text{Fe}(\text{bpy})_3^{2+}$ ,  $\text{Cu}(\text{bpy})_2^{2+}$ , and  $\text{Fe}(\text{CN})_6^{3-}$ .
- Development of portable instrumentation for fluorescence sensors for DOE sites initiated.
- Study of effects of components in Hanford subsurface water on sensor performance initiated with evaluation of effect of total ionic strength on sensitivity.

## Chromium Isotopes as Indicators of Hexavalent Chromium Reduction

Session: ERSP University-Led Research

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Stable isotope variations in chromium (Cr) may be used to quantify reduction of mobile, toxic Cr(VI) (hexavalent Cr) to the immobile, less toxic Cr(III) (trivalent Cr). This reaction is extremely important, since it is both the dominant mechanism of natural attenuation and the major means of *in situ* remediation for Cr(VI). Laboratory experiments show that reduction of Cr(VI) causes isotopic fractionation: The Cr(III) reaction product is enriched in lighter Cr isotopes, and the remaining Cr(VI) becomes progressively enriched in heavier isotopes as reduction proceeds. This enrichment is quantified as a shift in the  $^{53}\text{Cr}/^{52}\text{Cr}$  ratio; elevated ratios indicate Cr(VI) reduction. Work so far suggests  $^{53}\text{Cr}/^{52}\text{Cr}$  data should be highly effective as an indicator and possibly a quantifier of Cr(VI) reduction in groundwater systems; we are beginning to apply this approach in field studies.

We obtain high-precision  $^{53}\text{Cr}/^{52}\text{Cr}$  measurements on groundwater samples with concentrations as low as 0.001 mg/L using a multicollector ICP-MS method. Results are expressed as  $\delta^{53}\text{Cr}$  values, which are per mil deviations from NIST SRM-979. Precision is  $\pm 0.15\text{‰}$  (2s).

Our Idaho National Laboratory (INL) project is nearing completion. We have determined  $\delta^{53}\text{Cr}$  of Cr(VI) in 60 groundwater samples. Well USGS-065 is located in the fast-flowing Snake River Plain aquifer immediately downgradient from a known Cr(VI) contaminant source. It has the greatest Cr(VI) concentration (0.098 mg/L) of our sample set, and its measured  $\delta^{53}\text{Cr}$  is  $+0.30\text{‰}$ . This is nearly identical to the inferred  $\delta^{53}\text{Cr}$  of the contaminant; we conclude this water has had little or no Cr(VI) reduction. Nine other samples with greater than 0.015 mg/L Cr(VI) are assumed to contain contaminant Cr(VI). Their  $\delta^{53}\text{Cr}$  values range from  $0.3\text{‰}$  to  $1.8\text{‰}$ , and suggest the extent of Cr(VI) reduction ranges from essentially zero to roughly 40% of the original contaminant Cr(VI). The  $\delta^{53}\text{Cr}$  of natural background Cr(VI) in the aquifer ranged from  $1.0\text{‰}$  to  $+2.9\text{‰}$ ;  $\delta^{53}\text{Cr}$  of the basalt rock Cr source is almost certainly very close to  $0.0\text{‰}$ , so either Cr isotope fractionation occurs during weathering, or Cr(VI) released by weathering is subjected to partial reduction within the aquifer. Together, the results suggest that limited (<40% over a few decades) reduction of contaminant Cr(VI) occurs in some subsurface locations and not in others. Further data analysis will elucidate any spatial patterns in the reduction rate.

The second part of the project attempts to reveal Cr(VI) reduction patterns in a pilot-scale Calcium polysulfide permeable reactive barrier at Hanford 100-K. Breakthrough of Cr(VI) into the treated zone occurred in 2006, but has not yet spread beyond one well. In June 2006,  $\delta^{53}\text{Cr}$  was  $+3.93\text{‰}$  there, indicating the Cr(VI) did not merely bypass the treated zone, but was subject to strong reduction (we estimate >60% lost) as it moved through it. In May 2008, the concentration had increased three-fold to 0.025 mg/L, and  $\delta^{53}\text{Cr}$  had decreased to  $1.46\text{‰}$ , indicating weakening reduction over time.

## Processes Controlling Enhanced Transport of Plutonium in Unsaturated Conditions

Session: ERSP University-Led Research

Daniel Kaplan (lead PI), Christopher Bagwell—Savannah River National Laboratory, Aiken, SC; Ravi Kukkadapu, PNNL, Richland, WA; Fred Molz, Clemson University, Anderson, SC; Heino Nitsche, LBNL, Berkeley, CA; Brian Powell, Clemson University

In prior years, we showed that plutonium mobility was greatly influenced by resolubilization under oxidizing conditions. The mechanism for this resolubilization is unknown and the subject of recent, ongoing research. Progress has been made in (1) determining how various Fe phases affect Pu(V) reduction kinetics, and (2) using models to assess various coupled chemical, physical, and biological processes.

A laboratory study was conducted to determine which mineral phases in a Savannah River Site (SRS) sediment (pH 5, 1.6% total Fe) affected Pu(V) reduction. Using sediment fractions that were treated with various Fe dissolving solutions and that were characterized by XRD, TEM, and Mössbauer, Pu(V) reduction was determined not to be influenced by the removal of amorphous, poorly crystalline Fe-oxide phases. Dithionite citrate buffer (DCB) treatments resulted in the removal of essentially all Fe-oxides, but Pu(V) reduction rates *increased*, contrary to expectation. Preliminary Mössbauer analyses of the DCB treated sediments implied partial reduction of clay Fe(III). Apparently, the trace concentrations of Fe in the kaolinite and a muscovite-like mineral phases have the capacity to greatly increase Pu(V) reduction, even in the absence of surface Fe-oxide coatings. Pu(V) reduction for the whole sediment and for fractions with amorphous Fe-oxides removed were very similar,  $\sim 0.04 \text{ min}^{-1}$  ( $t_{1/2} = 20 \text{ min}$ ), whereas the rates for DCB-treated sediments were faster,  $\sim 0.1 \text{ min}^{-1}$  ( $t_{1/2} = 6 \text{ min}$ ). All aqueous Pu existed as Pu(V), and all solid phase Pu existed as Pu(IV) or Pu(V). Solid-phase Pu(V) quickly (in minutes) converted to Pu(IV), suggesting that adsorption followed by surface facilitated reduction was the mechanism responsible for Fe(V) sorption.

Eleven-year Pu field lysimeter experiments showed anomalous Pu-sediment distributions below the source, with significant upward migration above the source. The initial conceptual model of the transport process was based on variably saturated soil water movement, due to the natural climate coupled to kinetically controlled oxidation state transformations of two adsorbing Pu species. Simulations based on this model yielded reasonable below-source transport, but little above-source transport. The conceptual model was then modified to include Pu absorption by plant roots and upward movement in the transpiration stream. Modified simulations were then consistent with Pu activity concentrations in the top 20 cm of soil and suggested an accumulation of Pu on the soil surface due to annual grass dieback. Such a surface soil residue has now been verified by isotope ratio analysis by TIMS. A remaining failure of the conceptual model was a continuing inability to explain upward Pu migration within 5 to 10 cm of the source. This motivated a second modification to the initial conceptual model, wherein spreading in the immediate source vicinity is caused by a pseudo-diffusion process, perhaps a microbe-facilitated process resistant to advection. Simulations based on this model are data consistent and appear promising. New research has been initiated to identify uptake mechanisms and Pu behavior in plant tissue, with special attention directed to the probable occurrence of Pu chelation and the potential role of microbes in promoting more mobile forms of Pu.

## Quantifying Microbe-Mineral Interactions Leading to Remotely Detectable Induced Polarization Signals: Implications for Monitoring Bioremediation

*Session: ERSP University-Led Research*

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Recent laboratory results suggest that geophysical induced polarization (IP) measurements are sensitive to microbial activity in porous media. This presents the potential for remote, long-term monitoring of biogeochemical conditions using low-frequency electrical techniques. A gap in knowledge currently exists, however, in understanding the fundamental processes that lead to the biologic IP response. It is not yet clear which microbial mechanisms affecting the electrical properties of a porous medium—particularly those leading to alteration of the pore space and mineral surfaces—are responsible for the observed IP signatures. Discriminating between these mechanisms is a critical task if IP measurements are to be used for effective monitoring of complex processes like bioremediation.

Characterizing the biologic IP response is a quintessential scaling problem that spans nanometer to meter length scales. As such, our work couples surface-sensitive, nanoscale polarization and conductive force microscopy (PCFM) techniques with IP observations of bioactive columns at the lab scale. PCFM allows us to experimentally isolate microbial processes that are hypothesized to affect electrical properties at mineral-water-cell interfaces. In contrast, the IP measurements include both interfacial and bulk pore volume contributions. Comparing the electrical response at each scale will thereby provide initial qualitative inference of the underlying causes of the observed IP effect. Stochastic upscaling will link the two observation scales. Preliminary data show localized, nanoscale equivalents to traditional IP measurables, such as complex conductivity and apparent chargeability using PCFM. In particular, the frequency-dependent dispersions in the polarization forces measured on a bacterium correspond to modeled electrical permittivity dispersions. Moreover, frequency cut-off values for maximum polarization forces increase with increasing ion mobility in a pattern that is consistent with the modeled values. Comparing PCFM observations of the mineral-microbe interface with column IP measurements should provide much needed molecular-level insight into the bio-IP phenomena.

## Structure and Function of Subsurface Microbial Communities Affecting Radionuclide Transport and Bioimmobilization

Session: ERSP University-Led Research

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The overall goal of the proposed project is to closely couple cutting-edge microbiological and biogeochemical approaches to provide a mechanistic understanding of the functioning of subsurface microbial communities with a high bioremediation potential. Our focus is on sediments of the Oak Ridge Integrated Field Research Center (OR IFRC) in Oak Ridge, Tennessee, and the main objectives are to: (1) isolate and characterize novel anaerobic prokaryotes from subsurface environments exposed to high levels of mixed contaminants (U(VI), nitrate, sulfate); (2) elucidate the diversity and distribution of metabolically active metal- and nitrate-reducing prokaryotes in subsurface sediments; and (3) determine the biotic and abiotic mechanisms linking electron transport processes (nitrate, Fe(III), and sulfate reduction) to radionuclide reduction and immobilization. A number of novel strains of metal- and nitrate-reducing bacteria have been isolated, and environmental sequence data indicate that these species are abundant in the OR-IFRC subsurface. Based on phylogenetic analysis and phenotypic differences, strain FRC-32T is now described as a novel iron(III)- and uranium(VI)-reducing species of the genus *Geobacter* and proposed as *Geobacter daltonii* sp. nov. Analyses of both 16S rRNA gene and the *Geobacteraceae*-specific citrate synthase (gltA) mRNA gene sequences retrieved from ORIFRC sediments indicate that this strain is abundant and active in ORIFRC subsurface sediments undergoing uranium(VI) bioremediation. Five species of denitrifying bacteria from four phyla were isolated using an enrichment approach in which nitrate concentration and electron donor were systematically varied. The isolated bacteria belong to the genera *Afipia* and *Hyphomicrobium* (*Alphaproteobacteria*), *Rhodanobacter* (*Gammaproteobacteria*), *Intrasporangium* (*Actinobacteria*) and *Bacillus* (*Firmicutes*). A nitrate-reducing member of the *Pseudomonadaceae* was also isolated by direct sorting of single cells from Area 2 samples using flow cytometry. A total of 175 additional single-cell isolates were obtained from Area 2 samples via state-of-the-art direct cell sorting methods. These isolates are growing under nitrate-reducing conditions and are currently being identified. To track the activity and diversity of active uranium-reducing organisms *in situ*, mRNA was isolated from sediment cores sampled at a range of depths and contaminant levels. Using quantitative reverse transcription real-time PCR, gltA and dsrA genes were detected, indicating active populations in nearly all sediment samples analyzed. Clone library and phylogenetic analyses of gltA mRNA clone sequences demonstrated that groups closely related to *G. daltonii*, *G. uraniireducens*, and *G. metallireducens* are active and abundant in the OR-IFRC subsurface. Analysis of short dsrA mRNA clone sequences confirmed the specificity of the real-time PCR amplicons and suggested that groups closely related to *Desulfovibrio* and *Desulfotomaculum* species are active and abundant in the OR-IFRC subsurface. Further phylogenetic analyses using longer cDNA fragments are under way.



## Effects of Pore Structure Change and Multiscale Heterogeneity on Contaminant Transport and Reaction Rate Upscaling

Session: ERSP University-Led Research

W. Brent Lindquist (lead PI), Stony Brook University, NY; Michael Celia, Catherine Peters—Princeton University; Keith W. Jones, Brookhaven National Laboratory, NY; Mark Rockhold, Wooyong Um—Pacific Northwest National Laboratory, Richland, WA

This project addresses the interrelationships between pore-, core-, and field-scale reactive transport. Our focus is on the geochemical reactions driven by release of caustic, radioactive wastes. We target four issues central to the reactive transport scaling problem:

1. Water-rock interactions generally involve both dissolution and precipitation, the combination of which alters pore network structure in complex ways. Systematic study of the magnitude of impact of this phenomenon requires controlled laboratory experimentation at the core scale, combined with a description of pore structure.
2. To accurately model *in situ* dissolution and precipitation rates, which may occur in different regions of the pore network, careful accounting for pore-scale variation in physical and mineralogical properties, flow velocities, and wetting fluid/grain surface areas will be required.
3. Reaction rate parameterizations are usually developed from laboratory data derived from well-mixed, homogeneous systems. Random variations in heterogeneous geologic media imply that these rate laws may have limited relevance for field-scale modeling. Accurate upscaling of laboratory-based reaction rates will need to account for the pore-scale variations discussed in Issue 2.
4. It is a critical question as to the extent that the net result of the interplay between dissolution, secondary precipitation, and the effects in Issue 2 will amplify natural heterogeneity in bulk flow.

Our approach couples (a) measurements from reactive column experiments; (b) imagery generated from synchrotron X-ray computed microtomography (CMT), synchrotron micro X-ray fluorescence (XRF), backscattered electron (BSE) microscopy and energy dispersive X-ray (EDX) spectroscopy; and (c) an array of computational tools, including lattice Boltzmann, network flow, and continuum-based flow and reactive transport models.

In preliminary work, we have employed CMT with a flow-column experiment to capture and quantify snapshots in time of dissolution and secondary precipitation changes in the microstructure of a Hanford sediment column exposed to simulated caustic waste (bulk Peclet number  $\sim 0.3$ , Peclet-Damkohler  $\sim 10^{-5}$ ). Extensive analysis of the time sequence of images reveals that dissolution-induced changes included an increase in the number of larger pores. Precipitation-induced changes included reduction in the number of small pores and closure of small throats, with accompanying reduction in pore coordination numbers and reduction in the number of pore pathways. Tortuosities for pathways spanning the sample were, however, not dramatically affected.

In separate preliminary work, we have explored the use of CMT to provide 3D maps of *in situ* mineral phases. The mapping is coarse, but useful for improved modeling for reactive flow.

## Design and Application of Microbial Uranium Reduction Monitoring Tools

Session: *ERSP University-Led Research*

Robert Sanford, Craig Lundstrom, Thomas Johnson—University of Illinois at Urbana/Champaign, IL; Kenneth Kemner, Argonne National Laboratory, Chicago, IL; Kurt Pennell, Tufts University, Medford, MA; Kirsti Ritalahti, Frank Loeffler (lead PI)—Georgia Institute of Technology, Atlanta, GA

Uranium (U) contamination is a major remediation challenge at many DOE sites. While traditional site remedies present enormous costs to DOE, enhanced bioremediation technologies (i.e., biostimulation and bioaugmentation) combined with monitoring efforts are being considered cost-effective corrective actions to address subsurface contamination. This newly funded research project aims to enhance DOE remediation activities by developing novel tools with which microbial activity can be better understood and monitored, thus enabling science-based site management decisions that achieve contaminant detoxification, plume control, and long-term stewardship. The tools and knowledge generated will elucidate the underpinning mechanisms contributing to metal and radionuclide reduction, and will benefit the broad scientific community exploring the distribution, activity, and ecology of metal-reducing bacteria.

The overarching hypothesis is that the design, validation, and application of a suite of enhanced and novel molecular and biogeochemical tools to assess, predict, and monitor *in situ* reductive processes will guide decision making for environmental remediation and provide long-term stewardship at radionuclide-contaminated DOE sites.

Objectives: We will (1) continue to design nucleic acid-based approaches to elucidate the presence, abundance, dynamics, spatial distribution, and activity of metal- and radionuclide-detoxifying bacteria; (2) develop and apply cutting-edge proteomic workflows combining multidimensional peptide separations by two-dimensional nano liquid chromatography tandem mass spectrometry, nano electrospray ionization, and tandem mass spectrometry sequencing coupled with new generation bioinformatic protein identification tools; (3) monitor U isotopic fractionation using high-precision mass spectrometry to quantify U(VI) reduction for a range of reduction mechanisms and environmental conditions; (4) evaluate and refine these complementary approaches in continuous flow soil columns and synthesize the generated information into an integrated monitoring toolbox; and (5) validate the new tools using field samples from U-contaminated IFRC sites and demonstrate their prognostic and diagnostic capabilities in guiding decision making for environmental remediation and long-term stewardship.

To accomplish these goals, research activities build on previous achievements of the investigating team. Available genome information and U(VI)-reducing isolates will be used to study fundamental, molecular-level processes and understand cellular, population and community level interactions, functions, and dynamics. Culture-based studies will define and compare the nutritional and environmental requirements of U-reducing bacteria, including isolates we recently obtained from DOE IFRC sites. DNA-, RNA-, and proteome-based tools will monitor the presence, abundance, dynamics, spatial distribution, and activity of target organisms in the contaminated subsurface. Multi-collector-ICPMS will provide high-precision U isotope measurements to quantify U reduction reactions and provide *in situ* rate information. The new qualitative and quantitative tools will be tested, refined, and validated in continuous flow columns, and synchrotron-based techniques (XANES and XAFS) will confirm U redox state and complexation. Subsequently, the value of the integrated application of the new molecular and biogeochemical monitoring tools will be demonstrated through tests with materials from ongoing bioremediation projects at DOE IFRC field sites. Community-encompassing systems understanding provided by this comprehensive set of tools supplies the technical framework for selecting the most promising and cost-effective remediation technology, and promotes efficient implementation of bioremediation strategies for achieving predictable and verifiable outcomes (i.e., detoxification leading to site closure).

## Coupling *In Silico* Microbial Models with Reactive Transport Models To Predict the Fate of Contaminants in the Subsurface

Session: ERSP University-Led Research

Yilin Fang, Pacific Northwest National Laboratory (PNNL), Richland, WA; Srinath Garg, Kai Zhuang—University of Toronto, Ontario, Canada; Hanno Richter, Sarah Strycharz—University of Massachusetts, Amherst, MA; Radhakrishnan Mahadevan, U. of Toronto; Timothy Scheibe, PNNL; Derek Lovley, U. of Massachusetts, Amherst, MA (lead PI)

This research directly addresses the goal of the DOE Environmental Remediation Sciences Program to develop tools that will make it possible to predict the fate of subsurface contaminants under either natural attenuation or engineered bioremediation conditions. The objective is to couple genome-based *in silico* models of microbial metabolism with reactive transport models. We hypothesize that this integration will lead to a significant improvement in the predictive ability of numerical models that are used to simulate complex problems of contaminant fate and transport of interest to the DOE environmental mission. Initial studies are focusing on the *in situ* bioremediation of uranium contamination in groundwater. In the past year, we completed the first iteration of a coupled model that successfully described microbial growth and associated geochemical changes during the Fe(III)/U(VI) reduction phase of *in situ* uranium bioremediation at the Rifle, CO, site. This modeling approach was significantly more predictive than conventional rate models that require substantial “parameter fitting,” because it was based on first principles of microbial physiology and geochemistry. To further improve the modeling of U(VI) reduction by *Geobacter* species, a novel kinetic model was developed that represents the role of outer-membrane *c*-type cytochromes as temporary electron sinks. The results of the model provide important insights into the correlation of U(VI) removal from groundwater with high proportions of *Geobacter* species in a planktonic state. Given the apparent importance of planktonic *Geobacter* species, field and laboratory studies were initiated to further investigate the attachment of *Geobacter* species at the Rifle, CO, site. The surprising result was that ca. 90% of the *Geobacter* cells are planktonic during active Fe(III)/U(VI) reduction. Parameters for acetate uptake are key inputs to the modeling. Further analysis of acetate uptake by *Geobacter sulfurreducens* revealed three high-affinity acetate uptake systems likely to be important in natural attenuation, but also demonstrated that, at the high acetate concentrations typically employed during *in situ* uranium bioremediation, acetate uptake should be modeled as a diffusive process. *Geobacter* species compete with other organisms for resources both during natural attenuation of uranium and when anaerobic respiration is artificially stimulated. Recent availability of the genome-scale model of *Rhodospirillum rubrum* enabled improved modeling of the interactions between *Rhodospirillum rubrum* and *Geobacter* species, providing for the first time an explanation of why *Geobacter* species are so successful in outcompeting *Rhodospirillum rubrum* during acetate additions at the Rifle site, but why *Rhodospirillum rubrum* are often more abundant in zones in which there is natural attenuation of uranium. *Geobacter* must also compete with sulfate reducers for added acetate, and the outcome of this competition can have important consequences for the effectiveness of *in situ* uranium bioremediation, because sulfate reducers vary greatly in their capacity for U(VI) reduction. Results from initial attempts to incorporate sulfate reducers into the modeling will be presented.

These studies demonstrate that coupling a genome-level understanding of the physiology of subsurface microorganisms with well-known geochemical parameters and hydrological principals may make it possible to accurately predict the fate of contaminants in the subsurface.

## Molecular Analysis of the *In Situ* Metabolic State of Subsurface Microorganisms

Session: ERSP University-Led Research

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Predicting the fate and transport of contaminants in the subsurface and the rational design of bioremediation strategies requires an understanding of: (1) what microbial processes are taking place in the subsurface; (2) the rate of these processes; and (3) the factors controlling the rate and extent of these processes. The objective of our research is to determine whether quantifying levels of transcripts for key genes can provide an estimate of the rates of microbial metabolism in the subsurface and identify factors that might be limiting the rates of microbial reactions mitigating contaminant concentrations and/or transport in the subsurface, either under natural attenuation or engineered bioremediation strategies. Previous studies under this project focused on diagnosing the physiological status of the subsurface community of *Geobacter* species that predominate when U(VI) reduction is stimulated with the addition of acetate at the study site in Rifle, CO. Those studies demonstrated for the first time that quantifying the abundance of the appropriate gene transcripts in the subsurface could provide estimates for *in situ*: rates of metabolism; growth rates; limitations for iron, phosphate, or fixed nitrogen; and a diversity of environmental stresses. During the 2008 field study at the Rifle site, the focus shifted to diagnosing the sulfate-reducing community that competes with *Geobacter* species for acetate, but appears to be less effective in U(VI) reduction. Clone libraries based on the genes *dsrA* and *dsrB*, which encode subunits of the enzyme dissimilatory (bi)sulfite reductase, indicated that there were three major phylogenetic clusters of sulfate reducers at the site. *Desulfobulbaceae* were detected prior to the initiation of the bioremediation, but not during the active sulfate-reduction phase. *Firmicutes* were detected at constant numbers throughout the study, possibly reflecting the presence of spores. In contrast, *Desulfobacteraceae* were initially rare, but arose as the predominant sulfate reducers as sulfate reduction became an important process and U(VI) reduction was less effective. This is consistent with pure culture studies, which have demonstrated that members of the *Desulfobacteraceae* are capable of oxidizing acetate with the reduction of sulfate and do not reduce U(VI). The recovered *dsr* sequences were used to design primers to assess the metabolic state of the sulfate-reducing community from estimates of *dsr* transcript abundance. The results suggest that diversion of acetate flux from *Geobacter* species to *Desulfobacteraceae* is a likely explanation for the poor performance of the uranium bioremediation strategy under sulfate-reducing conditions. To better understand the succession from metal-reducing to sulfate-reducing conditions, this process was studied in detail in laboratory incubations of Rifle sediments under conditions that mimic those during *in situ* uranium bioremediation. Studies in which the availability of Fe(III) was manipulated suggested that the initial bloom of *Geobacter* species following acetate additions is the result of the faster intrinsic growth rate of *Geobacter* versus *Desulfobacter* species, rather than *Geobacter* initially outcompeting *Desulfobacter* for acetate. Once sulfate reducers are established, they effectively compete for acetate even if Fe(III) is available. Although measuring gene transcript abundance has proven to be a powerful tool for diagnosis of the metabolic status of microorganisms in the subsurface, the techniques for extracting mRNA and quantifying transcripts is difficult and expensive. Therefore, we have initiated a new approach of quantifying the abundance of key proteins with specific antibodies. Initial results suggest that this may be a simpler, more cost-effective strategy for elucidating the *in situ* metabolic status of subsurface microorganisms.

## Development and Spectroscopic Study of Catalytic DNA Biosensors for Radionuclides and Metal Ions

*Session: ERSP University-Led Research*

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We are developing novel catalytic DNA biosensors for simultaneous detection and quantification of bioavailable radionuclides (such as uranium) and metal contaminants (such as mercury and lead). The sensors are highly sensitive and selective, not only for different metal ions, but also for different oxidation states of the same metal ion (such as U(IV) versus U(VI)).

To achieve the goal, we are employing a combinatorial method called “*in vitro* selection” to search from a large DNA library ( $\sim 10^{15}$  different molecules) for catalytic DNA molecules that are highly specific for radionuclides or other metal ions through intricate 3-dimensional interactions. By labeling the resulting DNazymes with fluorophore/quencher, gold nanoparticles, or quantum dots, we have developed new classes of fluorescent and colorimetric sensors. Using this approach, we have obtained catalytic DNA sensors for Pb(II), Hg(II), Cu(II) and U(VI). The uranyl sensor has a detection limit of 45 pM or 11 parts-per-trillion, and selectivity of over one-million-fold over other metal ions. This sensitivity and selectivity rival those of instrumental analyses. Application of the sensor in detecting uranium in contaminated soil samples from ERSP’s Oak Ridge Field Research Center has also been demonstrated. A novel approach of using an inactive variant of DNazymes to tune the detection range of the sensors is also shown, and these sensors have been converted into simple “dipstick” tests for even more straightforward field applications.

In addition to sensor development for field applications, we are also using biochemical and biophysical techniques, including luminescence and EXAFS spectroscopy, to elucidate the structural elements responsible for high selectivity for radionuclides and metal ions. The knowledge gained can be useful for designing more selective sensors (as well as chelators) for radionuclides and metal ions in remediation.

The catalytic DNA sensors allow for on-site and real-time monitoring of concentration, speciation, and stability of radionuclides and metal contaminants at the DOE sites. The richer information will help researchers to better understand subsurface physical, chemical, and biological processes, and to carry out more accurate modeling to explain and predict remediation effects. Therefore, this project will not only help assess the effectiveness of science-based solutions for cleanup by researchers and engineers who perform the cleanup, but also contribute significantly to the long-term monitoring of DOE contaminated sites by DOE staff members, state and local regulation agents, and concerned citizens around the sites.

## Characterization of Microbial Communities in Subsurface Nuclear Blast Cavities of the Nevada Test Site

Session: *ERSP University-Led Research*

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From 1957 to 1992, the U.S. DOE conducted 828 underground nuclear tests at the Nevada Test Site (NTS). The resulting subsurface cavities were sterilized by temperatures and pressures sufficient to melt rock. Today, the juxtaposition of radionuclide contamination (e.g., Pu, Tc) with a range of rock types, environmental chemistries, and hydrologic conditions represents a unique natural laboratory for studies of legacy waste transport and microbial re-colonization of materials altered by nuclear detonations. Over the 2008 field season, in coordination with ongoing hotwell monitoring by the DOE Underground Test Area Program (UGTA), we obtained the first aqueous samples from nuclear detonation cavities to be characterized for microbiology.

Fluids from three cavities (GASCON, CHANCELLOR, and NASH) and one tunnel vent (U12N) were sampled by means of bailers or pumps. Microbial diversity was assessed by a combined molecular (16S rRNA gene libraries) and cultivation-based approach. Bacterial PCR amplifications were successful for GASCON, NASH, and the tunnel, verifying the presence of microorganisms in this extreme habitat. The one cavity from which we were unable to detect microorganisms (CHANCELLOR) may be hydrologically isolated, as it retains a very high temperature (~95°C) even after 35 years. Whereas all of the samples possessed significant diversity, several groups were common to all. Most conspicuous were predicted iron-oxidizing bacteria (most similar to *Siderooxidans lithoautotrophicus* and *Ferritrophicum raditicola*) and a cluster of sequences affiliated with a *Firmicutes* subphylum recently discovered in South African mines. Members of this group are thought to be deep biosphere specialists, able to subsist by sulfate reduction utilizing hydrogen and sulfate resulting from the radiolytic cleavage of water [1]. Other database sequences related to NTS clones included microorganisms capable of degrading phenolic (*Caulobacter* and *Phenylobacterium* spp.) and hydroaromatic compounds (*Azoarcus* and *Propionivibrio* spp.), as well as Deltaproteobacteria related to *Desulfovibrio*. Characterization of our growing NTS culture collection (nitrate-, sulfate-, and iron-reducers; fermentative microorganisms; and methanogens) continues, with special emphasis on metal reducers and anaerobes.

<sup>1</sup>Chivian, D., E.L. Brodie, E.J. Alm, et al. 2008. Environmental genomics reveals a single-species ecosystem deep within Earth. *Science* 322:275-278.

## Subsurface Uranium Fate and Transport: Integrated Experiments and Modeling of Coupled Biogeochemical Mechanisms of Nanocrystalline Uraninite Oxidation by Fe(III)-(hydr)oxides

Session: ERSP University-Led Research

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Uranium mobility and bioavailability in the subsurface is dependent on an intricate combination of abiotic, biotic, and coupled reactions that dictate oxidation state, aqueous complexation, and association with mineral surfaces. Despite the promise of reductive immobilization as a means of decreasing U(VI) concentrations in groundwater, emerging studies have revealed the propensity of biogenic  $\text{UO}_2$  to reoxidize in the presence of molecular oxygen, nitrate, and Fe(III). In several instances, Fe(III) (hydr)oxides, common constituents of soils and sediments, have been shown to reoxidize (and remobilize) biogenic  $\text{UO}_2$ . This reoxidation has also been shown to occur even under sulfate-reducing conditions; however, the mechanism of this process remains unresolved. Our main hypothesis is that *the oxidation of uraninite nanocrystals by Fe(III)-(hydr)oxides would occur through the interactions of uraninite nanocrystals with biogenic electron shuttling compounds or Fe(III)-chelating compounds (e.g., siderophores) which have solubilized Fe(III) from Fe(III)-(hydr)oxide solids.*

Under abiotic conditions, we have investigated the influence of electron shuttling and Fe(III)-chelating compounds on reoxidation of biogenic  $\text{UO}_2$  by ferrihydrite, goethite, and hematite-coated quartz sand in batch systems. Starting Fe and U concentrations were 2.5 and 0.12 mM, respectively. Results thus far indicate that desferroxamine B (DFB) and riboflavin added in concentrations of 0.1 mM promote formation of soluble U(VI) in solution following 7 days of incubation, while U(VI) is not detectable in unamended control systems.

In biotic systems, *Desulfovibrio desulfuricans* G20 was grown with hematite (15 and 0.89 mmol Fe/L),  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (0.89 mM). Some siderophore activity (equivalent to 9 mM DFB) was detected using the Chrome Azurol S (CAS) assay, after treatment with Zn acetate to remove sulfide interference. No siderophore was detected after 7 days; however, analysis after 15 days of incubation indicated limited siderophore activity. Additional characterization of U, Fe, and S mineral phases in terms of elemental composition (TEM-EDS), oxidation states of sulfur (XPS), and Fe(II) & Fe(III) ratios and Fe-oxide biotransformation (Mossbauer Spectroscopy; MS) will be presented.

Numerical simulations using the TOUGHREACT and PHREEQC codes were conducted to assess thermodynamic and kinetic controls on the abiotic  $\text{UO}_2$  reoxidation by Fe(III) (hydr)oxides. Because biogenic  $\text{UO}_2$  typically forms as nanometer-sized particles, the effect of particle size on  $\text{UO}_2$  solubility was taken into account. Using representative surface energy values, calculated low solubilities for 3 nm  $\text{UO}_2$  particles is near reported values for amorphous  $\text{UO}_2$ , whereas high-end values could be orders of magnitude greater. Accordingly, computed U concentrations upon reoxidation by Fe(III) (hydr)oxides vary by orders of magnitude, depending on the initial size of  $\text{UO}_2$  particles. Under equilibrium conditions, sulfide inhibits  $\text{UO}_2$  oxidation as it is preferentially oxidized by Fe(III) (hydr)oxides (i.e., reductive dissolution of Fe(III) (hydr)oxides by sulfide instead of  $\text{UO}_2$ ). Therefore, in natural sulfate-reducing environments, the relative rates of  $\text{UO}_2$  versus sulfide reoxidation (by Fe(III) (hydr)oxides) likely determines the overall tendency for  $\text{UO}_2$  reoxidation. Our simulations also confirm previous studies showing U(VI)- $\text{CO}_3$  and U(VI)-Ca- $\text{CO}_3$  as dominant species in the reoxidation of  $\text{UO}_2$ , raising questions on the effect of increased  $\text{HCO}_3^-$  concentrations in biostimulation systems designed to precipitate  $\text{UO}_2$ .

# Adopting Biophysics Methods in Pursuit of Biogeophysical Research: Advancing the Measurement and Modeling of Electrical Signatures of Microbe-Mineral Transformations Impacting Contaminant TRA

*Session: ERSP University-Led Research*

Camelia Prodan (lead PI), New Jersey Institute of Technology; Lee Slater, Dimitrios Ntarlagiannis—Rutgers-Newark (R-N), Newark, NJ

“Biogeophysics” is a rapidly evolving earth science subdiscipline studying the geophysical signatures of (1) microbial cells in the Earth, (2) the interaction between microorganisms and subsurface geologic media, and (3) alteration of the physical properties of geologic media as a result of microbial activity. Previous ERSP-funded research has conclusively demonstrated that electrical geophysical methods are sensitive to microbial-mineral transformations and are potentially deployable as a field-scale bioremediation monitoring technology. However, the modeling of these transformations has, as of yet, received little attention. We intended to advance the utility of electrical geophysical methods for understanding biomineral transformations by the adoption of (a) dielectric spectroscopy measurements, and (b) physics-based modeling protocols/concepts, developed by biophysicists rather than relying on traditional geophysical methods in the interpretation of broadband electrical data. We assert that this approach will improve (1) the sensitivity of electrical measurements to biomineralization transformations, and (2) the prediction of pore- and nano- (cell wall) scale transformations from electrical data.

The following hypotheses are formulated:

- H1.** Physics-based modeling of low-frequency dispersions (henceforth referred to as *alpha*) measured in broadband dielectric spectroscopy data can quantify pore-scale geometric changes impacting contaminant transport resulting from biomineralization.
- H2.** Physics-based modeling of high-frequency dispersions (henceforth referred to as *beta*) measured in broadband dielectric spectroscopy data can quantify rates of biomineral growth in/on the cell wall.
- H3.** Application of this measurement and modeling approach can enhance geophysical interpretation of bioremediation experiments conducted at the RIFLE IFRC, by providing constraints on bioremediation efficiency (biomass concentration, mineral uptake within the cell wall, bio-mineralization rate).



## Scale-Dependent Fracture-Matrix Interactions and Their Impact on Radionuclide Transport

*Session: ERSP University-Led Research*

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Matrix diffusion and adsorption within a rock matrix are widely regarded as important mechanisms for retarding transport of radionuclides and other solutes in fractured rock. Due to computational limitations and difficulties in characterizing complex subsurface systems, diffusive exchange between a fracture network and the surrounding rock matrix is often modeled using simplified conceptual representations. While these representations are useful and lead to tractable approaches for interpreting tracer tests and providing first-order estimates of field scale transport, there is significant uncertainty in the “effective” or “conceptual” parameters used in these models, such as the “effective matrix diffusivity”, and mass-transfer coefficients, which are calibrated parameters. Often, calibrated parameter values fall outside anticipated ranges, in large part because these interpretive models do not account for complex three-dimensional flow within fracture networks and matrix heterogeneity. There is very little fundamental understanding of the relationship between the effective parameters and underlying rock-mass characteristics, including network structure and matrix properties. There is significant recent evidence for an apparent scale-dependence in “effective matrix diffusion”—estimated values appearing to increase with scale and sometimes far exceeding typical molecular diffusivities. These observations raise additional questions as to whether fracture-matrix interaction parameters estimated from small-scale tracer tests can be used for predicting radionuclide fate and transport at the scale of DOE sites.

High-resolution three-dimensional Discrete-Fracture-Network-Matrix (DFNM) models based on well-defined local scale transport equations can help to address some of these questions. However, DFNM models have not been used extensively in the context of fracture-matrix interactions, because they are computationally demanding. However, due to tremendous advances in computational technology over the last 10 years, DFNM modeling in relatively large domains is now feasible.

The overarching objective of our research is to use DFNM modeling to improve fundamental understanding of how effective parameters in conceptual models are related to fracture network structure and matrix properties. An advanced three-dimensional DFNM model will be developed by incorporating efficient particle-tracking algorithms for fracture-matrix interaction into a recently developed parallel fracture-network flow simulator. The particle-tracking algorithms will allow complexity in flow fields at different scales, and track transport across fracture-matrix interfaces based on rigorous local approximations to the transport equations. This modeling approach can incorporate aperture variability within single fractures and among fractures, and matrix heterogeneity. We anticipate that the code can handle computational domains with about 1 billion nodes for flow, and simulate particle tracking for transport with about 1 billion particles. Extensive computational studies on computer-generated three-dimensional fracture networks will lead to a database for improving understanding of effective parameters and their scale-dependence, and the relative merits/disadvantages of various classes of upscaled transport equations. These results will also provide unique insights on (1) the relationship between effective fracture-matrix interaction parameters, network structure and matrix properties and (2) their scale dependence in different types of fractured rock environments. Our research plan will specifically target applications at the Oak Ridge Field Research Center, several former nuclear test sites in Nevada (e.g., the Shoal and Bullion tests) where recent tracer tests have been conducted to obtain fracture-matrix interaction parameters for site-scale models of radionuclide transport, and other field sites where reliable data sets exist (e.g., Mirror Lake). The nature of the underlying fracture network and rock-matrix properties at the above sites is vastly different and will thus provide a comprehensive backdrop for our investigations. Theoretical analyses will be pursued in parallel with the computational studies, to develop improved representations of field-scale fracture-matrix interactions in complex fracture networks and rock masses.

## Measuring Uranium Binding with Nanoparticulate Hematite and NOM

*Session: ERSP University-Led Research*

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Uranium can be released to the environment as a result of mining, milling, disposal, or natural causes. The mobility, bioavailability, and toxicity of uranium contamination largely result from the metal's speciation. While thermodynamic equilibrium constants are known for many inorganic solution complexes, U (VI) may also complex with naturally occurring nanoparticles, organic matter, and/or microbial exudates, for which binding constants are often conditional or nonexistent. This makes speciation modeling difficult and necessitates an analytical technique for measuring speciation with species encountered in environmental samples. We have coupled flow field-flow-fractionation, a technique that separates colloids by their diffusion coefficients, with inductively coupled plasma-mass spectrometry, which measures U concentrations in the parts-per-trillion range. Since a particle's diffusion coefficient can be related to its diameter by the Stokes-Einstein relationship, we are able to correlate concentrations of U with the associated size fraction.

We will present results of characterization studies of U sorption to synthesized hematite nanoparticles and U complexation with natural organic matter. Results to date show that measurements using FI FFF-ICP-MS compare well with measurements of partitioning using more traditional techniques. Furthermore, FI FFF-ICP-MS has advantages over other techniques including fewer measurement-induced artifacts and the ability to deal with samples containing polydisperse particles. We will also discuss the possibilities of using the method to better understand the mobility risks posed at sites where *in situ* recovery or milling operations occur.

## Advanced Self-Potential Inversion

*Session: ERSP University-Led Research*

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We are developing an advanced approach for inverting self-potential (SP) datasets, collected in a time-lapse manner, at the contaminated Oak Ridge National Laboratory Integrated Field Challenge (ORNL IFRC) Site in Tennessee. These datasets will be inverted both in terms of hydrological and geochemical properties. At this site, natural recharge creates large hydraulic and geochemical gradients, which disrupt geochemical equilibrium. Although understanding the impact of natural recharge on flow and geochemical processes is critical to assessing the sustainability of natural attenuation at this site and elsewhere, it is difficult to assess the spatiotemporal impacts of recharge on large subsurface systems using only wellbore data. The SP method is attractive for monitoring natural recharge processes because (1) it is a passive method that is cheap to implement and that can be used across a variety of spatiotemporal scales in real time; and (2) the SP signals respond to both groundwater flux and concentration gradients. The SP methods hold great potential for illuminating the spatiotemporal distribution of processes associated with natural recharge, and that information obtainable from this method can be explicitly coupled with reactive transport models to improve the prediction of complex processes at field relevant scales.

We have already developed a stochastic inversion framework that permits quantitative inversion of SP datasets in terms of both hydrological and geochemical processes. (In addition to SP data, we are working with spectral induced polarization, SIP, and seismoelectric data, SME, two other complementary geophysical methods.) We are working on coupling SP, SIP, SME and transport phenomena within the reactive transport model TOUGHREACT. The approach will be tested both through sandbox experiments and field-based datasets collected at the ORNL IFRC within the Bear Creek Watershed. Our goal is to combine this integrated approach for understanding transient hydrological-geochemical processes at field-relevant scales. The results from this proposed project should complement the ongoing investigations at the site, as well as promote the development of autonomous geophysical monitoring approaches that are needed to guide closure and long-term stewardship activities across DOE legacy waste sites. We have already completed a set of sandbox experiments, which illustrate the usefulness of our approach for remotely detecting contaminant plume migration and redox fronts in real time.

## Experimental Reactor Studies and Physiology-Based Modeling of Microbial Redox Metabolism in Biostimulated Uranium-Contaminated Subsurface Sediments

Session: ERSP University-Led Research

Eric Roden (lead PI), Santosh Mohanty, Evgenya Shelobolina—University of Wisconsin-Madison, WI; Darrell Chandler, Akonni Biosystems; Qusheng Jin, University of Oregon, Eugene, OR

A series of batch and flow-through reactor experiments have been conducted to examine the temporal progression of terminal electron-accepting processes (TEAPs), uranium speciation, and microbial community dynamics in ethanol-stimulated, uranium-contaminated sediment from Area 2 at the ORFRC. The goal of these experiments is to provide detailed reaction path and rate information required to parameterize bench- and field-scale reactive transport simulations of biostimulation at Area 2 and other DOE sites. Sediment from zones of maximum uranium contamination (native saprolite and gravel layer) was suspended in Pipes-buffered artificial groundwater (PBAGW) designed to match the groundwater chemistry in well GW835 at Area 2. The flow-through reactors were operated in “semicontinuous culture” mode, which involves removing ca. 30% of the aqueous phase of the reactor every 3–4 days, followed by addition of an equal volume of fresh PBAGW with or without 1 mM ethanol. Nitrate was completely consumed in the ethanol-amended (+ethanol) reactors, in contrast to the ethanol-free (-ethanol) systems, where only partial consumption of the incoming nitrate took place. Significant accumulation of dissolved Mn(II) was observed during the first few weeks in the +ethanol reactors, after which Fe(III) oxide reduction was the predominant TEAP. Detectable sulfate reduction began after ca. 40 days in the +ethanol reactors. No methane production was observed in any of the semicontinuous culture reactors. Dissolved and NaHCO<sub>3</sub>-extractable U(VI) concentrations were highly variable, and it was difficult to discern the response of uranium redox speciation to biostimulation. Results of culture-dependent and culture-independent microbial community analyses were in close agreement with one another. Both microbial enumerations and quantitative PCR (Q-PCR) analysis of 16S rRNA (*Eubacteria*, *Deltaproteobacteria*, *Geobacter*) and functional genes (*dsr*, *nirS*, and *nirK*) indicated that initial densities of ethanol- and acetate-utilizing nitrate-reducers were ca. 100-fold higher than Fe(III)- and sulfate-reducers, and that cell densities of all these groups increased by 3–6 orders of magnitude in response to biostimulation. T-RFLP targeting PCR-amplified reverse transcribed 16S rRNA showed that the abundance of restriction fragments belonging to *Geobacter*, *Ralstonia*, *Burkholderiaceae* increased over time in the +ethanol reactors. Gel element 16S rRDA array analyses were generally consistent with the microbial enumeration, Q-PCR, and T-RFLP results, indicating major stimulation of nitrate-, Fe(III)-, and sulfate-reducing taxa. Several strains of *Geobacter* and *Ralstonia*, as well as representatives of other physiological groups (nitrate- and sulfate-reducers) were isolated from the +ethanol reactors. These organisms can serve as models for determining U(VI) reduction capacity and kinetic parameters for electron donor and acceptor utilization.

A microbial physiology-based reaction model was developed to simulate the results of bench-scale reactor experiments en route to incorporation into field-scale reactive transport simulations of *in situ* biostimulation. The model accounts for direct and syntrophic metabolism of ethanol by various microbial functional groups. The rates of these processes are calculated according to a thermodynamically consistent rate law that includes kinetic control by electron donor and acceptor concentration, and thermodynamic control by time-dependent free energy availability in the reaction system. The model also considers various geochemical reactions that operate in parallel with microbial metabolism, including aqueous chemical speciation, sorption, and mineral precipitation and dissolution. The results of batch reactor simulations indicate that complete oxidation of ethanol by denitrification, incomplete oxidation of ethanol coupled to Fe(III) reduction and sulfate reduction, and acetoclastic methanogenesis were the dominant processes responsible for ethanol degradation. The developed model is currently being applied to simulation of the results of the semicontinuous culture reactor experiments.

## **Role of Desorption Kinetics and Physical Heterogeneity in Colloid-Facilitated Transport of Cesium and Strontium in Variably Saturated Porous Media**

*Session: ERSP University-Led Research*

Joseph N. Ryan (lead PI), Timothy M. Dittrich—University of Colorado at Boulder; James E. Saiers, Yale University, New Haven, CT

At several U.S. Department of Energy sites, colloid-facilitated transport has been documented as an important process in the migration of contaminants in the subsurface environment. For colloid-facilitated transport to be important, the kinetics of desorption must be slow relative to the time scale of transport. In the vadose zone, colloid-facilitated transport will also be affected by physical heterogeneity of the media (e.g., macropores). Our research has examined the role of desorption kinetics and physical heterogeneity in the colloid-facilitated transport of contaminants in variably saturated porous media. The cations cesium and strontium were used as the contaminants, and illite was used as the colloids. Cesium and strontium were monitored by liquid scintillation as  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$ . The porous medium was composed of quartz sand of two grain sizes—the smaller size as matrix sand and the larger size as a central macropore. The transport experiments were conducted in saturated and variably saturated homogeneous and heterogeneous porous medium columns. In both saturated and unsaturated homogeneous porous media, the importance of facilitated transport was greater for cesium than for strontium, owing to the stronger binding of cesium to illite. In the unsaturated porous media, the difference in colloid-facilitated transport between cesium and strontium increased with decreasing moisture content, owing to lower flow velocity and longer residence times in the columns. In the heterogeneous saturated and unsaturated columns, the presence of a preferential flow path, the central macropore, resulted in more rapid transport of the cations and no significant difference between the extent of colloid-facilitated transport of cesium and strontium.

## Colloid and Cs-137 Mobilization and Transport in Vadose-Zone Sediments under Transient-Flow Conditions

Session: *ERSP University-Led Research*

James Saiers (lead PI), Tao Cheng—Yale University, New Haven, CT; Joseph Ryan, U. of Colorado

Several studies have demonstrated that contaminants present within the vadose zone of U.S. Department of Energy (DOE) sites are associated with mineral colloids. These associations may stabilize contaminants in the aqueous phase and facilitate their transport through the vadose zone and into underlying groundwaters. Our work is aimed at exploring the coupled processes that govern the facilitated transport of radionuclides by mineral colloids. In particular, we seek to elucidate the effects of perturbations in porewater flow on the mobilization of mineral colloids and radionuclides within variably saturated sediments. Measurements made during simulated rainfall onto laboratory columns packed with sediments contaminated with Cs-137 reveal that high concentrations of *in situ* colloids were mobilized with the passage of drying and wetting fronts, but that colloid mobilization was slow during periods of steady porewater flow. The mobilization of Cs-137 occurred concomitantly with colloid release, and Cs eluted from the columns existed predominantly in the colloid-associated form. Although the concentrations of Cs-137 adsorbed to the eluted colloids (Cs-137 mass per colloid mass) were independent of porewater flow velocity and ionic strength, effluent colloid concentrations, and hence total effluent Cs-137 concentrations (Cs-137 mass per effluent volume), were affected by these factors. When taken together, these results suggest that porous-medium imbibition and drainage trigger colloid release, and the migration of Cs-137 in vadose-zone sediments is controlled by colloid mobility. We used the laboratory observations on colloid migration to test a new model for the mobilization, transport, and re-deposition of particles. This model is based on the assumptions that colloids are mobilized from those pores that undergo snap-off (i.e., pores that fill with water during soil imbibition or empty of water during soil drainage) and that the snap-off response, and thus colloid release, is distributed according to the water-retention characteristics of the porous medium. Comparison of modeled and measured results reveal that colloid mobilization rates declined precipitously with increases in porewater ionic strength, were less sensitive to porewater velocity, and, for a given ionic strength, were greater during soil drainage than during soil imbibition. Findings from this work, then, provide sorely needed data on colloid-associated radionuclide mobilization and transport within real sediments and a means for quantifying the phenomena.

## Geochemical and Microbial Controls of Iodine-129 Mobility in Groundwater Relevant to Long-Term Stewardship of DOE Sites

*Session: ERSP University-Led Research*

Peter H. Santschi (lead PI), Robin Brinkmeyer, Kathleen A. Schwehr—Texas A&M University, Galveston, TX; Daniel I. Kaplan, Chris M. Yeager—Savannah River National Laboratory, Aiken, SC

I-129 is among the top three risk drivers for waste disposal at the Yucca Mt., Hanford, and Savannah River Sites. I-129 is of major concern because of its perceived mobility in the environment, excessive inventory, high toxicity, and long half-life (~16 million yrs). Iodine is a biophilic element that exhibits a relatively complex chemistry, with iodide, organo-iodine, and to a much lesser extent iodate, as the common chemical forms detected in groundwater. The various isotopes of iodine can be strongly bound to macromolecular organic matter, which can significantly decrease or increase its transport, bioavailability, and transfer to man, depending on the molecular weight and physico-chemical properties of the resulting iodine-organic matter species. We contend that the chemical form of iodine, as iodide or organo-iodine (e.g., in the form of iodinated, i.e., covalently bound fulvic and humic acids), is significant with respect to the mobility of I-129 in groundwater. Thus, a key to improved risk assessment and remediation strategies for I-129 groundwater contamination is a better understanding of iodine interactions with natural organic matter in the subsurface. The objective of our experiments is not only to determine the chemical speciation (iodide, iodate, organo-iodine) of I-129 and I-127 in selected groundwater samples from two I-129-contaminated DOE sites, but also to investigate the extent that organic matter content, microbial cells and exopolymeric substances affect the speciation and mobility of iodine. Three hypotheses will be tested through detailed environmental characterization paired with controlled laboratory experiments: (H1) The chemical form of iodine in groundwater is dependent not only on abiotic, but also biotic processes—importantly, these processes must be investigated using environmentally relevant iodine and ligand(s) concentrations; (H2) Iodine mobility through sediment is highly dependent on its speciation—organo-iodine mobility can be enhanced or retarded depending on the organic moiety, whereas inorganic iodine mobility is largely dependent on its oxidation state; (H3) Naturally occurring microorganisms present in the Hanford Site and Savannah River Site subsurface environment can alter the chemical form of iodine. Our experimental approach will determine how microbial activity, concentrations and chemical speciation (iodide, iodate, and organo-iodine) of I-129 and I-127, as well as organic carbon (OC), in selected groundwater samples from contaminated Hanford and Savannah River Site locations affect iodine mobility. Controlled experiments will assess the extent and mechanisms of iodine uptake by microbial cells and exopolymeric substances. Methods include 16S rRNA gene sequencing, FISH, MICRO-FISH, and species-specific DNA probes for microbiology, HPLC, CHN, TOC, GC-MS, NMR and AMS for iodine and organic iodine characterization, and use of I-125 radiotracers. So far, our experiments with aquifer sediments from the Savannah River Site reveal that iodine interactions are highly dependent on iodide (I<sup>-</sup>) concentrations added to sediment slurries, the time of equilibration, and the OC content of these sediments, with particle-water partition coefficients ( $K_d$ ) becoming an inverse power function of the iodide concentration. After equilibrating a sediment suspension with ambient concentrations of iodide, organo-iodine was formed and constituted a major fraction of the total iodine. As the total concentration of amended iodide increased, the fraction of organo-iodine in the system decreased. This trend was attributed to OC becoming the limiting factor. Past laboratory experiments conducted at elevated iodide concentrations that are more convenient for analytical detection have underestimated or altogether missed the detection of organo-iodine formation. These findings suggesting that subsurface iodine may exist as organo-iodine more commonly than previously believed has important implications on our present conceptual biogeochemical model and our perceived risk associated with radioiodine.

## Optimizing Metalloporphyrin-Catalyzed Reduction Reactions for *In Situ* Remediation of DOE Contaminants

Session: *ERSP University-Led Research*

M.A. Schlautman (lead PI), E.R. Carraway, S.L. Estes, R. Zhang—Clemson University, SC

Past activities conducted by the U.S. DOE have resulted in a legacy of contaminated soil, groundwater, and other environmental media at DOE facilities nationwide. Uranium and hexavalent chromium have consistently been ranked among the most frequently encountered and highest-priority metal and radionuclide contaminants present in groundwater and soil at DOE installations. Chemical or biological reduction of U(VI) to U(IV) and Cr(VI) to Cr(III) at contaminated DOE facilities can be beneficial because the reduced metal species are less toxic and less mobile. Although direct biological remediation or natural attenuation have been reported for U(VI) and Cr(VI), the reactions can be slow due to unfavorable environmental conditions. For example, high concentrations of contaminants or co-contaminants present at DOE sites may inhibit microbial activity or even be lethal to the microorganisms. In addition, the unusual conditions of pH, ionic strength, redox potential, etc., often encountered at contaminated DOE sites may also result in microbial inhibition. Under such conditions, a promising approach for the *in situ* remediation and immobilization of some DOE contaminants is to utilize abiotic reductants, together with redox catalysts when needed, to chemically reduce the contaminants to less mobile and toxic forms.

Our research results to date have shown that several common electron donors are capable of reducing Cr(VI) in acidic to slightly alkaline aqueous solutions, such as sodium dithionite (pH 6.0), *L*-ascorbic acid (vitamin C) (pH 2.5, 6.5, and 8.5), and microsized zero-valent iron (ZVI) (pH 5.5 to 8.0). Cr(VI) reduction by sodium dithionite is sufficiently rapid such that no catalyst is required. The reduction rate of Cr(VI) by vitamin C decreased with increasing pH and with increasing concentrations of  $Mg^{2+}$  or  $Ca^{2+}$  at constant pH. Currently, it is hypothesized that the observed decrease in rate is a result of complexation of  $Mg^{2+}/Ca^{2+}$  by vitamin C, possibly coupled with vitamin C oxidation by oxygen. Additional studies are under way to test these hypotheses. Microsized ZVI demonstrated only moderate Cr(VI) reduction rates, again with the reduction rate decreasing with increasing pH. Therefore, Cr(VI) reduction with both vitamin C and microsized ZVI shows potential benefit from the use of metalloporphyrin catalysts. Preliminary investigations using vitamin B<sub>12</sub>, a corrin (i.e., porphyrin derivative) with a Co metal center, in conjunction with microsized ZVI demonstrated increased Cr(VI) reduction rates compared to the ZVI alone. UV-Vis absorption spectra showed that vitamin B<sub>12</sub> was reduced from Co(III) to Co(II) during the reaction, consistent with the theory that metalloporphyrin catalysts work as electron shuttles.

Addition of the reductants sodium dithionite and vitamin C to acidic uranyl solutions resulted in decreases in the steady-state luminescence intensity of U(VI). However, neither reductant led to the expected visual precipitation of U(IV). Because complexing agents can also potentially lead to the luminescence quenching of U(VI), the possible complexation of U(VI) by dithionite, sulfate, or other unknown dithionite decomposition products was investigated. Stern-Volmer analyses revealed, however, that the decrease in U(VI) luminescence upon addition of sodium dithionite was not consistent with uranyl complexation. In fact, addition of sulfate resulted in an increase in U(VI) luminescence. Additional studies are being designed to determine the presence or absence of U(VI) complexation in these experimental systems. For example, investigations utilizing techniques such as luminescence lifetime measurements and competitive ligand reactions are under way to elucidate whether uranyl reduction or complexation is responsible for the observed luminescence decrease. In the presence of the redox catalyst vitamin B<sub>12</sub>, a decrease in U(VI) luminescence was also observed. In this case, however, we determined that the decreasing uranyl luminescence was consistent with the Stern-Volmer model after correcting for inner-filter effects, which indicates that only a single type of quenching, either static or dynamic, is occurring.

Future work includes the investigation of nanosized ZVI, which is hypothesized to increase reduction rates significantly, as well as additional work identifying Cr(VI) and U(VI) complexes that may enhance or inhibit reduction of these metals. The efficacy of additional reduction catalysts, such as free and sol gel immobilized forms of uroporphyrin, coproporphyrin, and protoporphyrin, will also be investigated.



## Integrated Hydrogeophysical and Hydrogeologic Driven Parameter Upscaling for Dual-Domain Transport Modeling

*Session: ERSP University-Led Research*

John Shafer (lead PI), Michael Waddell, Camelia Knapp—University of South Carolina, Columbia, SC; Gregory Flach, Margaret Millings, Mary Harris—Savannah River National Laboratory, Aiken, SC; Susan Hubbard, Michael Kowalsky—Lawrence Berkeley National Laboratory, Berkeley, CA

Our research is motivated by the observations that conventional characterization approaches capture only a fraction of the heterogeneity affecting field-scale transport, and that conventional modeling approaches, which use these sparse data, typically do not successfully predict long-term plume behavior with sufficient accuracy to guide remedial strategies. The P-Area at the Savannah River Site serves as our field site for testing data integration and dual-domain modeling approaches intended to lead to better understanding field-scale transport processes. During the third year of research, work continued on the development of the multiscale characterization framework and numerical experiments with multidomain transport. We also installed three additional characterization wells at our field site in which geophysical and hydrogeologic tests were performed to bolster our understanding of the multiscale heterogeneity controlling plume migration.

We have developed a linked data integration-transport modeling approach for long-term contaminant transport predictions and collected a variety of field-scale hydrogeophysical data at the P-Area to test the approach. We are using a simplified transport model that can be parameterized mechanistically with multiscale characterization data. We employ a 1D dual-domain transport model with the assumption that it can adequately reproduce the transport behavior of a 2D aquifer containing low permeability inclusions. Transport between the mobile domain (i.e., high permeability regions) and the immobile domain (i.e., low-permeability regions) is explicitly accounted for using geometrical features inferred in the data integration approach. Specifically, the transport model requires characterization of the geometry of the immobile blocks (surface areas and nodal distances between the immobile inclusions and the aquifer), the spatially varying volume fraction throughout the modeled region, and a mass-transfer parameter. The required information is obtained through a Bayesian approach that integrates multiple data types (e.g., geophysical and hydrological data) collected at different scales, such as wellbore and surface-based geophysical data. In particular, a pseudo 3D surface seismic reflection dataset collected at the site reveals subsurface stratigraphy that is expected to control permeability zonation, and thus both short and longer term plume behavior.

High-resolution numerical experiments also have been performed to investigate nondimensional relationships between multidomain transport parameters (i.e., porosities, mass transfer coefficient, dispersivity) and practical measures of field-scale heterogeneity. The simulations involved 30 realizations of 32 different normalized discrete and continuous heterogeneity configurations. Optimal dual-domain model parameters were determined by empirical fitting of breakthrough curves. Four transport formulations were considered: (1) traditional advection-dispersion, (2) dual-domain (mobile-immobile porosities) without dispersion, (3) triple domain (mobile-immobile-nonparticipating porosities) without dispersion, and (4) triple-domain with dispersion. We are presently relating these optimal values to practical field-scale characterization information, such as median and effective permeability, spatial correlation length(s), flow field orientation, and contaminant mass transfer and exposure time scales. Our interpretations suggest that optimal dual-domain values can be approximately defined from the types of field characterization data described above.

## Toward Quantifying Kinetics of Biotic and Abiotic Metal Reduction with Electrical Geophysical Methods

Session: ERSP University-Led Research

Kamini Singha (lead PI), Aaron Regberg—Penn State University, State College, PA; Flynn Picardal, Indiana University, Eric Roden, University of Wisconsin-Madison, Susan Brantley, Penn State University

We conducted three sets of batch experiments with goethite as the electron acceptor, increasing the complexity to understand the effect of iron reduction on fluid electrical conductivity ( $\sigma_f$ ). Our goal is to identify contributions from changes in ionic strength, redox, biofilm formation, permeability, and precipitation from the electrical methods in conjunction with geochemical measurements to quantify the associated biogeochemical processes through time at the flask and column scale. The simplest experiments (abiotic), designed so that reduction occurred without a biological catalyst, contained ascorbic acid as the electron donor. In vitro experiments, conducted to include a biogeochemical reaction without the complication of whole cells, contained Na-formate as the electron donor and total membrane fractions (TM) from *Shewanella oneidensis* MR-1, a known iron reducer. The abiotic, in vitro, and in vivo Fe reduction batch experiments were characterized by different rates of change of  $\sigma_f$ , pH, and dissolved Fe(II) concentrations ([Fe(II)]) over hours. These data were interpreted using composite chemical reactions validated with chemical measurements. We found that surface area-normalized reduction rates of goethite increase from abiotic to in vivo to in vitro reduction for similar concentrations of electron donor and acceptor, and the electrical geophysical measurements were key to developing meaningful composite reactions. We determined that certain diagnostic reactions (reductive dissolution, acid-base reactions, and surface adsorption) control changes in electrical conductivity regardless of the reaction mechanism. To scale up in complexity, we conducted column experiments where we measured electrical conductivity in flow-through reactors with ongoing iron reduction. The columns were filled with iron-bearing sediments and inoculated with an environmental culture. We fabricated and inserted electrodes in the column. Sterile PIPES-buffered artificial groundwater was amended with acetate to encourage iron reduction. Acetate consumption and the reduction of iron oxides observed in the column effluent did not correspond to changes in bulk electrical conductivity ( $\sigma_b$ ).  $\sigma_b$  during Fe reduction was observed to increase more rapidly than predicted based upon in vivo flask experiments or measured fluid conductivity in the column: during the last two months of the experiment,  $\sigma_b$  increased by a factor of 3. [Fe(II)] does not show a similar trend. We currently hypothesize that biofilm growth causes the increase in  $\sigma_b$ , and new column reactions are currently being built at Penn State to explore this phenomena. In addition to the diagnostic reactions outlined in the batch experiment, we note that biosynthesis likely needs to be considered in these systems. Simple, well-controlled experiments building up in complexity are the key to understanding both column and field experiments.

## Geophysical Characterization and Monitoring of Groundwater-Surface Water Interaction in the Hyporheic Corridor at the Hanford 300 Area

*Session: ERSP University-Led Research*

Lee Slater, Rutgers-Newark (lead PI), NJ; Fred Day-Lewis, U.S. Geological Survey; Andy Ward, Pacific Northwest National Laboratory (PNNL), Richland, WA; John Lane, U.S. Geological Survey; Roelof Versteeg, Idaho National Laboratory, Idaho Falls, ID; Dimitrios Ntarlagiannis, Kisa Mwakanyamale—Rutgers-Newark, Carole Johnson, U.S. Geological Survey; Christopher Strickland, PNNL

We are performing geophysical research to characterize the Hyporheic Corridor (HC) at the Hanford 300 site and determine how aquifer architecture and sediment characteristics interact with temporally and spatially variable hydraulic forcing to (1) dictate the size of the HC, and (2) regulate hydrologic exchange with, and contaminant transport to, the Columbia River. Geophysical measurements performed during Year 1 of the project primarily focused on characterization of the hydrogeological framework of the zone of interaction between uranium-contaminated groundwater and river water along an approximately 3 km stretch of the Columbia River centered on the 300 Area Integrated Field Research Challenge (IFRC). Extensive waterborne resistivity, induced polarization (IP), seismic and ground penetrating radar (GPR), supplemented with focused land-based resistivity, IP, and GPR were performed during Year 1 of this project. Inversion of the large waterborne resistivity/IP dataset has revealed the distribution of the Hanford and Ringold units along the hyporheic corridor, as well as the depth to the Hanford-Ringold contact. The IP measurements proved particularly effective at imaging the distribution of these units, and the depth to the contact, as the fine fraction of the Ringold formation imparts a high polarizability relative to the overlying coarse-grained Hanford formation.

The results of the waterborne resistivity/IP survey are generally consistent with recent estimates of regions of the Columbia Riverbed, where the transmissive Hanford formation is in direct contact with the riverbed (as based on planar projections from wells onland, as well as direct push tests on the riverbed). However, the resistivity/IP survey identifies additional locations on along the hyporheic corridor where the Hanford unit is in direct contact with the riverbed. Such estimates provide important insight into the distribution of the Hanford unit in contact with the riverbed, which in turn is critical to determining the likely contributing source zone of uranium contamination of Columbia River water. Waterborne GPR and seismic surveys provided limited stratigraphic information, primarily due to the poor signal penetration arising from scattering by pebbles/boulders on the riverbed, along with the relatively high electrical conductivity associated with the Ringold formation that is in direct contact along a significant portion of the hyporheic corridor. Scattering/reflection of energy from the riverbed did provide useful qualitative information on the variability in the physical characteristics of bed material along the hyporheic corridor. Land-based GPR was able to resolve the Hanford-Ringold contact in some locations along the ~3 km zone of the river corridor adjacent, and central to, the 300 IFRC. Perhaps most significantly, land-based GPR has revealed compelling evidence for the location of paleochannels incised below the Ringold-Hanford contact and potentially proving conduits for accelerated transport of contaminated groundwater into the Columbia River. Unlike earlier geophysical studies conducted at the site, our work has identified at least one paleochannel in direct contact with the Columbia River. This hydrogeologic characterization was used to help constrain the location of a fiber optic distributed temperature sensor (DTS) system along the channel reach. Cables were deployed, and the system calibrated in late 2008; continuous data collection started in December 2009. Analysis of these data is ongoing and will be a focus of our Year 2 efforts. Autonomous resistivity and self-potential surveys are anticipated shortly.

## Field Investigations of Microbially Facilitated Calcite Precipitation for Immobilization of Strontium-90 and Other Trace Metals in the Subsurface

Session: ERSP University-Led Research

Robert W. Smith (lead PI), U. of Idaho, Moscow, ID; Yoshiko Fujita, Idaho National Laboratory, Idaho Falls, ID; Timothy R. Ginn, U. of California at Davis, CA; Susan S. Hubbard, LBNL

Subsurface radionuclide and trace metal contaminants throughout the U.S. Department of Energy (DOE) complex pose one of DOE's greatest challenges for long-term stewardship. One promising stabilization mechanism for divalent trace ions, such as the short-lived radionuclide strontium-90, is coprecipitation in calcite. We have previously found that calcite precipitation and co-precipitation of Sr can be accelerated by the activity of urea hydrolyzing microorganisms, that higher calcite precipitation rates can result in increased Sr partitioning, and that nutrient additions could stimulate ureolytic activity. We are conducting integrated field, laboratory, and computational research to evaluate the relationships between urea hydrolysis and calcite precipitation rates and trace metal partitioning under environmentally relevant conditions, and investigating the coupling between flow/flux manipulations and precipitate distribution and metal uptake.

Our first field campaign was conducted within a 5-spot well-field dedicated to the project and located at the Vadose Zone Research Park (VZRP), an uncontaminated site with subsurface hydrogeologic characteristics similar to the nearby <sup>90</sup>Sr contaminated Idaho Nuclear Technology and Engineering Center of the Idaho National Laboratory. Well-to-well experiments using conservative tracers, molasses, and urea were conducted to estimate *in situ* ureolytic activity at the VZRP, and to conduct a preliminary assessment of the ability of geophysical and molecular biomarker methods to detect subsurface urea hydrolysis. Samples were collected from the extraction wells for chemical and biological analysis. In addition, cross borehole tomographic radar, seismic, and complex electrical data were collected before, during, and after urea injection to characterize baseline *in situ* heterogeneity and to monitor the spatiotemporal distribution of biogeochemical transformations associated with the urea hydrolysis. Analyses of the extracted water confirmed the conversion of urea to ammonium in the subsurface, and a first-order urea hydrolysis rate constant of 0.034 d<sup>-1</sup> was estimated. This was more than an order of magnitude higher than rate constants estimated aboveground using water samples spiked with <sup>14</sup>C-labeled urea, suggesting that attached microbial communities may play a significant role in *in situ* urea hydrolysis. The seismic data collection at the VZRP was determined to be ineffectual, but the radar and ERT data appear to be of good quality, and the analyses of those data are ongoing. Also ongoing are the analyses of microbial biomarkers for urea hydrolysis (urease genes and transcripts).

In support of the field investigations, targeted laboratory studies and flow and reactive transport modeling development are under way. In synchrotron microtomography and laboratory flow-through column experiments, conducted to date the observed distributions of calcite precipitates, have been sparse, but the geophysical monitoring results suggest that complex resistivity measurements are sensitive to the biogeochemical transformations associated with stimulated calcite precipitation. Also in the laboratory, we are conducting cation exchange studies, to develop a site-specific ion exchange model for the VZRP. The ion exchange model will be incorporated into the multicomponent reactive transport model of ureolysis and induced calcite precipitation and strontium co-precipitation. The reaction component of the model includes product-inhibited enzymatic ureolysis kinetics, and has been tested against data from the literature, the column experiments, and data from (NSF-funded collaborator) J. DeJong at UC Davis. The model is embedded in two separate modeling platforms, grid-based and streamtube-based, for dual analyses of the *in situ* ureolysis experiments, affording cross-validation of modeling results. Currently, the model is being used to support the analysis of the well-to-well tracer tests for characterization of VZRP hydraulic properties.

## Uranium Immobilization by the Activities of Microbial Phosphatases

*Session: ERSP University-Led Research*

Patricia A. Sobecky (lead PI), Robert J. Martinez, Melanie J. Beazley, Kathleen Salome—Georgia Institute of Technology, Atlanta, GA; Cindy Wu, Terry Hazen, Gary Andersen—Lawrence Berkeley National Laboratory, Berkeley, CA; Samuel M. Webb, Stanford Synchrotron Radiation Laboratory, Menlo Park, CA; Martial Taillefert, Georgia Institute of Technology, Atlanta, GA

Our previous funded project (DE-FG02-04ER63906) demonstrated that the natural phosphatase activities of subsurface microbes, isolated from contaminated Oak Ridge, TN FRC (ORFRC) subsurface soils, released inorganic phosphate ( $\text{PO}_4^{3-}$ ) extracellularly during growth in the pH range 5-7. The amount of  $\text{PO}_4^{3-}$  liberated from an exogenous organophosphorus substrate, provided as the sole carbon and phosphorus (P) source, was sufficient to precipitate >95% of uranium [U(VI)] as low solubility uranium-phosphate minerals even at pH 5. Presently, the range of phosphatase activities in subsurface microbial communities exposed to long-term metal and radionuclide contamination is unknown, and their potential role in immobilizing metals and radionuclides remains poorly characterized. Moreover, the geochemical conditions regulating the rates of formation and the stability of these phosphate precipitates in soils and groundwater have not been examined. In this project, a multiphasic approach is used to determine if microbial phosphatase activities present in natural subsurface communities can liberate sufficient phosphate to promote the precipitation of U(VI) under both oxic and anoxic growth conditions. One component of this work entails demonstrating the formation of U-phosphate minerals in subsurface soils. Soils from ORFRC are incubated with different types of P source, in the presence or absence of dissolved oxygen, and over a wide pH range to investigate nonreductive U biomineralization activities. The mineral product of these incubations is analyzed using X-ray absorption spectroscopy, and its stability is studied at different pH's and in the presence of carbonates or other P sources. A second component of the project examines microbial community structure in soil slurry incubations maintained under oxic or anoxic conditions as well as acidic (pH 5.5) and circumneutral (pH 6.8) pH regimes. Oxic slurry incubations demonstrate that organophosphate hydrolysis rates are greater at low pH. Anoxic soil slurry incubations initiated with 10 mM glycerol-2-phosphate and 15 mM nitrate amendments under acidic anoxic conditions reveal denitrifying and organophosphate hydrolyzing activities. Total DNA extractions from soil slurry incubations are currently analyzed via high-density oligonucleotide microarray (PhyloChip) in collaboration with LBNL investigators. Preliminary data suggests that under oxic conditions, the microbial community structure is enriched at low pH compared to the diversity of unamended soils.

## Chromate Reduction under Nitrate-Reducing Conditions in *Geobacter metallireducens*, *Sulfurospirillum barnesii*, and *Desulfovibrio desulfuricans*

Session: ERSP University-Led Research

Peter Chovanec, Courtney Sparacino, Partha Basu, John F Stolz (lead PI)—Duquesne University, PA

The focus of our ERSP investigation has been to determine how respiratory nitrate reduction to ammonia impacts Cr(VI) transformation. The goal is to elucidate the specific mechanism(s) that limits Cr(VI) reduction in the presence of nitrate, and to use this information to develop strategies that enhance Cr(VI) reduction and thus immobilization and detoxification. Our central hypothesis is that nitrate impacts the biotransformation of metals and metalloids in three ways: (1) as a competitive alternative electron acceptor (inhibiting transformation); (2) as a co-metabolite (i.e., concomitant reduction, stimulating transformation); and (3) as an inducer of specific proteins and pathways involved in oxidation/reduction reactions (stimulating transformation). We have identified three model organisms, *Geobacter metallireducens* (Mechanism 1), *Sulfurospirillum barnesii*, (Mechanism 2), and *Desulfovibrio desulfuricans* strain 27774 (Mechanism 3).

Mechanism 1. *G. metallireducens*: We have found that growth on nitrate is actually inhibited by the presence of Cr(VI). Activity assays indicate that Cr(VI) inhibits nitrite reduction (e.g., Nrf activity). Proteomics analysis indicates that aldehyde oxidoreductase and an iron-containing alcohol dehydrogenase are up-regulated upon Cr(VI) exposure. This may be interpreted as a response to oxidative stress. The inhibition, nevertheless, could be attenuated by amending the medium with ferric citrate (the higher the concentration of ferric citrate, the greater and faster the attenuation).

Mechanism 2. *S. barnesii*: We confirmed that this organism can simultaneously reduce Cr(VI) while respiring nitrate. Activity assays, however, indicate that Cr(VI) inhibits nitrite reduction (again being preferentially reduced by Nrf); thus, physiologically, there must be a separate mechanism for Cr(VI) reduction. Activity assays have shown that Cr(VI) is reduced by RarA, a protein that is overexpressed under these growth conditions (as determined by proteomics analysis). We also have found that a redox active secondary metabolite (possible a ketoquinone) is secreted into the medium during growth and are investigating its reactivity with Cr(VI).

Mechanism 3. *D. desulfuricans* 27774: This organism first reduces any Cr(VI) in the medium prior to respiratory growth on nitrate. Activity assays also showed inhibition of Nrf and the accumulation of nitrite. Comparison of the proteome of cells grown in the presence of Cr(VI) versus those grown on nitrate alone have revealed a suite of proteins that are differentially expressed.

These results have provided new insights into the dynamics of Cr(VI) on nitrate metabolism in the three organisms. They suggest that Cr(VI) is a preferred substrate for Nrf and thus interferes with nitrite reduction.

## Identifying Biomarkers and Mechanisms of Toxic Metal Stress with Global Proteomics

Session: *ERSP University-Led Research*

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Data suggest that Hg(II) is toxic in two ways. At low levels, Hg damages membrane-bound respiration, causing a burst of reactive oxygen species (ROS) that further damage the cell. At higher Hg concentrations, Hg(II) may also enter the cytosol, directly inactivating cysteine-containing proteins. Our first objective is to identify, quantify, and compare ROS- and Hg-damaged proteins under various Hg concentrations to test this model for two mechanisms of Hg toxicity. Our second objective is to define the cellular roles of the ubiquitous mercury resistance (*mer*) locus. We know much about how *mer* proteins transform reactive ionic Hg(II) and organic RHg(I) to less toxic, fully reduced, volatile, monoatomic Hg(0), but much less about how they protect other cell proteins from Hg damage. We use mass spectrometric global proteomics both to identify Hg- and RHg-damaged cellular proteins and follow their recovery with and without the *mer* operon. We focus initially on adducts of monovalent organomercurials, phenylmercury (PhHg), and ethylmercury (EtHg) as safer surrogates for methylmercury.

Since RHg-modified proteins cannot be enriched by affinity chromatography, the Miller lab devised a computational filter based on the seven stable isotopes of Hg to reveal RHg-modified peptide signatures in mass spectra. This algorithm can confirm putative hits identified by SEQUEST and has successfully identified adducts in RHg-treated pure protein samples and bulk cell extracts (see below). For the *in vivo* RHg exposure work, the Summers lab treated mid-log phase cultures of *E.coli* MG1655 with PhHg and EtHg, also treated or not with IAM, and supplied these cells to the Lipton group for mass-spectrometric total-proteome analysis. The Summers group also benchmarked each prep for total and protein-bound thiols, protein carbonylation, essential metals content, and free Fe. The hierarchy of damage to total and protein thiols is Hg(II)>>PM>MT. Surprisingly, brief mercurial exposures (15 minutes) resulted in neither increased protein carbonylation nor changed metal pools. However, EPR shows that Hg(II) releases Fe(II) from Fe-metalloproteins, amounting to nearly 75% of total cellular Fe. Proteome analysis of cells, prepared under 12 variations of mercurial and IAM treatment, demonstrated that blocking other thiols with IAM immediately after RHg exposure preserves RHg-adducts in whole cell preparations. In analyzing the fragmentation pattern of pure proteins treated with phenylmercury, the Miller group also confirmed the value of IAM blocking of free thiols to stabilize RHg-adducts on peptide cysteines. Recently, the Miller group observed some loss of Ph/EtHg-peptide adducts during sample processing for mass spectrometry and are working with the Lipton group to rectify this. Future work will evaluate RHg-adduct stability, analyze MS proteomic data for evidence of oxidized peptides, and analyze *in vitro* vulnerability of cysteines in select proteins identified in whole cells. The Lipton lab has completed mass spectrometric analyses on 40 total cell or pure protein experimental preparations from the Miller and Summers groups. These followed 99 pilot runs for optimization of procedures to improve recovery of total peptides and proteins and detection of mercurial adducts. Typically, ~8000 peptides per preparation are recovered, yielding identification of 1100 proteins, comparing well with typical peptides and proteins identified by mass spectrometry in other bacteria. Nearly 50 RHg-adducted cysteines have been identified in 41 highly abundant proteins using SEQUEST and filtering for stable Hg isotopes. The Summers group is analyzing RHg-modified positions in protein structures and comparing them with unmodified cysteines in the same and related proteins. Mapping RHg-vulnerable proteins to KEGG pathways identifies candidate biomarkers for Hg exposure. The remarkably wide reach of RHg toxicity is obvious in the functions of the abundant proteins targeted by PhHg or EtHg; multiple targets lie in glycolysis, the TCA cycle, the translational apparatus, redox damage control, and respiration. Future work will add pre-MS treatments to enrich less abundant, but important proteins, including transcriptional regulators.

## Integrating the Molecular Machines of Mercury Detoxification into Host Cell Biology

Session: *ERSP University-Led Research*

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The bacterial mercury (Hg) resistance (*mer*) operon functions in Hg biogeochemistry and bioremediation by converting reactive inorganic [Hg(II)] and organic [RHg(I)] mercurials to relatively inert monoatomic mercury vapor, Hg(0). Its genes regulate expression (MerR, MerD, MerOP), import Hg(II) (MerT, MerP, and MerC), and demethylate (MerB) and reduce (MerA) mercurials. We focus on these components' interaction with each other and with the host cell to allow cells to survive and detoxify Hg compounds.

*Regulation of mer Operon Expression:* By calorimetry, free and DNA-bound MerR differ kinetically and thermodynamically in binding Group 12 metal ions. DNA-bound MerR strongly prefers Hg(II), consistent with NMR observations on the DNA-dependence of metal-specific allostery. On binding Hg(II), MerR-MerOP becomes more compact and monodisperse. In DNA binding buffer (with additional Mg(II) and K(I) ions) MerR coordinates Hg(II) with 3 sulfurs at 2.44Å, just as in free MerR. In contrast, free MerR's binding of Zn(II) has both both exo- and endothermic phases, an overall stoichiometry of >6, and is not well fit by any model; XAFS reports Zn(II) has 3 sulfur ligands at 2.32Å on free MerR. The MerR-MerOP complex binds Zn(II) 14-fold less well than Hg(II), and thus Zn(II) is lost in dialysis during XAFS preparation, precluding observation of Zn(II) coordination in this complex. Both free and DNA-bound MerR aggregate when bound to Zn(II). Cd(II) binding to free MerR is also multiphasic, exo- and endothermic, but can be fit to a 4-site sequential model with 2 high and 2 low affinity sites. Cd(II)-provoked aggregation is also indicated by two hydrodynamic measures. Calorimetry and XAFS work on MerR-Cd(II) and MerOP-MerR-Cd(II) are in progress. MerR also contacts RNA polymerase (RNAP); we've used cross-linking to show MerR domains that abut various (RNAP) subunits. MerD is a MerR antagonist that deactivates operon transcription. MerD's insolubility has compromised *in vitro* work on it, but we have overcome that problem using metabolic induction.

In the year ahead, we will examine the anti-activator, MerD, alone and complexed with MerR, MerOP, RNAP by crosslinking, footprinting, as well as various hydrodynamic measures of size and shape with our ORNL collaborators. Proteolysis of MerA: From 10-30% of MerA is proteolytically truncated *in vivo* with or without Hg(II). We've ruled out autolysis and activity of any single ATP-dependent soluble protease. Core does not bind the membrane, but a small fraction of full-length MerA is membrane-bound, so we examined mutants in the membrane-bound ATP-dependent protease FtsH and found that loss of FtsH eliminates MerA truncation. FtsH's normal role is to remove damaged membrane proteins. That MerA is a substrate for FtsH suggests it suffers redox damage while binding the membrane to access electrons for Hg(II) reduction.

Future work will assess the effects of mutants in other components of the FtsH complex on MerA, and we'll also examine MerA for oxidative damage. MerA Core Interactions with NmerA: Initial NmerA-Hg(II)-Core crosslinked complex, made to examine contacts between domains, was heterogeneous, so we're making this complex by a new strategy for use in small angle x-ray and neutron scattering work with ORNL collaborators. We also have a new strategy to obtain pure full length (untruncated) MerA for interaction and structural studies such as x-ray scattering with the ORNL group.

Novel protein motion in MerA mechanism: Structural bioinformatics work, including a structure of a MerA triple Cys-to-Ala mutant (determined with previous DOE funding), identified a novel interaction between an active site lysine and an arginine in the mutant protein, with a possible role in catalysis. Mutation of Arg to Met in wild type MerA yields a new inactive mutant that stabilizes an intermediate that normally occurs only transiently in the catalytic reaction. The results provide evidence for a novel transient motion controlling the catalytic mechanism. MerB interacts preferentially with NmerA: Stopped flow kinetics using fluorescence of the active-site proximal Trp95 as a reporter show that NmerA rapidly and completely removes the Hg(II) product from MerB, but cellular concentrations of glutathione react more slowly and do not go to completion. These results clearly show that direct Hg(II) transfer to MerA provides a more efficient route for rapid turnover of RHg(I).

In summary, our work covers the interactions of the four macromolecules involved in *mer* regulation—MerR, MerOP, RNAP, and MerD—and the interactions with the two enzymes MerB and MerA (and the latter with a host membrane protease). Future work will add interactions of MerA with the *mer* membrane Hg(II) transporter, MerT.



## Impact of Microbial Mn Dynamics on Uranium Bioremediation

*Session: ERSP University-Led Research*

Bradley M. Tebo (lead PI), Satyavathi Chinni—Oregon Health & Science University, Portland, OR; Daniel Giammar, Washington University St. Louis, St. Louis, MO; Rizlan Bernier-Latmani, Jonathan O. Sharp—Ecole Polytechnique Federale de Lausanne, Switzerland; Kai-Uwe Ulrich, Washington University St. Louis; Harish Veeramani, Ecole Polytechnique Federale de Lausanne

Bioreduction of U(VI) to U(IV) has been proposed as a strategy for the stabilization of U contamination in the subsurface. For such approaches to be effective, the products need to be stable with respect to reoxidation or remobilization under groundwater chemical conditions. One ubiquitous but largely overlooked subsurface dynamic involves microbially catalyzed Mn redox cycling. Catalytic oxidation of groundwater  $Mn^{2+}$  by microbes produces the powerful oxidants Mn(III) and  $MnO_2$  under suboxic (low oxygen) conditions.  $Mn^{2+}$  is present in groundwater at US DOE sites at concentrations of 1–20  $\mu M$ . However, under reducing conditions, the microbial reduction of  $MnO_2$  occurs along with the reduction of U(VI), leading to the accumulation of much higher (mM range)  $Mn^{2+}$  concentrations in the groundwater. Thus, when  $O_2$ -containing groundwater comes into contact with the reduced zone following bioremediation, it is likely that  $Mn^{2+}$  oxidation will occur at the interface of these two zones, and U(IV) reoxidation could then be driven by oxidized Mn products. Ironically, biogenic  $MnO_2$  is a strong sorbent for heavy metal cations, due to its high surface area, malleable structure, and tendency to coat other minerals. Thus, while Mn oxides catalyze the oxidative dissolution of  $UO_2$ , they can also retard the transport of U(VI). In spite of the clear importance of these processes, little is known about their dynamics and mechanisms in the subsurface. Our research to date has focused on the kinetics and mechanisms of  $UO_2$  oxidation by Mn(II)-oxidizing bacteria and by synthetic and biogenic Mn oxides. Using Mn(II)-oxidizing spores of *Bacillus* sp. strain SG-1 as a model system, we have investigated the effect of different concentrations of U and Mn on the coupled biogeochemical oxidation-reduction reactions of U and Mn. In the presence of 5% headspace oxygen  $UO_2$  oxidation is 30-50 times faster in the presence of Mn oxides as compared with Mn oxide free controls (i.e., 5%  $O_2$  alone). As a consequence of  $UO_2$  oxidation, Mn oxides are reduced to Mn(II), which can then be re-oxidized by SG-1 spores. SG-1 spores cannot directly oxidize  $UO_2$ , but oxidation of synthetic  $UO_2$  proceeds rapidly with Mn(II) concentrations of  $<5 \mu M$ . Thus, only catalytic amounts of Mn(II) are required for the rapid oxidation of  $UO_2$ . Kinetic studies show that increasing concentrations of  $UO_2$  and U(VI) decrease the rate of Mn(II) oxidation in different ways by inhibiting the Mn(II)-oxidizing enzyme. Experiments with biogenic  $UO_2$  show a similar behavior as the synthetic  $UO_2$ . Mn(II)-reacted biogenic  $UO_2$  was overall more stable than unreacted biogenic  $UO_2$ . However, oxidation of Mn(II)-reacted  $UO_2$  accelerated after an initial lag phase, as some Mn(II) was released from the mineral and oxidized by the spores. These results emphasize the need to consider the impact of Mn(II)-oxidizing bacteria when predicting the potential for  $UO_2$  oxidation in the subsurface.

## Optimization and Directed, Natural Evolution of Biologically Mediated Chromate Reduction in Subsurface Soil Microcosms

Session: ERSP University-Led Research

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Scientifically sound, effective strategies for the bioremediation of contaminated Department of Energy waste sites depend on knowledge of the structure, dynamics, and activity of the autochthonous microbial community. Mass spectrometry (MS)-based shotgun proteomics has tremendous potential for unraveling the metabolic profiles and protein signatures indicative of active metal-reducing soil microbial communities. When coupled with the careful use of ssu-rRNA-based techniques, it is possible to characterize both the extent of microbial diversity within the community and the expressed activity of that community. As such, we are taking a bicameral approach to characterizing the microorganisms and their attendant protein expression profiles in chromate [Cr(VI)]-contaminated soil microbial communities. DOE Hanford Site subsurface soils were used to construct benchtop microcosms to study the effect of various carbon sources and *Pseudomonas putida* F1 (a chromate reducer) on the indigenous microbial community. Triplicate microcosms amended with glucose, lactate, additional Cr(VI), or *P. putida*, as well as combinations of these, were incubated for 36 days, during which time the evolved CO<sub>2</sub> was monitored in order to assess the physiological response (microbial activity) of the community in terms of aerobic respiration. At the end of this period, one microcosm from each treatment was sacrificed, and subsets of each were chosen for parallel molecular phylogenetic and HPLC-MS/MS analyses. The respiration data indicated that in the absence of exogenous *P. putida* F1, respiration levels were highest for glucose-amended microcosms. Addition of *P. putida* to lactate- or glucose-treated microcosms initially yielded higher respiration levels with lactate, only to be surpassed later by respiration on glucose. In the absence of added *P. putida*, glucose stimulated CO<sub>2</sub> production in microcosms to a higher level than did lactate. Phylogenetic analysis of the autochthonous microbiota revealed stark contrast between the microbial community composition of contaminated versus uncontaminated Hanford Site soils. The microbial communities in uncontaminated soils were dominated by *Actinobacteria*, including *Arthrobacter*; *Verrucomicrobiae*; *Bacteroidetes*; and alpha-, beta-, and delta-*Proteobacteria*, while contaminated soils comprised most notably *Firmicutes* and gamma-*Proteobacteria*. Most striking was the presence of several *P. putida* relatives such as *Pseudomonas mendocina*, *Pseudomonas pseudoalcaligenes*, and *Pseudomonas nitroreducens*, as well as other uncultivated *P. putida* close relatives to name a few. None of these phylotypes, or any other members of the gamma proteobacterial subphylum, have been found in the uncontaminated soils. The phylogenetic analysis indicated that the degree of species richness (i.e., number of different phylotypes) in both the Cr(VI)-contaminated and uncontaminated soil samples is ideal for development and application of MS-based proteomics to soil: the species richness is high enough to present a realistic challenge, but not so high as to be an insurmountable task. It is also clear that the choice of *P. putida* as a seed organism was prescient. Our phylogenetic analysis indicated that close relatives of Cr(VI)-reducing *P. putida* are natural and significant members of the chromate-contaminated microbial community that we are studying, and that the ambient conditions in the soil are sufficient to support the growth of this model microorganism. Currently, we are in the process of applying phylotype-specific fluorescent rRNA probes to the microcosms. These probes will enable the relative enumeration and quantification of the mixed populations of microbes in the microcosms. The data obtained will serve as an indicator of which organism-specific proteins should be easiest to identify by MS-based proteomics and a measure of the sensitivity of the method in identifying proteins from the less abundant populations in the community.

## Technetium Reduction and Permanent Sequestration by Abiotic and Biotic Formation of Low-Solubility Sulfide Mineral Phases

Session: *ERSP University-Led Research*

Paul Tratnyek (lead PI), Bradley Tebo—Oregon Health & Science University; Jim Szecsody, PNNL, Richland, WA; Jim McKinley, PNNL

Technetium-99 is a highly mobile subsurface contaminant at U.S. Department of Energy (DOE) sites, and its mobility gives it one of the highest risks of exposure. The predominant and mobile form of technetium in the subsurface is the  $\text{Tc}^{\text{VII}}$  oxyanion pertechnetate ( $\text{TcO}_4^-$ ). Under reducing conditions,  $\text{TcO}_4^-$  is readily reduced to  $\text{Tc}^{\text{IV}}$ , which forms highly insoluble oxides such as  $\text{TcO}_2 \cdot n\text{H}_2\text{O}$ . Many studies have investigated the reduction of  $\text{TcO}_4^-$ , by abiotic and biotic means, on the premise that this could be used for long-term immobilization of the contaminant for remediation activities at DOE sites. However, a difficulty with this strategy is that (re)oxidation of  $\text{Tc}^{\text{IV}}$  oxides is relatively facile and therefore remobilization is possible.

One way to minimize the prospect of remobilization is to effect reduction under sulfidogenic conditions, so that most  $\text{Tc}^{\text{IV}}$  will be immobilized as  $\text{Tc}_2\text{S}_7$ , which should remain relatively insoluble even under oxic conditions. For this purpose, a feasible and practical way to create well-poised sulfidogenic conditions is the injection of fine-grained zero-valent iron ( $\text{Fe}^0$ )—or related materials composed of various combinations of  $\text{Fe}^0$ ,  $\text{S}^0$ ,  $\text{Fe}^{\text{II}}$ , and  $\text{S}^{\text{II}}$ . These materials will react directly with  $\text{Tc}^{\text{VII}}$ , yielding a degree of immobilization by reduction and precipitation, but the  $\text{Fe}^0$  also will scavenge dissolved oxygen and nitrate, and generate significant concentrations of dissolved  $\text{H}_2$ , thereby stimulating sulfate- and sulfur-reducing bacteria (collectively referred to as SRB), which will favor sequestration of Tc in less reoxidizable forms (esp.,  $\text{Tc}_2\text{S}_7$ ).

Fine-grained  $\text{Fe}^0$  and related chemical reductants—ranging in size from 10's of microns to 10's of nanometers—are available and suitable for introduction into the deep subsurface (vadose and saturated zones) by a variety of injection technologies. This makes the approach proposed here applicable for *in situ* chemical reduction at sites like Hanford, where the vadose zone and groundwater contamination by Tc is 80–120 m deep. The outcome of such scenarios, however, will depend on a complex mixture of multiscale processes, where the spatial distribution of the injected material will have heterogeneous effects on the *in situ* biogeochemistry, which—in turn—will determine what mixture of fundamental processes control the overall (im)mobilization of Tc.

The overall objective of this work will be to provide the fundamental understanding necessary to evaluate the feasibility of reductive immobilization of  $\text{TcO}_4^-$  in the vadose zone or groundwater by controlled application of  $\text{Fe}^0$ ,  $\text{Fe}^0/\text{S}^0$  (and sulfate or organic carbon, if necessary) to stimulate microbial sulfate reduction. This proposed work is focused on these biogeochemical mechanisms of interaction between  $\text{Fe}^0$ ,  $\text{S}^0$ , and Tc (not the technology to emplace  $\text{Fe}^0$  and  $\text{Fe}^0/\text{S}^0$  in the vadose or saturated zones).

## The Reaction Specificity of Nanoparticles in Solution: Interactions between Iron Core/Shell Nanoparticles, Their Coatings, and the Environment

Session: ERSP University-Led Research

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The general objective of this project is to understand how the environmental fate and effects of iron/iron oxide nanoparticles (NPs) (including transformations of NPs by aging and transformations by NPs of solutes such as DOE priority contaminants) are controlled by reactions within the NPs (i.e., between the core, shell, and coatings), and interactions between the NPs and the geochemical milieu consisting of water, major solutes (inorganic anions), and minor solutes (contaminants). The overall project includes four activities (synthesis, *ex situ* characterization, *in situ* characterization, and modeling), which are shared among investigators at PNNL, OHSU, and Univ. of Idaho. Results from *in situ* characterization that has been performed at OHSU will be presented here.

Using powder disk electrodes (PDEs) made with iron/iron oxide NPs, we can make precise and quantitative *in situ* measurements of the electrochemical properties of these materials. Corrosion potentials ( $E_{\text{corr}}$ ) measured by chronopotentiometry (CP) exhibit diagnostic transitions that can be fit to a quantitative model for autocatalytic breakdown of the passive film. CP data also show the complex effects of solution species such as carbonate and natural organic matter (NOM)—which can be both corrosive and passivating, depending on time scales and conditions. Values of  $E_{\text{corr}}$  measured on materials with varying degrees of “aging” in solution correlate with rates of  $\text{CCl}_4$  degradation and  $\text{H}_2$  production rate. These measures of aging all suggest increased reactivity in the first 1-2 days after immersion, followed by a gradual decline in reactivity. Additional studies of NP aging are under way, using alternative probes and more environmentally realistic conditions.

The potential effects of NOM are of particular interest to this project, because they range from insulating the passive film from interactions with solutions (passivation), to local acid/base or chelation effects on the oxide film (corrosivity), to catalyzing electron transfers (shuttle effects), to stabilization vis-à-vis inter- and intraparticle attachment. Based on our most recent column and batch experiments, it appears that NOM dramatically decreases attachment of iron/iron-oxide NPs, which greatly increases their mobility in porous media. The effects of NOM on NP reactivity appear to be more complex, however, with electrochemical and batch experiments showing mostly subtle effects, suggesting competing corrosive and passivating processes.

While our main interest is NPs in solution, some procedures require recovery of the particles from solution before analysis. The potential for artifacts related to the recovery process is especially high for iron/iron-oxide NPs because of their reactivity. Some of these effects can be quantified using the electrochemical methods mentioned above. We have used these methods to evaluate methods of NP recovery and identify recommended protocols for minimizing changes during recovery and subsequent storage.

## Influence of Natural and Synthetic Organic Ligands on the Stability and Mobility of Reduced Tc and U

*Session: ERSP University-Led Research*

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It is imperative to clearly understand the fate and transport of reduced uranium [U(IV)] and technetium [Tc(IV)] in order to design better strategies for the remediation of contaminated DOE sites, where a number of natural and synthetic organic ligands commonly co-exists with the contaminant. Our primary objectives are (1) to quantify the interactions of organic ligands with Tc(IV) and U(IV) through the generation of thermodynamic and kinetic parameters needed to assess and predict the mobility of reduced Tc(IV) and U(IV) at DOE contaminated sites; and (2) to determine the impact of organic ligands on the mobility and fate of reduced Tc(IV) and U(IV) under field geochemical conditions. The first year of this project is dedicated to the study of Tc(IV) and its interactions with organic ligands. Our initial work was to set up an atmosphere-controlled glove-box to conduct experiments in absence of oxygen and therefore maintain Tc in the tetravalent state. We successfully completed the chemical reduction of technetium from Tc(VII) to Tc(IV), producing a soluble  $\text{TcCl}^{62+}$  complex solution, confirmed by UV-visible spectrophotometry. Experiments were then conducted to ensure an appropriate replication of published Tc(IV) complex stability constants. The stability constant of the Tc(IV)/oxalate complex, determined by solvent extraction, was found to be  $\log \beta_{101} = 5.9 \pm 0.1$  at 0.5 ionic strength and pCH 1.9. This result is in good agreement with the published value of  $\log \beta_{101} = 5.76 \pm 0.01$  at pCH 1.46 for the same ionic strength. Confident in the validity of our experimental protocol, we are starting similar experiments to determine the stability constants of Tc(IV) with acetate ligand, at different ionic strengths. Within one to two years, we will determine the stability constants of Tc(IV) with a variety of organic ligands at different ionic strengths and perform the dissolution and transport studies of reduced Tc(IV) in contaminated sediments obtained from the Oak Ridge Integrated Field Challenge Site. All data obtained from these studies will then be used to calculate parameters (SIT or Pitzer) to be integrated in geochemical speciation codes. The research is anticipated to provide new thermodynamic data on the behavior of reduced Tc(IV) and U(IV) with organic ligands and ultimately our improved predictive capabilities in the long-term stability and mobility of these contaminants at DOE contaminated sites.

## **Influence of Wetting and Mass Transfer Properties of Organic Chemical Mixtures in Vadose Zone Materials on Groundwater Contamination by Nonaqueous Phase Liquids**

*Session: ERSP University-Led Research*

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Previous studies have found that organic acids, organic bases, and detergent-like chemicals change the properties of organic chemical mixtures and surface wettability. Wastewater and NAPL mixtures discharged at Hanford contain such chemicals, and their proportions likely change over time due to reaction-facilitated aging. Our specific objectives are to determine (1) the effect of organic chemical mixtures on surface wettability, (2) the effect of organic chemical mixtures on  $\text{CCl}_4$  volatilization rates from NAPL, and (3) the migration, entrapment, and volatilization of organic chemical mixtures.

Representative organic mixtures (MIX1-MIX6) were created from chemicals discharged at the Hanford site. The organic mixtures include different compositions of carbon tetrachloride (CT), DBBP, TBP, DBP, and lard oil (LO). A representative wastewater was also prepared. The addition of DBBP, TBP, DBP, and LO components caused a considerable lowering of both the interfacial tension with water and the surface tension of a wastewater phase in equilibrium with the NAPL, but had a minimal effect on the surface tension of the DNAPL itself. The interfacial tension of biologically aged MIX1 decreased slightly, indicating that the biological activity may not significantly affect the interfacial tension of CT mixtures due to the presence of surface active compounds already in the mixtures. For pure CT, contact angles from different pre-equilibration fluids (i.e., water and MIX1) were similar for both quartz and calcite slides. However, MIX1 does change the wettability of the quartz surface from water wetting to neutral wetting and the wettability of the calcite surface from water wetting to oil wetting.

The homogeneous micromodels were fabricated and then used to perform NAPL entrapment and dissolution experiments for pure CT and the MIX1 NAPL. After NAPL entrapment, the initial NAPL area of MIX1 was slightly lower than that of pure CT, while initial perimeter length of MIX1 was higher than that of pure CT. Thus, initial NAPL dissolution was higher for MIX1 than for pure CT. Since MIX1 has more small NAPL blobs than CT, overall NAPL dissolution rate was faster in MIX1 until a large fraction of high solubility components (DBBP and CT) in MIX1 dissolved. Later, the NAPL dissolution rate in MIX1 decreased dramatically, probably due to a high fraction of TBP and LO remaining because of their lower solubility relative to DBBP and CT, while the dissolution rate in pure CT decreased steadily.

We conducted four flowcell experiments in a 100 cm long, 80 cm high, and 5.5 cm-wide 2D flowcell to account for the impact of the organic mixtures and wastewater on DNAPL distribution and migration in a layered vadose zone, containing a fine lens surrounded by coarser sand. After packing, either the organic mixture with the lowest interfacial tension or pure CT was released into the flow cell. After the NAPL distribution reached equilibrium conditions (no apparent movement), soil vapor extraction (SVE) was applied. For one experiment with pure CT and wastewater, the CT penetrated the bottom of the fine sand lens and reached the water-saturated zone. For another experiment with the organic mixture and wastewater, the mixture did not migrate beyond the fine sand lens. For SVE experiment with pure CT, numerical modeling using STOMP with no fitting parameters matched experimental results well. This showed that local equilibrium assumptions can be used to simulate CT volatilization in the layered system, in which a majority of NAPL was initially located in a lower permeability zone, provided permeability and initial NAPL distributions are known.

During the coming year, we will complete NAPL entrapment and mass transfer experiments in micromodels using pure CT and water, as well as fresh NAPL mixtures and wastewater. Modifications to incorporate multicomponent mass transfer into the STOMP simulator will be completed, which will be validated with micromodel experimental results. We will compare NAPL infiltration, NAPL redistribution, and soil vapor extraction results from flowcell experiments with STOMP simulations. STOMP will also be used to simulate NAPL migration and NAPL entrapment at the 3D field scale.

## The Role of Nanopores on U(VI) Sorption and Redox Behavior in U(VI)-Contaminated Subsurface Sediments

Session: *ERSP University-Led Research*

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Most reactive surfaces in clay-dominated sediments are present within nanopores (pores of nanometer dimension). The behavior of geological fluids and minerals in nanopores is significantly different from those in normal non-nanoporous environments. The effect of nanopore surfaces on U(VI) sorption/desorption and reduction is likely to be significant in clay-rich subsurface environments. Recent studies of ethanol-stimulated reduction of U(VI), associated with weathered shale saprolite sediments from the Oak Ridge National Laboratory Field Research Center (ORFRC), indicate that only ~50% of solid-associated U(VI) is subject biotic or abiotic reduction. The redox speciation of the residual, nonreducible U(VI) cannot be explained based on standard models of U(VI) redox speciation. We propose that both water property changes in the nanopores and surface energy changes in nanopore surfaces result in a very strong physical-chemical affinity for U(VI) in the nanopores, and thereby control the reactivity of a large fraction of solid-associated U(VI). Our research objective is to test the hypothesis that U(VI) sorption on nanopore surfaces can be greatly enhanced by nanopore confinement environments. To test this hypothesis, we will carry out the following five tasks: (1) synthesize and characterize nanoporous alumina and goethite as analog materials; (2) investigate U(VI) sorption and desorption on nanopore surfaces in controlled carbonate-bearing model systems; (3) investigate U(VI) sorption and desorption of natural sediments from the FRC site (4) investigate the effects of nanopore sizes on the susceptibility of the sorbed U(VI) to reduction by a range of commercially available quinones with well-defined redox potentials, as well as well-characterized dissimilatory metal-reducing bacteria (e.g. *Geobacter sulfurreducens*); (5) carry out EXAFS studies of U(VI) sorbed on normal surfaces, on nanopore surfaces, and in post-reduction samples, in order to understand chemical environments of the sorbed U(VI) at the molecular level. To isolate the effect of nanopore surfaces, we will also conduct parallel experiments on nonporous bulk materials; the measured chemical reactivity of nanoporous versus nonporous bulk materials will be compared. With the experimental data, the relationship between nanopore size and chemical affinity of sorbed uranium will be established.

Our preliminary FTIR study shows that vibration modes in the range of ~1,625 to ~1,640  $\text{cm}^{-1}$  from nanopore water are different from that from bulk water (1,643  $\text{cm}^{-1}$ ). Recent molecular dynamics simulations show that the dielectric constant of water confined in a nanodimensional spherical cavity is much lower than that of bulk water. The dielectric constant of water will affect Born solvation energy of both cations and anions. The solute water in nanopores may reduce uranyl and/or uranyl-carbonate (with very large molecular dipole moment) solvation, and therefore increase U(VI) sorption and chemical affinity (i.e., significantly lower effective redox potential). The energy difference between the surface with negative curvature and the surface with positive curvature can further or limit/decrease desorption of U(VI) from the nanopore surfaces. The influence of nanopore surfaces on coupled uranium sorption/desorption and reduction processes is likely to be significant in virtually all subsurface environments.

## Improvements to GeoChip 3.0 and Application for Microbial Community Analysis

Session: ERSP University-Led Research

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GeoChip 2.0 is a functional gene array containing probes (50mer oligonucleotides) for >24,000 genes involved in the geochemical cycling of C, N, P, and S, metal resistance and reduction, and organic contaminant degradation. Due to exponential increases in the number of genes and gene sequences reported, a new generation of array, GeoChip 3.0, has been developed, which has several new features. First, GeoChip 3.0 contains ~25,000 probes and covers ~47,000 sequences for 292 gene families. Second, the homology of automatically retrieved sequences by key words is verified by HMMER, using seed sequences so that unrelated sequences are removed. Third, a universal standard has been implemented so that data normalization and comparison of different microbial communities can be conducted. Fourth, a genomic standard is used to quantitatively analyze gene abundance. In addition, GeoChip 3.0 includes phylogenetic markers, such as *gyrB*. Finally, a software package has been developed to facilitate data management, analysis and updates. Examples of GeoChip analysis of microbial communities at DOE ERSP sites during bioremediation processes are presented.

(1) Microbial communities within a pilot-scale *in situ* biostimulation of U(VI) reduction in the subsurface by injection of ethanol were examined. Sediment from eleven different sampling wells, representing two different treatment zones within this system, was evaluated. The results showed that different microbial communities were established in different wells, and high gene overlap was observed from wells within the same treatment zone. Higher microbial functional gene number, diversity and abundance were observed within the active bioremediation zone. The microbial community structure was highly correlated with the hydraulic flow rate and geochemical conditions of the treatment zone, especially pH, manganese concentration, and electron donor level.

(2) In a different study of the same system, functional community dynamics were examined during a period of oxidation by nitrate. Diversity and gene number decreased after nitrate exposure, and while recovery appeared to begin, the gene numbers were still low even 100 days after nitrate exposure. Principal component analysis of detected genes indicated a shift in community structure after nitrate exposure. Nitrate exposure appeared to result in long-term changes to the overall community.

(3) In the third study from the FRC, analysis of groundwater monitoring wells along a contamination gradient revealed less overlap between wells with different levels of U and NO<sub>3</sub> contamination. While diversity of nitrate-fixation genes decreased in NO<sub>3</sub>-contaminated wells, the diversity of metal reduction and resistance genes did not correlate with metal concentrations. Signal intensity did, however, increase in heavily contaminated wells, indicating a larger percentage of organisms with metal-related genes. Sulfate-reduction genes had greater diversity and greater signal intensity in more contaminated wells. Individual principal component analyses (PCA) of the gene diversity and geochemistry of the wells separated them in similar ways. CCA indicated that pH was an important variable that correlated with gene diversity in the lowest-contamination well, while NO<sub>3</sub> and U correlated with the most contaminated well.

Overall, contaminant level appears to have significant effects on the functional gene diversity along the contaminant plume at the FRC. These studies demonstrate the analytical power of the GeoChip in examining microbial communities and its ability to provide direct linkages between microbial genes/populations and ecosystem processes and functions.



## **ERSP Federal Agency Research**



## Multiscale Assessment of Prediction Uncertainty in Coupled Reactive Transport Models

*Session: ERSP Federal Agency Research*

Gary Curtis, U.S. Geological Survey (lead PI); Ming Ye, Florida State University; Philip Meyer, Pacific Northwest National Laboratory; Steven Yabusaki, Pacific Northwest National Laboratory; Derrick R. Rodriguez, Colorado School of Mines

Reactive transport models provide an important framework for the integration of hydrologic and biogeochemical conceptual process models into a quantitative description of subsurface behaviors. The ultimate goal of these modeling analyses, however, is to assess risk and remediation performance for waste management decision-making. Achieving this goal will require an additional and commensurate effort to characterize and quantify uncertainty in the model predictions and assessments. Experience in groundwater hydrology has demonstrated that the total uncertainty of a model simulation consists of both the uncertainty of the parameters and the uncertainty in the conceptual model that represents the scientific understanding of a particular hydrogeologic environment. Moreover, it has been demonstrated that for groundwater flow simulations, the conceptual uncertainty dominates the total simulation uncertainty. This project will assess the both the parametric and the conceptual model uncertainty of hexavalent U(VI) transport at both the Naturita UMTRA site and the Rifle FRC. The parameter and conceptual model uncertainty will be evaluated using a maximum likelihood formulation of Bayesian model averaging. The focus of the work at the Naturita site will be to investigate how conceptual model uncertainty varies across spatial scales ranging from column tests to the plume scale. Preliminary results of assessing uncertainty in uranium adsorption models at the column scale will be presented. These results will provide a fundamental understanding of the uncertainty associated with upscaling models from laboratory conditions to a field setting. The research at the Rifle site will evaluate the parameter and conceptual model uncertainty for highly complex biogeochemical reaction networks that result from the stimulated bioreduction of U(VI) by microbial processes. At both sites, the uncertainty analyses will be extended to assess the value of new data in reducing prediction uncertainty. Together, the research at these two sites will provide complementary results, which together can provide a quantitative understanding of how parametric and conceptual uncertainty at various experimental scales and levels of hydrogeochemical complexity impact the prediction uncertainty of future plume-scale behavior.

## Upscaling of Long-Term Uranium(VI) Desorption from Pore-Scale Kinetics to Field-Scale Reactive Transport Models

*Session: ERSF Federal Agency Research*

James A. Davis, U.S. Geological Survey (USGS) (PI); Gary P. Curtis, USGS; Derrick R. Rodriguez, Colorado School of Mines, Golden, CO; Andy Miller, Colorado School of Mines; Bruce D. Honeyman, Colorado School of Mines; Kelly J. Johnson, USGS; Matthias Kohler, Colorado School of Mines; Carl I. Steefel, Lawrence Berkeley National Laboratory; Douglas B. Kent, USGS

Upscaling of reactive transport models was studied to develop a quantitative understanding of how processes occurring over a range in scales can be incorporated in plume-scale model simulations. The approach taken was to obtain a detailed understanding of U(VI) desorption rates from contaminated sediments at three scales: bench, intermediate, and field.

Uranium-contaminated aquifer sediments were collected at the Naturita (CO) UMTRA site, with grain sizes ranging from silt to gravel. A <2 mm composite sample was prepared and sieved into 6 size fractions, each of which was studied intensively. The rate of desorption measured in the size fractions increased with decreasing size fraction. For the smallest fractions, U(VI) desorption was initially rapid, exceeding 80% of the equilibrium value after 8 hr, followed by slow desorption that required 30 days to reach equilibrium. Conversely, U(VI) desorption by larger size fractions did not have a significant rapid release of U(VI), but still required 30 days to reach equilibrium. Column experiments showed slow U(VI) desorption, with rates increasing with bicarbonate concentration. Complete desorption was rate-limited, as shown by U(VI) effluent concentrations below those predicted by chemical equilibrium and by increases in U(VI) concentrations during stop-flow events. The bench-scale results were consistent with a conceptual and numerical model in which U(VI) desorption is limited by diffusion of U(VI) through coatings on grain surfaces and/or the intragranular space of grains and aggregates.

Two intermediate-scale experiments (2.4 m length) were completed that examined U(VI) desorption in a homogeneously and a heterogeneously packed 2-D tank. The two experiments were packed with the same sediments except that one tank was packed homogeneously with <2 mm sediments; the second tank introduced physical and chemical heterogeneity by splitting the <2 mm sediments into >250 and <250  $\mu\text{m}$  dry-sieved fractions. The two size fractions were placed in alternating layers of different thicknesses, with a “block” of <250  $\mu\text{m}$  fraction at the upgradient end. Spatial gradients of U(VI) were variable in time and space and differed in each tank. Although solid-phase U(VI) content in the two tanks was similar, U(VI) release rates differed significantly, e.g., 7.3 (homogeneous) versus 4.9 moles/L (heterogeneous) during the first pore volume of elution. Reactive transport modeling is being conducted to evaluate the effects of heterogeneity on U(VI) release rates.

Small-scale field experiments were conducted under both natural- and forced-gradient conditions to evaluate the role of groundwater velocity on U(VI) desorption rates. Experiments were conducted by injecting uncontaminated groundwater (with Br tracer) into the aquifer. Breakthrough was observed in 6 multilevel wells located 1.7, 2.1, and 2.5 m downgradient. The velocities observed in the natural-gradient experiment varied from 0.1–0.4 m/d, with several wells exhibiting highly nonideal behavior (e.g., double Br peaks). U(VI) concentrations exhibited complex behavior, with several reaction fronts that coincided with the arrivals of Br and a dissolved Ca front that resulted from ion exchange. Groundwater velocities were 5 times faster in the forced-gradient experiments, with chemical results still being determined. Reactive transport simulations of the natural-gradient experiment were successful using a surface complexation model to simulate U(VI) adsorption, including ion exchange reactions that impact dissolved Ca and U(VI) aqueous speciation.

Two multicontinuum modeling approaches are being evaluated for incorporating bench-scale desorption kinetics into intermediate scale, tracer-test scale, and plume-scale U(VI) desorption simulations.

## **Joint ERSP-NSF-Funded EMSI Research**



## Heterogeneous Chemistry of Water and Carbon Dioxide on Magnesium Oxide Surfaces under Ambient Conditions

*Session: Joint ERSP-NSF-Funded EMSI Research*

John T. Newberg, David. E. Starr, Soren Poosgard—Lawrence Berkeley National Laboratory (LBNL), CA; Susumu Yamamoto, Sarp Kaya—Stanford Synchrotron Radiation Laboratory, Menlo Park, CA; Erin R. Mysak, LBNL; Tom Kendelewicz, Stanford University, CA; Anders Nilsson, Stanford Synchrotron Radiation Laboratory; Miquel Salmeron, LBNL; Gordon E. Brown, Jr., Stanford University, CA; Hendrik Bluhm, LBNL (lead PI)

This work was performed in the framework of the Stanford Environmental Molecular Science Institute (EMSI). The goal of the Stanford EMSI is to create fundamental molecular-level understanding of environmental interfaces and the important chemical and biological processes occurring there. Using synchrotron-based spectroscopies under ambient temperatures and relative humidities, we probe the coverage and chemical speciation of molecules, in particular water, at surfaces under realistic thermodynamic conditions. Oxide surfaces have a particular relevance in environmental science, since water-oxide interactions play an important role in chemical, environmental, and biological systems. Even though these systems have been extensively studied using vacuum-based surface science techniques, the nature of the water-oxide interface under ambient conditions remains poorly understood. Using ambient pressure photoemission spectroscopy and near-edge x-ray absorption fine structure spectroscopy, we have investigated a number of relevant oxides surfaces, e.g., MgO(100). Our experiments focus on the basic mechanism for water adsorption and bonding on oxide surfaces, in particular the role of hydroxyls in determining the hydrophilicity of the surface, and the thickness of the water layer as a function of relative humidity. We consistently find that on oxide surfaces, water adsorbs molecularly at relative humidities below 1%, i.e., even in the driest environments on earth, molecular water will be present at oxide surfaces. The adsorption of water is always preceded by the formation of hydroxide groups at the oxide surface. We will also present recent results related to the influence of water adsorption on the reactivity of oxide surfaces towards carbon dioxide, an important subject in view of the potential large scale use of oxides in carbon sequestration.

## Reduction of U(VI) by Magnetite: Observed Oxidation States and Calculated Rates of Electron Transfer

*Session: Joint ERSF-NSF-Funded EMSI Research*

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Our team uses the computational and experimental capabilities of the Environmental Molecular Sciences Laboratory (EMSL) at PNNL to provide detailed understanding of electron-transfer processes across interfaces under study in the Stanford EMSI. Our focus since FY08 has been on understanding mechanisms and rates of heterogeneous electron transfer from magnetite ( $\text{Fe}_3\text{O}_4$ ) to  $\text{U(VI)}_{(\text{aq})}$ . The reductive adsorption of U(VI) to magnetite ( $\text{Fe}_3\text{O}_4$ ) surfaces prospectively retards radionuclide transport in some subsurface environments, including locations such as the Hanford site and in scenarios of compromised iron-based materials for subsurface storage of spent nuclear fuel. Previous investigations assert that magnetite reduces U(VI) to U(IV) but with incomplete reduction, leaving mixed-valent uranium on the magnetite surface. The thermodynamic stabilities of possible reaction products at the surface are completely unknown. Here, we describe the results of our experimental and computational molecular investigation into the heterogeneous electron-transfer process at the interface. Natural magnetite crystals were cleaved approximately parallel to the (100) surface and exposed to uranyl nitrate solution at pH 4.5 under anoxic conditions for ~90 hours. X-ray photoelectron spectroscopy (XPS) was used to characterize metal oxidation states and their relative proportions before and after exposure. Results indicate a significant amount of U(VI) sorption on all samples, but samples that differed in their initial surface  $\text{Fe}^{2+/3+}$  ratios showed different propensity to reduce U(VI). A sample with an initially stoichiometric  $\text{Fe}^{2+/3+}$  ratio showed partial reduction of U(VI) to stable U(V) products. No U(IV) was observed. A sample that was partially oxidized initially showed no evidence of U(VI) reduction. These results indicate that reduction is dependent on the availability of lattice  $\text{Fe}^{2+}$  at the oxide surface, which depends in part on the electron resupply rate from the underlying bulk to the surface.

Quantum-mechanical calculations were used to first predict the charge distribution of lattice  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  at magnetite (100) surfaces, and to calculate rates of electron resupply via the  $\text{Fe}^{2+/3+}$  valence interchange transfer on the octahedral sublattice. Predicted rates of electron transfer were not surprisingly found to be strongly dependent on surface environment and local structure. At the surface, rates span four orders of magnitude from  $10^9$ – $10^{12}$  hops/second. Measured electron transfer rates for bulk magnetite powders are in the range of  $10^{12}$  hops/second. Therefore, rates near the surface are generally two to three orders of magnitude slower than in the bulk. Surprisingly, the presence of adsorbed water does not have a significant effect on the charge distribution nor electron-transfer rates in the surface. The findings suggest that relatively slow  $\text{Fe}^{2+/3+}$  interchange rates at the surface could be rate-limiting for reductive U(VI) adsorption if reduction involves similar or faster rates of electron transfer. However, quantum mechanical cluster models of inner-sphere bidentate uranyl surface complexes suggest that electron transfer from surface  $\text{Fe}^{2+}$  to adsorbed U(VI) is much slower ( $\sim 10^6$  hops/second) than electron resupply. Furthermore, the calculations predict reduction of U(VI) will end at thermodynamically stable adsorbed U(V) and not to U(IV), in agreement with the XPS observations. Increasing the number of coordinating ligands for the  $\text{U}^{4+}$  cation to configurations comparable to that in crystalline  $\text{UO}_2$  did not increase the endergonicity of conversion to U(IV). If less  $\text{Fe}^{2+}$  is available at the surface, reduction to U(V) is less favorable, indicating that the locally available concentration of  $\text{Fe}^{2+}$  is the key for reduction rate and extent at the magnetite surface. Collectively, these observations shed new molecular-level insight into chemical processes important for predicting uranium stability in subsurface environments.



## Water Adsorption on Silica with Deposited Alkali Halide Particles: *In Situ* Atomic Force Microscopy and Photoelectron Spectroscopy studies

*Session: Joint ERSP-NSF-Funded EMSI Research*

Miquel Salmeron (lead PI), Lawrence Berkeley National Laboratory (LBNL); Kenta Arima, Department of Precision Science and Technology, Japan, Peng Jiang, LBNL; Albert Verdaguier, Centre d' Investigació en Nanociència i Nanotecnologia, Bellaterra, Spain; Hendrik Bluhm, LBNL

We have studied the adsorption of water on silica surfaces, both bare and covered with nanometer thick deposits of alkali halides. The goal is to understand molecular-level chemical and biological processes that are important in environmental interfaces. We use laboratory-based Scanning Probe Microscopies, such as STM and AFM, to image the structure and wetting properties of model surfaces, including real mineral surfaces. The properties studied include water growth and structure, ionic solvation, ionic mobility, dissolution and ion exchange between solution and mineral surface. In parallel, we use synchrotron-based spectroscopies (XPS, XAS) under ambient temperatures and relative humidities, to probe the chemical speciation of adsorbed molecules in the presence of water vapor under realistic environmental conditions of pressure and temperature.

In this work, we will present recent examples that illustrate the possibilities of our two techniques. Using noncontact mode AFM (Scanning Polarization Force Microscopy), we followed the nanometer-scale topography of silica surfaces before and after deposition of small amounts of alkali halides. The adsorption of water up to nanometer-thick films was followed to determine the growth of water and the solvation of ions of the salt deposits. Solvation of ions gave rise to large increases in the dielectric response. The mobility of the solvated ions can be studied with frequency-dependent force response. Mobile ions diffuse on the surface, giving rise to Ostwald ripening of the salt particles. Dissolution of the salts was accompanied by selective segregation of anions at the liquid-gas interface in the case of KI, KBr, KCl and NaCl, which gave rise to a negative contact potential. In the case of KF, positive ions segregated instead. These results are interesting because they show that the presence of the liquid-solid interface does not alter the segregation known to occur in bulk solutions.

*In situ* XPS studies confirmed spectroscopically the findings from the AFM experiments and also revealed chemical shifts associated by the solvation of the ions, with differences between surface and sub-surface species.

## Kinetics of Fe(II)-Catalyzed Transformation of Ferrihydrite under Anaerobic Flow Conditions

*Session: Joint ERSP-NSF-Funded EMSI Research*

Carl I. Steefel, Li Yang (lead PI), Matthew Marcus—Lawrence Berkeley National Laboratory, Berkeley, CA; John Bargar, Stanford Synchrotron Radiation Laboratory, Menlo Park, CA

The readsorption of ferrous ions produced during the abiotic and microbially mediated reductive dissolution of iron oxides drives a series of transformations of the host Fe-hydroxide minerals. These transformations play a critical role in regulating the mobility of soil and groundwater contaminants. To better understand the mechanisms by which these transformations occur and their kinetics within a microporous flow environment, flow-through experiments using capillary tubes packed with ferrihydrite-coated glass spheres were conducted in which inorganic Fe(II) solutions were injected anaerobically under circumneutral pH conditions at 25°C. Synchrotron X-ray powder diffraction techniques were used to identify the secondary phase(s) formed and to provide data for quantitative kinetic analysis. At concentrations above 1.8 mM Fe(II) in the injection solution, magnetite was the only secondary phase formed (no intermediates were detected), with complete transformation requiring 30 hours and 150 hours of reaction at 18 and 1.8 mM Fe(II), respectively. Rather than occurring at a constant rate, the kinetics of the transformations to magnetite, as revealed by the time series of X-ray diffraction peak area data, were best described by a power rate law, with rate constants of  $1.31 \times 10^{-5}$  and  $2.14 \times 10^{-6} \text{ s}^{-1}$  for 18 and 1.8 mM Fe(II), respectively. However, when the injection solution consisted of 0.365 mM Fe(II), goethite was the predominant reaction product, and only minor magnetite was formed. In this case, the transformation occurred much more slowly and followed a linear rate law that gave a rate constant of  $3.12 \times 10^{-7} \text{ s}^{-1}$ . The kinetic phenomenon observed in this study is not compatible with the conventional dissolution-recrystallization mechanism, since it cannot explain the inhibition of magnetite and the switch to a goethite-dominant pathway at 0.36 mM Fe(II) injection when the system was still far oversaturated with respect to magnetite, nor the much more rapid transformation rate to magnetite observed at 18 mM than at 1.8 mM Fe(II) when there was no dramatic difference in thermodynamic driving forces and sorption of Fe(II) is essentially the same. Rather than a simple dissolution and recrystallization mechanism, the results obtained from this study suggest that the transformations involved an electron-transfer reaction between the aqueous Fe(II) and ferrihydrite acting as a semiconductor. The rapid and complete conversion to magnetite without the formation of an intermediate phase indicated a mechanism involving the direct solid state reduction of ferrihydrite by fast electron injection from the high concentration of Fe(II) aqueous solution. With an injection of 0.36 mM Fe(II), the electron-transfer rate was apparently not rapid enough to keep up with the destabilization of ferrihydrite structure, and the transformation process became dominated by a reconstructive dissolution-recrystallization mechanism resulting in the formation of goethite. The results obtained from this study further highlighted the importance of semiconductor properties of iron oxides and electron-transfer reactions as an important mechanism for their transformations in subsurface environments.

## **Joint BER-EPA-NSF-Funded Nanoparticulate Research**



## Microbially Mediated Transformation of Metal and Metal Oxide Nanoparticles

*Session: Joint BER-EPA-NSF-Funded Nanoparticulate Research*

M. J. Doktycz (lead PI), D.A. Pelletier, C. K. McKeown, S. D. Brown, B. Gu, W. Wang, Oak Ridge National Laboratory, TN; D.P. Allison, University of Tennessee, Knoxville, TN; D.C. Joy, University of Tennessee, Knoxville/Oak Ridge National Laboratory, T. J. Phelps, Oak Ridge National Laboratory

Engineered nanostructures have a central role in energy conservation strategies and economic growth. One of the most significant impacts of engineered nanostructures is for effecting heterogeneous catalysis as required for fuel transformation, energy storage, polymer production and chemical synthesis. Metal and metal oxide nanoparticles are often used. They possess high surface areas and the ability to selectively mediate chemical transformations. The size and composition of the particle affects performance and may similarly affect nanoparticle fate and transport in the environment. The transformation of such nanoparticle catalysts in the environment is likely to proceed through interactions with bacteria. Several bacterial species are well known to interact with nanoparticles. Nanoparticle production, nanoparticle toxicity, nanoparticle binding and incorporation with bacteria have all been observed. However, basic knowledge that would allow prediction of the probable interaction between an engineered nanoparticle and bacteria is lacking. Our efforts seek to quantify and characterize interactions between engineered metal and metal oxide nanoparticles and selected microbial species. The effect of size and chemical composition of nanoparticles that are currently considered for various applied uses are being studied. Initial efforts are focused on the effects of cerium oxide nanoparticles on the growth, viability, and genetic response of *E. coli*. Well-characterized CeO<sub>2</sub> nanoparticles of various sizes have been prepared and presented to bacterial cells in a dose-dependent manner. Advanced imaging techniques are used to evaluate the binding and fate of the nanoparticles and the bacterial cell. The results of these studies will provide a basis for understanding how nanoparticle size and composition influence their interactions with microorganisms, and how microorganisms may alter the fate and transformation of engineered nanoparticles in the environment.

## Palladium(0) Nanoparticle Formation by *Clostridium* sp. BC1 Provides an Effective Biocatalyst for Hexavalent Chromium Remediation

Session: Joint BER-EPA-NSF-Funded Nanoparticulate Research

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This project addresses the need to understand the catalytic activity, reactivity, and transport of palladium metal nanoparticles in soils and aquifers. The rapid increase in the use of palladium-containing catalysts, which results in their incidental release to water resources and their deliberate release for environmental remediation, necessitates fundamental *a priori* knowledge of their environmental fate and impact to properly assess related societal implications. The project has two main objectives: (1) determine the biogeochemical transformations that alter the bioavailability and transport of palladium metal nanoparticles in soils, and (2) apply this knowledge to develop a novel *in situ* remediation strategy for the immobilization and/or degradation of U.S. Department of Energy priority contaminant metals, radionuclides, and organics. The results presented at this meeting will demonstrate the use of Pd and Cr in a novel, yet generally applicable remediation strategy.

The remediation strategy involves co-injecting aqueous palladium and a carbon source to stimulate sulfate-reducing and fermentative soil bacteria that in turn produce palladium nanoparticles and hydrogen within an active aquifer treatment zone or bioreactor. In the presence of palladium nanoparticles, the hydrogen acts as the electron donor to reduce and immobilize soluble chromate ions. This remediation scheme is an extension of recently demonstrated strategies used to treat metal-plating wastes, and it is intended to extend nanoparticle-based *in situ* treatment to deep aquifers, where traditional reactive barriers cannot be implemented.

Our results show an extremely efficient method for synthesizing Pd(0) nanoparticles using members of the genus *Clostridium*. Aqueous Pd(II) ions are readily reduced to produce uniform Pd(0) nanoparticles associated with the microbial cell surface. The Pd(0) nanoparticles act as effective biocatalysts for the removal and reduction of hexavalent chromium in both batch and advective flow sand column experiments, most likely via a reduction mechanism using biologically produced hydrogen as an electron donor. Microbial community derived 16S rRNA gene sequencing from within the treatment zone will also be reported. This study has significant implications for both the fate and transport of nanoparticles in the environment, and the *in situ* generation and use of Pd(0) nanoparticle biocatalysts for contaminant remediation.

## ***In Situ* Measurements of Functionalized Nanoporous Particle Transport in Porous Media**

*Session: Joint BER-EPA-NSF-Funded Nanoparticulate Research*

Chongxuan Liu (lead PI), Zheming Wang, Kake Zhu, Juan Li, Jun Liu, John M. Zachara—  
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Various nanoporous materials have been developed for research and industrial applications such as in energy storage and fuel cell technology, catalysis, photonics, biology and bioengineering, and environmental remediation. These nanoporous particles, which could eventually enter subsurface environments either intentionally or unintentionally, may affect groundwater quality. This project aims to understand the fate and transport of engineered nanoporous particles in subsurface porous media, and their effects on the reactive transport of radionuclides at DOE cleanup sites.

A functionalized material of engineered nanoporous silicate particles (ENSP) has been synthesized, its internal pore surface covalently bonded with the metal-chelating ligand salicylamide. Salicylamide was selected because of its strong, selective adsorption affinity for U(VI) in groundwater and its fluorescence-producing properties, enabling *in situ* monitoring. Two sizes of nanoporous particles have been synthesized (80 nm and 200 nm) with internal pore diameter of about 2 nm. Various synthetic approaches have been evaluated to minimize the aggregations and improve the stability of nanoporous particles during porous silicate structure formation and ligand assembling processes. An optimal synthesis approach has been developed.

Particle transport is typically studied in column systems by varying experimental conditions and observing the responses of particle concentrations in column effluents. *In situ* measurements of particle transport, while important for understanding the physics of particle interaction with porous media, are rarely performed because of the technical difficulty in detecting particles in porous media. Here, we have developed a scanning optical fiber-laser excitation fluorescence-profiler system that can track, *in situ*, the transport of nanoporous particles in a column system. An array of optical fibers is installed at different locations within the column, and the excitation laser beam is sequentially directed into the optical fibers to incident on the porous media inside the column. The emitted fluorescence is collected by the same fibers, and after they are dispersed through a spectral graph, the fluorescence spectra are recorded by an intensified CCD camera.

The *in situ* measurements revealed the complex behavior of ENSP fate and transport in the column system. The complex behavior apparently resulted from the combinational effects of various factors, including preferential flow, mass transfer into slow mobile domains, time-dependent retention/release properties, and sediment surface electrostatic properties that are affected by solution chemical composition. Research is under way to evaluate the role of these factors in contributing to the observed transport behavior.

This project is a part of the Nanotechnology Research Program: Investigating Fate, Transport, Transformation, and Exposure of Engineered Nanomaterials: A joint research program involving EPA, NSF, and DOE.

## Fate and Transport of Engineered Nanoparticles after Their Release into Soils and Groundwaters

*Session: Joint BER-EPA-NSF-Funded Nanoparticulate Research*

Susan Habas, Yongman Kim, Saeed Torkzaban, Taleb Mokari, Tetsu Tokunaga, Jiamin Wan (lead PI)—  
Lawrence Berkeley National Laboratory

The rapid development of nanotechnology has generated considerable interest in the application of engineered nanoparticles (ENPs) for renewable energy technologies, medicine, and life sciences. At the same time, the unevaluated risks of ENPs to human health and ecosystems have raised considerable speculation and could have very costly impacts. This research project aims to advance the DOE's proactive decision to further fundamental understanding on the environmental impacts of ENPs, thereby helping guide the future development of safe nanomaterials. ENPs will most likely enter the subsurface through soils; thus their mobility through the unsaturated zone will control their entry into underlying groundwater. Subsequently, the stability and mobility of ENPs in aquifers will determine their long-range subsurface transport to aquatic environments and drinking water supplies. The overall objective of this project is to address the crucial question: "Can we predict the stability and mobility of ENPs after their releasing into soils and groundwaters?" We address this question from three aspects: (1) understanding to what degree ENPs retain their original sizes and surface properties after they enter the subsurface environments, (2) understanding mobility of ENPs in unsaturated soils and testing the applicability of the unsaturated filtration model developed for colloids (Wan and Tokunaga, 1997) to ENPs, and (3) quantifying mobility of ENPs under a range of transport conditions representative of aquifers, and testing the applicability of classic filtration theory for ENPs.

Cadmium selenide (CdSe) quantum dots (QDs) were chosen for initial investigations because of their unique nanoscale optical properties, which make them excellent candidates for devices and commercial applications. These nanocrystalline semiconductors (4–6 nm in diameter) were synthesized and stabilized in water using different types of surface stabilizing ligands. Studies on the kinetic stability of ligand stabilized CdSe QDs as a function of pH, ionic strength, ion type, and particle concentration are in progress. Concurrent investigations into the transport of the CdSe QDs are being conducted. The variables being tested include water saturation of the porous media, flow rate, grain surface properties of the porous media, and solution chemistry. Our preliminary findings will be summarized in our poster presented during the workshop. We anticipate that these studies will significantly improve our understanding and predictions of the stability and mobility of ENPs (QDs) after their release into the subsurface.



## The Fate of Organic Nanoparticles Used for Heavy Metal Remediation

*Session: Joint BER-EPA-NSF-Funded Nanoparticulate Research*

Paul M. Welch (lead PI), Kim Rasmussen—Los Alamos National Laboratory; Jerred Chute, UC Santa Barbara/Theoretical Division, Los Alamos National Laboratory; Nikolaos Voulgarakis, Lu Yang, Marc Cawkwell—Los Alamos National Laboratory

More than six decades of R&D and production of nuclear materials at DOE sites have resulted in a staggering legacy of environmental contamination. Pollutants, including radioisotopes, metals, and organic solvents, are present at over 100 individual DOE sites. The estimated cost for cleaning up the many landfills, surface spills, and water reservoirs exceeds \$200 billion. Fortunately, new technologies are in development that promise to improve the economic efficiencies of current remediation methods. Organic nanoparticles such as dendrimers and core-shell polymers demonstrate remarkable capabilities to sequester radioisotopes, anthropogenic solvents, and metals. However, the fate of these exotic materials in nature and the design principles needed to improve their function in environmental settings remains unknown. This is unacceptable from a regulatory stance, since we may be compounding the contamination with their use. Moreover, our current lack of knowledge with respect to the chemical and physical behavior of these materials once deployed limits our ability to optimize their effectiveness. We are pursuing a course of research to rectify this poor understanding by examining their molecular level behavior and decomposition in various soil and fluid environments.

To facilitate informed decisions about the use of nanoparticles for heavy metal remediation and to improve upon their capabilities, we seek to answer four questions: (1) Under what conditions will the nanoparticles aggregate, and what does that do to their ability to sequester their targets? (2) How do they interact with mineral surfaces? (3) How do they interact with microbial cell membranes? (4) How do they chemically degrade, and what are their decomposition products when left in aqueous and mineral surroundings? We are answering these questions using a combination of theoretical and computational tools, including coarse-grained simulations, atomistic molecular dynamics, reactive molecular dynamics, semi-empirical methods for dealing with photo-degradation, and self-consistent mean field theory.

In this poster, we will present the results from ongoing efforts to address these questions. Specifically, we will present results on the physical properties of tectodendrimers, core-shell polymers, and dendrimers in different environments, as well as the behavior of various nanoparticles in the presence of environmental substrates.



## SciDAC Research



## **PFLOTRAN: Next-Generation Model for Simulating Reactive Flow and Transport at the Petascale**

*Session: SciDAC Research*

Peter Lichtner (lead PI), Los Alamos National Laboratory; Glenn Hammond, PNNL; Richard Mills, ORNL; Bobby Philip, LANL; David Moulton, LANL; Barry Smith, ANL; Albert Valocchi, UIUC; Steve Yabusaki, PNNL

As the U.S. Department of Energy continues to make investments in high-performance computing as a means of accelerating scientific discovery, there is great opportunity to leverage this capability in the simulation of subsurface flow and transport to both improve understanding of the fundamental physical and chemical processes in the subsurface and apply this knowledge to real-world applications. As part of DOE's SciDAC-2 program, we are continually advancing the development of the massively parallel code PFLOTRAN, a multiscale, multiphase, and multicomponent reactive flow and transport code founded upon established and well-known DOE computational libraries (i.e., PETSc, SAMRAI). A comprehensive overview of PFLOTRAN's capabilities is presented, including a description of the object-oriented Fortran9X coding paradigm implemented in PFLOTRAN, parallel I/O through HDF5, and adaptive mesh refinement (AMR) through SAMRAI C++.

Leveraging PETSc provides access to a suite of sophisticated preconditioner/solver implementations in PETSc, as well as providing interfaces to those from several external packages (e.g., Hypre, Trilinos, etc.). Through careful design and algorithmic development, the original PETSc-based structured grid implementation of PFLOTRAN has been extended to allow for structured AMR that requires the use of multiple grids at different levels of resolution. Developments related to the implementation of discretizations, vector and matrix operations, nonlinear and linear solvers, and multilevel preconditioners on AMR grids will be described. PFLOTRAN has been designed to enable applications to be run on structured, structured AMR, or unstructured grids while using the same chemical/physical process kernels. With such a degree of modularity, the code is very extensible both with respect to the domain science and the solver framework.

PFLOTRAN's chemical algorithms include homogeneous speciation equilibria, mineral precipitation and dissolution using a kinetic formulation of reaction rates, and a mechanistic local equilibrium description of sorption through surface complexation and ion exchange. Features currently being implemented in the code include unstructured grids, multiple interacting continua, colloid-facilitated transport, and solid solutions. A novel multirealization capability within PFLOTRAN enables the execution of thousands to tens of thousands of realizations simultaneously, where each realization may run on one or more processor cores. Performance and scalability studies demonstrating the exceptional scalability of PFLOTRAN to tens of thousands of processor cores on leadership-class supercomputers are also presented.

## PFLOTRAN: Application to Uranium Migration at the Hanford 300 Area

*Session: SciDAC Research*

Peter Lichtner (lead PI), Los Alamos National Laboratory; Glenn Hammond, PNNL; Richard Mills, ORNL; Bobby Philip, LANL; David Moulton, LANL; Barry Smith, ANL; Albert Valocchi, UIUC; Steve Yabusaki, PNNL

This work is a collaborative exercise between the DOE ERSP Hanford 300 Area IFRC and the DOE ASCR SciDAC-2 program, where PFLOTRAN, a multiscale, multiphase and multicomponent reactive flow and transport code designed for execution of any range of computing platforms (i.e., laptop to petascale), is being developed and applied to simulate real-world problem scenarios on DOE's latest supercomputing architectures.

Results are presented for the geochemical transport of uranium at the Hanford 300 Area incorporating surface complexation and mineral dissolution and precipitation, using the massively parallel subsurface reactive flow code PFLOTRAN developed under the SciDAC-2 program. The Hanford 300 Area presents many challenges with regard to simulating radionuclide transport. Aside from the many conceptual uncertainties in the problem such as the choice of initial conditions, rapid fluctuations in the Columbia River stage, which occur on an hourly basis with several meter variations, can have a dramatic impact on the size of the uranium plume, its migration direction, and the rate at which it migrates to the river. Due to the immense size of the physical domain needed to include the indispensable river boundary condition, the grid resolution required to preserve accuracy, and the number of chemical components simulated, 3D simulation of the Hanford 300 Area would be unsustainable on a single workstation, and thus high-performance computing is essential. In this preliminary formulation, multiscale processes are incorporated through an effective dissolution rate for the uranium source in the form of metatorbernite  $[\text{Cu}(\text{UO}_2)_2(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}]$ . Model results for the cumulative flux of uranium into the Columbia River are compared for cases with and without sorption.

## Upscaling Multi-Physicochemical Processes in Porous Media from Pore to Continuum Scales

*Session: SciDAC Research*

Peter Lichtner (lead PI), Qinjun Kang, Moran Wang—Los Alamos National Laboratory, NM

Pore-scale modeling can provide insight towards understanding the underlying physics of transport and reaction in the submicron and micron length scales. The objective of this work is to investigate the role of pore-scale heterogeneity on macro-scale continuum representations of multiphysicochemical processes in porous media. To this end, multiple processes, including fluid flow, diffusion and advection, electrochemistry, adsorption-desorption reactions, and other chemical reactions, as well as the evolution of pore geometry due to dissolution/precipitation, are simulated at the pore scale using the lattice Boltzmann (LB) method. Heterogeneous reactions are incorporated into the LB method through boundary conditions imposed at the mineral surface, with an augmented triple-layer model to account for complexation between the ions in the solution and the active sites covering the solid surface. The LB models can provide detailed information on local fields, such as fluid velocities, electric potential, solute concentrations, mineral compositions and amounts, as well as the evolution of pore geometry due to chemical reactions. Provided sufficient resolution of the pore-scale geometry can be obtained, the pore-scale model can be used to determine the form of continuum formulation (single, dual, or multiple continua) that best fits the upscaled pore-scale simulation and, simultaneously, provide parameters needed for constitutive relations appearing in the multiscale continuum formulation. It is demonstrated, however, that for species-dependent diffusion, the rates of homogeneous reactions cannot be replaced by mass action relations in the LB implementation, necessitating the solution of all aqueous species with a very large kinetic rate constant for local equilibrium conditions. For many systems, this is prohibitive numerically, and in this case alternative numerical methods are needed to solve the pore-scale reactive transport equation.

As an example, we apply the multi-component reactive transport LB model to modeling the injection of a CO<sub>2</sub> saturated brine into various porous media structures at temperature T=25 and 80°C. In the various cases considered, the porous medium consists initially of calcite with varying grain size and shape. A chemical system consisting of Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, H<sup>+</sup>, CO<sub>2</sub>(aq), and Cl<sup>-</sup> is considered. Flow and transport by advection and diffusion of aqueous species, combined with homogeneous reactions occurring in the bulk fluid, as well as the dissolution of calcite and precipitation of dolomite, are simulated at the pore scale. The effects of the structure of the porous media on reactive transport are investigated. The results are compared with a continuum-scale model, and the discrepancies between the pore- and continuum-scale models are discussed. It is shown that for both T=25 and 80°C, dolomite only precipitates, but calcite both dissolves and reprecipitates. It is found that for the higher temperature, a sharp reaction front is formed. The front moves at an approximately constant velocity that is found to be dependent on the calcite surface area, in contrast to a conventional continuum formulation where the front propagation rate is independent of kinetics. This effect is caused by armoring of the calcite surface with dolomite. The front velocity is roughly inversely proportional to the volume fraction of the fraction of reactive calcite. The numerical results presented here are still subject to confirmation by high-resolution microscopic experiments. Nevertheless, these results may help understand the fundamental physicochemical processes during CO<sub>2</sub> sequestration in particular, and reactions in heterogeneous media in general.

## Development of a Framework for Subsurface Simulation Using the Common Component Architecture

*Session: SciDAC Research*

Bruce Palmer (lead PI), Yilin Fang, Vidhya Gurumoorthi, Glenn Hammond, James Fort—  
Pacific Northwest National Laboratory, Richland, WA

The goal of this project is to develop a component-based framework for performing simulations of subsurface flows, specifically targeting two codes. The first is designed to do pore-scale simulations of reactive flows using a smoothed particle hydrodynamics algorithm (SPH) that uses Lagrangian particles to model the behavior of fluid flow. The second code is a continuum reactive subsurface flow simulator based on the STOMP suite of codes (Subsurface Transport Over Multiple Phases). Both these codes are in the process of being divided into individual components in a way that encapsulates different numerical, physical, or computer science functionalities in individual components. This encapsulation is designed to promote ease of maintenance, reuse of core technologies, easier upgrades to new technologies, and easier inclusion of new physical and chemical processes. The use of the Common Component Architecture (CCA) toolkit to create this framework provides additional benefits, including automatic language interoperability and runtime configuration of applications.

A nearly complete set of components for performing parallel SPH simulations has been developed. These are being used to validate SPH flows in three dimensions and to begin looking at pore-scale transport using systems containing on the order of 7 million particles. Preliminary results on a study of advective porosity will be presented. Work is ongoing to improve the parallel IO performance of these codes so that larger simulations can be performed, and a reactive chemistry component is being developed to model the behavior of uranium transport in subsurface porous media in collaboration with the PNNL Science Focus Area effort.

Work on componentization of the STOMP subsurface simulation code is less developed than for the SPH code, but significant progress has been made. Most importantly, the grid has been abstracted from the rest of the STOMP code, recast with an interface that uses an unstructured grid syntax, and encapsulated as a separate component. Most of the remaining STOMP code has been reconfigured to use this unstructured syntax. Although the existing grid component still uses a rectangular grid under the interface, it should be possible to develop other versions of the grid component that create unstructured grids and plug these into the existing STOMP component with minimal modification. Work is ongoing to develop such a component in collaboration with the ITAPS SciDAC Center for Enabling Technology. Additional work is planned to further componentize the remaining STOMP core, including isolating IO routines, solvers, chemistry, and input as separate components.

A longer term goal of the framework is to develop components that can be used to couple both the SPH and STOMP simulations together to create multiscale hybrid simulations of reactive flows that are coupled at significantly different scales. These components will be based on methods currently being developed and tested under the related SciDAC Groundwater Science Application.



## Hybrid Numerical Methods for Multiscale Simulations of Subsurface Biogeochemical Processes

*Session: SciDAC Research*

Tim Scheibe (lead PI), Alexander Tartakovsky, Phil Long—Pacific Northwest National Laboratory, Richland, WA; George Redden, Paul Meakin—Idaho National Laboratory, Idaho Falls, ID; Daniel Tartakovsky, University of California San Diego, CA; Scott Brooks, Oak Ridge National Laboratory, TN

Many subsurface flow and transport problems of importance today involve coupled nonlinear processes that occur in media exhibiting complex heterogeneity. In particular, reactive transport involving biological mediation of reactions, precipitation, or dissolution of mineral phases, and/or diffusion-limited mass transfer falls into this class of problems. Recent experimental research has revealed important details about the physical, chemical, and biological mechanisms that control these processes from the molecular to laboratory scales. We are developing a hybrid multiscale modeling framework that combines discrete pore-scale models (that explicitly represent the pore-space geometry at a local scale) with continuum field-scale models (that conceptualize flow and transport in a porous medium without a detailed representation of the pore space geometry). At the pore scale, we have implemented a parallel three-dimensional Lagrangian model of flow and transport using the Smoothed Particle Hydrodynamics (SPH) method and performed test simulations using millions of computational particles on the supercomputer at the Environmental Molecular Sciences Laboratory. We have also developed methods for gridding arbitrarily complex pore geometries and simulating pore-scale flow and transport using parallel implementations of grid-based computational fluid dynamics methods. Within the multiscale hybrid framework, we have directly linked pore- and continuum-scale models to simulate coupled diffusive mixing, reaction, and mineral precipitation, and compared the results with conventional continuum-only and pore-scale-only simulations. Quantitative conditions under which a hybrid modeling approach is necessary have been mathematically defined and numerically tested. We have developed a number of application problems that serve as both numerical testbeds for methods development and demonstrations of the applicability of hybrid methods. This project is funded under a partnership between the Environmental Remediation Sciences Program (ERSP) and the Scientific Discovery through Advanced Computing (SciDAC) program, and is closely coordinated with two SciDAC Science Application Partnership projects described in separate abstracts (Bruce Palmer, PI and Karen Schuchardt, PI). Under these partnerships, our reactive transport codes are being developed using advanced high-performance component architectures and efficient parallel solvers, and integrated into a component-based workflow environment to facilitate seamless integration of codes operating at multiple scales with different physical, biological, and chemical conceptualizations appropriate to the needs of specific simulation problems. The hybrid multiscale modeling framework also draws on a number of SciDAC enabling technologies, including the Common Component Architecture, advanced solvers, grid technologies, scientific workflow tools, and visualization technologies.

## An Integrated Framework for Subsurface Simulation and Analysis

*Session: SciDAC Research*

Karen Schuchardt (lead PI), Jared Chase, Jeff Daily, Todd Elsethagen, Vicky Freedman, Timothy Scheibe—Pacific Northwest National Laboratory, Richland, WA

The goal of this project is to develop a framework that integrates data management, analysis and visualization, and workflow technologies into an extensible environment that simplifies the process of conducting subsurface modeling studies encompassing hundreds to thousands of simulations. Key areas where such a framework can impact the science include: tracking provenance of the process and exposing the relationship between individual simulations and analyses, management of the data inputs and outputs associated with each simulation perhaps performed across many machines, automated movement of large data sets to and from archives, and desktop visualization utilizing remote parallel visualization tools. The current framework supports running two models: the Subsurface Transport over Multiple Phases (STOMP) continuum code, and the Common Component Architecture (CCA)-based Smooth Particle Hydrodynamics (SPH) code, which is currently under development. However, the framework is not specific to these codes; any simulator can be supported and a new hybrid model that combines continuum and particle scales will be supported when available. Two visualization tools have been integrated to date: TecPlot and General Mesh Viewer (GMV). Support for the parallel visualization tool VisIt is in progress and will enable desktop visualization of large data sets residing on compute servers. The framework is exposed to modelers through a python-based user interface tool called the Organizer. This tool provides access to tools that have been integrated through a registry. This includes tools such as simulators, visualization tools, data translation scripts, grid viewers, editors, etc. In principle, any tool can be integrated. As the modeler invokes tools, the inputs and outputs of each task are recorded, along with the relationships between tasks, thus creating a directed graph representing the full provenance record of the research process. Each recorded task can be annotated and “tagged” to capture context and knowledge.

The Organizer presents two primary views of the provenance record. The first is a graph-based representation where the layout is provided by the GraphViz program. This view shows the full provenance record, provides access to all inputs and outputs of each task, and can be used to initiate new tasks. The second is a simulation view, which shows a table of simulations and key information known about each, such as where it was run, its status, and the start and finish times. This view can also provide a timeline view, but does not show relationships between simulations. Simulation execution is supported through a workflow developed using the Kepler workflow system. This workflow takes a set of job requests, along with a set of target machines, and distributes these jobs to the machines while balancing CPU load. Workstations, clusters, and supercomputers are supported.

## **Integrated Field-Scale Subsurface Research Challenges (IFRC)**



## Multiscale Mass-Transfer Processes Controlling Natural Attenuation and Engineered Remediation: An IFRC Focused on Hanford's 300 Area Uranium Plume

*Session: Hanford IFRC (Principal Investigator: John Zachara)*

John Zachara, PNNL, Bruce Bjornstad, PNNL, John Christensen, LBNL, Mark Conrad, LBNL, Jim Fredrickson, PNNL, Mark Freshley, PNNL, Roy Haggerty, Oregon State University, Glenn Hammond, PNNL, Doug Kent, USGS, Allan Konopka, PNNL, Peter Lichtner, LANL, Chongxuan Liu, PNNL, Jim McKinley, PNNL, Mark Rockhold, PNNL, Yoram Rubin, University of California, Berkeley, Vince Vermeul, PNNL, Roelof Versteeg, Idaho National Laboratory, Andy Ward, PNNL, Chunmiao Zheng, University of Alabama

The Integrated Field-Scale Subsurface Research Challenge (IFRC) at the Hanford Site 300 Area uranium (U) plume addresses multiscale mass transfer processes in a complex hydrogeologic setting. A series of forefront science questions on mass transfer are posed for research which relate to the effect of spatial heterogeneities; the importance of scale; coupled interactions between biogeochemical, hydrologic, and mass-transfer processes; and measurement approaches needed to characterize and model a mass-transfer dominated system. The project was initiated in February 2007, and considerable progress was made installing the saturated zone experimental site within the footprint of the South Process Pond during FY 08. The site has 35 instrumented wells and an extensive monitoring system. It included a deeper borehole for microbiologic characterization that sampled the entire thickness of the unconfined 300 A aquifer and that will be used for future downhole biogeochemical research. The well field yielded over 1000 sediment samples, and a characterization program is under way to define physical, chemical, and electrical/geophysical properties as necessary to construct a 3D geostatistical model of the IFRC experimental domain. Geophysical field characterization is employing crosshole electrical resistance tomography that will provide information on sediment properties and structures over the 10 m distances between wells. An extensive hydrologic testing campaign was completed, and a large nonreactive tracer injection experiment was conducted during November 2008. The results display and quantify the hydrologic complexity of the site, but also define the presence of variable hydraulic conductivity zones conducive to the evaluation of our mass-transfer scientific theme. The project modeling team is developing a robust hydrogeologic site model that can be expanded to include mass transfer, geochemical, and biogeochemical processes, and site-wide properties distributions as documented. Studies of uranium adsorption/desorption and mass transfer have been initiated with contaminated sediments from the site to parameterize process-specific modules for field-scale application. The project moves into CY 2009 with ambitious plans for a series of well-planned saturated zone field experiments and is seeking opportunities for collaboration with other ERSP investigators.

This research is part of the ERSP Hanford IFRC at Pacific Northwest National Laboratory.

## Uranium Isotope Systematics in the 300 Area (Hanford, WA): The Relationship Between the U Groundwater Plume and U-Contaminated Vadose Zone Sediments

*Session: Hanford IFRC (Principal Investigator: John Zachara)*

John N. Christensen, Lawrence Berkeley National Laboratory, Mark E. Conrad, Lawrence Berkeley National Laboratory, James P. McKinley, Pacific Northwest National Laboratory, P. Evan Dresel, Pacific Northwest National Laboratory, Donald J. DePaolo, Lawrence Berkeley National Laboratory/UC Berkeley, John M. Zachara, Pacific Northwest National Laboratory

The 300 Area at the Hanford Site in Washington is situated along an ~2 km stretch of the Columbia River. Past operations in the 300 Area included the disposal of chemical and radioactive waste derived from fuel rod fabrication and other experimental processes into a series of settling ponds and disposal trenches. These waste fluids included U, Zr, and Cu, as well as a mixture of acidic and basic solutions. This resulted in a persistent groundwater U plume in the 300 Area (that communicates with the Columbia River), with U concentrations reaching greater than 100 ppb. Despite removal of the contaminated pond sediments and trench material starting in 1995 (completed by 2004), the U plume has not dissipated on the predicted time scale. The persistence of the groundwater U plume is likely due to replenishment from slow release from contaminated vadose zone sediments influenced by the dynamic groundwater chemistry, resulting from incursions of Columbia River water due to highly variable river stages (see review in Zachara et al., 2007, and references therein). The 300 Area IFRC was established to conduct field-scale experiments and modeling regarding U mobility in a hydrologically dynamic environment. Here, we report and discuss our U isotopic studies of the groundwater of the 300 Area, with a particular focus on the IFRC experimental well array, and of vadose zone sediment samples both from beneath the process ponds and within the IFRC array. These data not only provide insights into the sources and temporal dynamics of the U plume, but also aid in the planning of experiments within the IFRC array (e.g., identification of wells with similar and contrasting U isotopic compositions to the IFRC array). Planned experiments will involve adsorption/desorption of U within the IFRC experimental array that will be monitored in part through U isotopic analysis of groundwater samples from the array. Groundwater U and sediment leachate U isotopic compositions fall along similar arrays, indicating mixtures derived from processed enriched U fuels, processed natural U fuels, and in some cases depleted U. Significant U isotopic differences are evident between different process ponds (with subpond sediments from the North Process Ponds characterized in part by higher  $^{236}\text{U}/^{238}\text{U}$  than from the South Process Ponds, each designated NPP1, SPP1, etc., for individual samples), along with U isotopic variations with depth. Groundwater within the IFRC experimental plot appears to be effected by SPP2 U and a second source (perhaps SPP1), while groundwater nearer shore, represented by wells 2-1 and 3-1, appears to have been affected by both SPP2 U and the source for Well 1-17a, possibly the 316-5 trenches (yet to be characterized). The spatial and temporal variations we observe in the U isotopic composition of 300 Area groundwater allows tracking of water masses independent of U concentration, and identification of possible sources of U contamination to the Columbia River. This research is part of the ERSP Hanford IFRC at Pacific Northwest National Laboratory.

## Experimental Investigation of the Effect of Transient Groundwater Flow on U(VI) Transport in the Hanford 300 Area

*Session: Hanford IFRC (Principal Investigator: John Zachara)*

Jun Yin, Oregon State University, Roy Haggerty, Oregon State University (lead PI), Jonathan D. Istok, Oregon State University, Doug Kent, U.S. Geological Survey, Mark Rockhold, Pacific Northwest National Laboratory

The U(VI) transport at the Hanford 300 Area is affected by stage changes of the Columbia River. Continuously changing hydraulic gradient in the 300 Area produces a complicated transport situation, with interactions of high- and low-alkalinity waters that strongly affect U(VI) transport. Alkalinity gradients are preserved in immobile and low-permeability zones, increasing the contact area between high- and low-alkalinity waters. We report results of ongoing column experiments investigating the effects of transient flow (including gradient reversals with high- and low-alkalinity waters) on U(VI) mass transport. In the column experiments, artificial solutions are prepared to represent the clean/contaminated Hanford groundwater (SGW) and Columbia River water (SCRW). The clean SGW is first injected into the column to desorb the labile U and obtain clean sediments. The contaminated SGW (with a standard U concentration of 100 ppb) is then injected into the “clean” sediment to observe the sorption-only process. In a transient flow experiment, contaminated SGW is injected into the clean sediment, followed by flow reversals (using SCRW injected at the opposite end of the column), according to the oscillation period of Columbia River. The effects of the transient flow are investigated by comparing the transient flow U(VI) breakthrough curve to a breakthrough curve obtained with steady (nonoscillating) flow. Column experiments will provide controlled results that will be used to test the ability of a solute transport model to represent U(VI) transport in a geochemically complex environment, and in the presence of oscillating and changing pore-water geochemistry. This research is part of the ERSP Hanford IFRC at Pacific Northwest National Laboratory.

## Variability in Adsorbed Uranium Concentrations in Saturated-Zone Sediments from the IFRC Tracer-Test Well Array in Hanford's 300 Area Uranium Plume

*Session: Hanford IFRC (Principal Investigator: John Zachara)*

Douglas Kent, U. S. Geological Survey, Deborah Stoliker, U.S. Geological Survey, John Zachara, Pacific Northwest National Laboratory

The objective of this research is to characterize the distribution, chemical forms, and reactivity of sediment-bound U(VI) contamination across the Hanford 300 area IFRC field experimental domain. Research to be reported on here focuses on the distribution of sediment-bound U(VI) contamination in the saturated zone of the IFRC tracer test well array (TTWA) and the variation in U(VI) adsorption over the range of groundwater chemical conditions observed across the 300 Area.

Concentrations of adsorbed U are being determined on samples taken from sites in the TTWA where three-well clusters were emplaced. A shallow (near the water table) and deep (near the base of the Hanford formation) sample from two of the three well locations in each cluster are being investigated. Grab samples collected from adjacent depths were composited to obtain a sufficient quantity of the <2 mm size fraction for the characterization work. In addition to these samples, experiments were conducted on sediments (<2 mm size fraction) previously collected at Site C5708, which is the location at which IFRC Well 2-5 was emplaced.

The time scale for U desorption was determined by extracting sediments with a sodium bicarbonate/carbonate buffer at pH 9.1. Time required for desorption was ~1,000 hours, in agreement with the time required for U desorption from deep vadose-zone sediments from the South Process Pond (SPP) determined previously. This time scale is consistent with rate control by intragrain diffusive mass transfer.

Total adsorbed U concentration varied with depth from approximately 1 to 17 nanomoles/g. Peak adsorbed U concentrations were observed in the smear zone. Based on this limited set of samples, there appears to be significant spatial heterogeneity in adsorbed U concentrations in the smear zone over both the minimum and maximum spatial scales represented by this sample set. Adsorbed U concentrations and spatial variability are much lower in samples collected near the base of the Hanford formation. Total U concentrations in the sediments, determined by alpha spectrometry, follow similar vertical and horizontal trends. The adsorbed U concentrations for the four shallow sediments with the highest adsorbed U concentrations comprised 39-44 percent of the total U. For most of the deep sediments, adsorbed U constituted 4-15 percent of the total U.

The concentration of adsorbed U extracted by artificial groundwater is a strong function of alkalinity. Results show that most of the adsorbed U on these sediments can be desorbed as the alkalinity reaches the upper range of values typically observed in 300-area groundwater.

This research is part of the ERSF Hanford IFRC at Pacific Northwest National Laboratory.



## Geochemical Characterization of the Hanford Site 300 Area IFRC Groundwaters and Sediments

*Session: Hanford IFRC (Principal Investigator: John Zachara)*

James P. McKinley, Pacific Northwest National Laboratory (PNNL), Richland, WA, John M. Zachara, PNNL, Richland, WA, Andy L. Ward, PNNL, Richland, WA

The Hanford IFRC is located at a site where persistent residue from reactor fuels fabrication contributes to a groundwater contaminant plume migrating into the Columbia River. The well array provided sediment samples at spacing sufficient to characterize the distribution of reactive phases within the test plot, and groundwater sampling can be adapted to experimental need. The array included 35 wells in a triangular plan. Twenty-six wells were screened (~6 m) through the saturated Hanford Fm. A subset of wells, in three three-well clusters, was completed to discretely sample the upper, middle, and lower few feet of this interval. Sediments were systematically collected from the ground surface to the uppermost Ringold Formation. Within the limitations imposed by drilling results and operations, the sediments were either collected as continuous core (7 wells), or as grab samples taken every 0.6 m: more than 700 samples were collected in total. Characterization measurements include analyses of groundwaters and sediments. Groundwater samples are collected using an automated pump system, and include onsite measurements of conductivity, pH, temperature, oxidation-reduction potential, and dissolved oxygen. Groundwater sampling is conducted monthly or as experimentation permits; samples are analyzed for cation, anion, carbon, and uranium concentrations. Preliminary results showed temporal and lateral variations in groundwater compositions. Sediment samples were catalogued during drilling. For a baseline estimation of reactive properties and distribution, all the grab samples from one well of each three-well cluster were analyzed. Analyses included total U, bicarbonate-extractable (labile) U, single-point U Kd using a synthetic groundwater formulation, NH<sub>4</sub>OH-HCl extractable Fe(III), BET surface area, spectral gamma counting on bulk and size-separated materials, and U isotopic analyses of extraction solutions. Groundwater compositions varied in total dissolved solids over the fall of 2008. For example, from September to December, the long-screen samples (derived from water pumped from the middle of the saturated zone), had Cl and U concentrations that decreased by approximately 25% (U concentrations fell from 50 µg/L to 37 µg/L, while Cl concentrations fell from 24.4 to 18.6 mg/L). Shallow and deep groundwaters each showed declines in U of ca. 20 µg/L (from 70 and 50 µg/L, respectively), but intermediate waters remained approximately constant at 50 µg/L. Labile U and Kd measurements also reflected stratification: bicarbonate-extractable U was highest near the water table and near the bottoms of the sediment column, and the Kd estimated from bicarbonate extractions and sediment washing varied in parallel. These preliminary results suggest that seasonal mixing between groundwater and river water causes significant variation in U concentrations, and that significant stratigraphic variation in reactivity is superimposed on the hydrologic system. This research is part of the ERSP Hanford IFRC at Pacific Northwest National Laboratory.

## Hydrologic Characterization and Results from the First Tracer Experiment at Hanford's 300 Area IFRC Site

*Session: Hanford IFRC (Principal Investigator: John Zachara)*

Mark Rockhold, Pacific Northwest National Laboratory, Vince Vermeul, Pacific Northwest National Laboratory, Chris Murray, Pacific Northwest National Laboratory, John Zachara, Pacific Northwest National Laboratory

An array of 35 instrumented groundwater-monitoring wells was installed in the summer of 2008 within the footprint of a former disposal pond for uranium process wastes in Hanford's 300 Area. Field hydraulic characterization activities that have been performed to date at the site include short-duration, constant-rate injection tests in 14 of the wells, and electromagnetic borehole flow meter (EBF) testing in all 35 wells. The first tracer test at the field site was also performed in November 2008. The average horizontal saturated hydraulic conductivity of the Hanford Fm aquifer at the site is approximately 7000 m/d. Geostatistical analyses of the hydraulic conductivity data indicate horizontal and vertical correlation lengths on the order of 25–30 m and 1–2 m, respectively. Numerical modeling of the first tracer experiment has also been performed using the STOMP simulator. Summaries of the field hydraulic characterization data, geostatistical analyses of these data, and comparisons of observed and simulated plume behavior for the first tracer test are presented. This research is part of the ERSP Hanford IFRC at Pacific Northwest National Laboratory.

## The Method of Anchored Distributions (MAD) for Integration and Inversion of IFRC Hydrogeological Data and for Establishing a Geostatistical Site Model

*Session: Hanford IFRC (Principal Investigator: John Zachara)*

Yoram Rubin, Haruko Murakami, Xingyuan Chen, Hang Bai—  
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This presentation will focus on the efforts undertaken by our team to meet our tasks in this project. We are charged with developing a geostatistical model of the Hanford IFRC's site by integrating the multiple types of information, direct and indirect, local and no-local, that are in the process of being acquired at the site, such as various types of pumping tests, tracer tests, measurements of pressure and hydrogeophysical surveys. Geostatistics is emphasized because it provides a rational approach for modeling uncertainty due to spatial variability and data scarcity. Using multiple sources of information enhances our inverse modeling capabilities by examining the subsurface form multiple perspectives.

The central devices of MAD are a new concept called "anchors" and a succinct classification of all relevant data. Anchors are localized, statistical distributions of the variables of interest (e.g., conductivity) that are conditioned on the indirect and nonlocal information. Ideally, the anchors would capture all the relevant information that is contained in all the available information. For example, anchors can be used to interpret a tracer test and extract from it the information that is relevant for improved characterization of the conductivity field. Or they can be used for joint interpretation of large-scale pump tests and borehole flowmeter tests, again in terms of statistical distributions of conductivities at key locations (hence, anchors). Generally this would require selecting several anchors at the area affecting the tests and identifying the statistical distributions of the conductivity at these anchors, conditional on data. Capturing the relevant information that is encapsulated in complex experiments in terms of a few anchors instead of a detailed spatial distribution of the parameters is where the computational savings are.

The data classification scheme makes MAD modular and extensible by classifying data in an abstract, nondisciplinary way. Data is defined by the way it could be represented by anchors and not by physical relationships. The physical models, in the form of flow, transport, or geophysical numerical models, are integrated into MAD through independent, plug-in modules. Model developers can plug in their codes into the MAD computational platform without having to rewrite an inversion code or to develop new inversion concepts. The significance of this step is in engendering an "open-source" environment. Such an environment is important for broad adoption of MAD, but more importantly, for continuity of its development as a community resource.

In this talk, we will present the MAD concept in detail, and we will demonstrate its application to Hanford-like synthetic studies and initial applications to the IFRC site. This research is part of the ERSP Hanford IFRC at Pacific Northwest National Laboratory.

## An Initial Nonreactive Tracer Experiment at the Hanford 300 Area IFRC Site

*Session: Hanford IFRC (Principal Investigator: John Zachara)*

Vince Vermeul, Brad Fritz, Rob Mackley, Mark Rockhold, John Zachara—  
Pacific Northwest National Laboratory, Richland, WA

The U.S. Department of Energy—Environmental Remediation Sciences Division is supporting an Integrated Field Research Challenge (IFRC) Site at Hanford's 300 Area. This site, immediately adjacent to the Columbia River, is the location of a groundwater uranium plume that resulted from past discharges of liquid effluent to unlined disposal ponds and trenches. An IFRC site has been established in the area of one of the former disposal ponds to address a series of forefront science questions on mass transfer posed to evaluate the effect of spatial heterogeneities; the importance of scale; coupled interactions between biogeochemical, hydrologic, and mass transfer processes; and measurements/approaches needed to characterize and model a mass-transfer dominated contaminant system. As an initial step in the hydrologic characterization of this field research site, a nonreactive tracer experiment was conducted in November 2008. The objectives of this tracer experiment were to assess transport processes and formational heterogeneities present in the saturated zone, to refine the site conceptual model and associated numerical models, and to test operational procedures. A solution containing a conservative tracer (~56 mg/L Br<sup>-</sup>) was injected into a single injection well and tracer arrival was monitored in surrounding wells. A tracer solution volume of ~160,000 gal was injected at a rate of 180 gpm, for a total injection phase duration of ~900 minutes (15 hrs). The test was run for a sufficient duration to fully describe the arrival response at the three closest monitoring wells, at which time the injection was stopped and the tracer plume was allowed to drift under natural gradient conditions. The location of the injected tracer plume within the well field was tracked by sampling selected monitoring wells over time and monitoring with downhole probes. Bromide concentrations were measured using Ion Selective Electrodes (ISE), both in a bench-top flow-through cell for analyzing aqueous samples and *in situ* using downhole ISE probes. Archive samples were also collected and submitted to PNNL laboratories for verification of Br<sup>-</sup> concentration by ion chromatography. Tracer concentrations were monitored for several weeks following the injection until tracer concentrations within the well field had declined to near background levels. The objectives of this initial nonreactive tracer experiment were met, providing for an improved understanding of the formational heterogeneities and flow system characteristics at the IFRC site.

This research is part of the ERSP Hanford IFRC at Pacific Northwest National Laboratory

## Geophysical Characterization of the Hanford IFRC Site

*Session: Hanford IFRC (Principal Investigator: John Zachara)*

Andy Ward, Pacific Northwest National Laboratory, Roelof Versteeg, Idaho National Laboratory, Timothy Johnson, Idaho National Laboratory, Christopher Strickland, Pacific Northwest National Laboratory

Pleistocene-age cataclysmic flood deposits are particularly heterogeneous, and their transport properties can vary over a wide range of spatial scales. An integrated hydrogeophysical investigation surface and borehole geophysical survey is being conducted to classify the dominant sedimentary facies and map architectural elements including possible channels. Prior to installation of the IFRC well field, a surface-geophysical investigation was conducted as part of a preliminary assessment of the extent of contamination, subsurface structure, and the location of conductive lithologic layers. Magnetometer, 2D resistivity, inductive terrain conductivity, ground-penetrating radar, and seismic methods were used to characterize the south process pond and surrounding areas. Magnetometer gradient anomalies were generally small. Terrain conductivity, resistivity, and induced polarization showed a conductive, high-chargeability that was avoided in site selection. Resistivity profiles also showed significant structure with incisions into the conductive Ringold Formation in-filled with resistive sediments. Seismic walkaway tests suggest that common midpoint reflection profiles would be useful for mapping structure and stratigraphy at the site. Twenty-eight of 35 wells at the IFRC site were instrumented to measure temperature and resistivity. Pre-completion borehole neutron and gamma logs show evidence of small-scale facies transitions with three main architectural units, and a strong correlation between K, U, and T and grain-size statistics. Core resistivities also show three dominant facies, with resistivities ranging from 1-2 kohm-m in the Hanford formation to 100 kohm-m in the Ringold. Following well completion, 35 wells were logged with conventional tools, including acoustic televiewer, electromagnetic induction, borehole deviation, crosshole radar, and gross natural gamma activity. Conductivity in the vadose zone was sensitive to the permanent sensors and cannot be used to deconvolve lithologic effects. However, saturated zone conductivity and radar measurements showed significant structure within the architectural elements. Collectively, these data are being used to characterize facies distributions, to constrain resistivity inversions, and to develop correlations with facies abundance. The integrated approach to data collection, analysis, and interpretation allows the site conceptual model to be refined iteratively as additional spatial geophysical data and time-dependent transport data are collected. This research is part of the ERSP SFA at Pacific Northwest National Laboratory.

## Modeling Field-Scale Uranium Mass Transfer at the Hanford IFRC Site

*Session: Hanford IFRC (Principal Investigator: John Zachara)*

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Microscopic and spectroscopic analysis of contaminated sediments, batch and flow-through laboratory experiments, and numerical modeling have collectively implied that the fate and transport of uranium at the Hanford IFRC site is controlled by complex coupling of uranium geochemical reactions and mass-transfer processes at various scales. Our objective is to: (1) incorporate laboratory research results into a field-scale reactive transport model and (2) identify important parameters and processes that control the reactive transport of uranium under field-relevant hydrogeochemical conditions.

A multirate, surface complexation model (SCM) that has been proposed to describe the coupling of uranium geochemical reactions and mass-transfer processes (Liu et al., 2008, *Water Resources Research*) is applied to a selected vertical section across the Hanford IFRC site located in the Hanford 300 Area adjacent to the Columbia River. The model is intended to assess the hypothesized importance of multirate processes on the fate of uranium at the Hanford IFRC site, and to more generally evaluate the effect of variable geochemical conditions caused by dynamic river stage fluctuations. In this presentation, we will discuss: (1) the implementation of the multirate SCM in the existing multicomponent reactive transport simulator PHT3D based on MT3D and PHREEQC-2; (2) the sensitivities of key model parameters in the multirate SCM under field relevant conditions; and (3) pre-experimental scenarios of the fate and transport of uranium at the Hanford IFRC site.

The importance of the multirate mass-transfer processes has been assessed by numerically comparing the multirate SCM against a correspondent equilibrium SCM. In this comparison, a U(VI) bearing solution was introduced as a short-term point source at approximately 150 m from the Columbia River. In both models, river-water intrusion results in complex spatio-temporal variations of the water chemistry and uranyl speciation that generally decreases aqueous uranium concentration and enhances uranium adsorption. In the case of the multirate SCM, U migration is much more dynamic and in sync with the groundwater flow field. Even though the general extent of the U plume does not change significantly after five years, the plume's high concentration zone remains fairly dynamic, while in the simulations with the equilibrium SCM, the plume shows very limited seasonal movement.

Overall, the results from the multirate SCM appear to be more consistent with field observations of dynamic behavior of uranium plumes. The reactive transport simulation results indicate that with both the multirate SCM and equilibrium SCM, the U plume is strongly adsorbed by aquifer sediments. Uranium breakthrough curves at selected observation points and the mass balance (integrated mass versus time) indicate that uranium adsorption/desorption never attains equilibrium, as a result of both the highly dynamic flow field and the chemistry variations caused by the intrusion of river water. Thus, the multirate SCM model appears to be a crucial feature for future reactive transport simulations of uranium at the 300A site.

This research is part of the ERSF Hanford IFRC at Pacific Northwest National Laboratory.

## Research Highlights and Future Directions of the Oak Ridge Integrated Field Research Challenge Project

*Session: Oak Ridge IFRC (Principal Investigator: Philip Jardine)*

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This project is a DOE ERSD funded Integrated Field Challenge (IRC) located on the Oak Ridge Reservation in eastern Tennessee. The project encompasses an integrated multidisciplinary, multi-institutional research program that provides an improved scientific understanding and predictive capability of subsurface contaminant fate and transport at scales ranging from the molecular to the watershed. The Oak Ridge Field Research Facility (ORFRC) is being used by the IFRC as the field laboratory for investigations of contaminant attenuation rates and mechanisms using a combination of geophysical, chemical, microbial, and hydrological analytical tools. The influence of coupled processes on contaminant migration are being quantified along numerous distinct flow pathways and within transition zones throughout the watershed, particularly processes associated with natural attenuation of contaminant nitrate by denitrification and of U/Tc by natural immobilization on soil/rock. These investigations are a unique blend of macroscopic geophysical, isotopic, and hydrogeochemical monitoring observations at the watershed scale, coupled with smaller-scale laboratory studies and high-resolution surface interrogation technologies. The various observation scales are being integrated with ongoing numerical studies to provide an accurate predictive capability for long-term site performance, and to determine if targeted manipulation is required to impede contaminant migration. Pilot-scale manipulations are therefore being tested which include sustained *in situ* bioreduction for U/Tc immobilization, controlled adjustment of soil-groundwater pH to precipitate U/Tc with aluminum hydroxides, and organophosphate and oleate amendments to enhance U/Tc adsorption/precipitation. The influence of spatially and temporally variable groundwater recharge on contaminant fate and transport processes is also being investigated at a variety of scales, since the ORIFRC resides within a climate regime that receives ~1400 mm rainfall/y, of which 50% contributes to groundwater and surface water recharge. The ORIFRC is heavily involved with a variety of outreach activities, including site-specific sample dissemination to DOE investigators, EM and site contractor technology transfer, public information releases via technical and nontechnical presentations and news articles, and peer-reviewed publications. Active participation in the DOE ORR Closure Project Team by the ORIFRC manager and principal investigators will assure remediation planning needs are addressed and technical insights are transferred into DOE remediation efforts. The project will deliver multiscale predictive monitoring and modeling tools that can be used at sites throughout the DOE complex to inform and improve the technical basis for decision making, and to assess which sites are amenable to natural attenuation and which would benefit from source zone remedial intervention.

## Distinctive Shifts in Subsurface Microbial Community Structure Correlate with Uranium Redox Phases during *In Situ* Field Manipulation at the Oak Ridge Integrated Field Research Challenge (OR-IFRC)

Session: Oak Ridge IFRC (Principal Investigator: Philip Jardine)

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Long-term field manipulation experiments investigating the effects of subsurface redox conditions on the fate and transport of soluble uranium(VI) were conducted at the OR-IFRC over a 3-year period. In the highly contaminated Area 3, introduction of ethanol to the subsurface stimulated native denitrifying, sulfate-reducing, iron-reducing, and fermentative microorganisms and reduced U to below 0.03 mg/L. Subsequently, oxygen and nitrate were experimentally re-introduced into the subsurface to examine the potential for re-oxidation and re-mobilization of U(IV). Introduction of oxygen or nitrate caused changes in subsurface geochemistry and re-oxidation of U. After reoxidation, the subsurface experienced several months of starvation conditions before ethanol injection was restored to reduce the treatment zone. Subsurface microorganisms were characterized by community fingerprinting, functional gene arrays (GeoChip), targeted population analyses, and quantitative PCR of key functional groups in 50 samples taken during multiple phases of field manipulation. Statistical analysis confirmed the hypothesis that the microbial community would co-vary with the shifts in the subsurface geochemistry. Multidimensional scaling plots reveal distinctive shifts in microbial community structure during ethanol amendment, nitrate reoxidation, and starvation phases of field manipulation. Principal components analysis further shows that community structure correlates with the accumulation of reductants (Fe[II], sulfide) during ethanol treatment and oxidants (nitrate, U[VI]) during reoxidation. U(VI) concentrations were correlated with elevated nitrate and negatively correlated with ethanol amendments in the absence of nitrate. Ethanol and nitrate+ethanol amendments yielded significant shifts in microbial community structure; however, the effect of ethanol alone was small relative to differences by sampling well and depth. Conversely, nitrate+ethanol treatment and starvation selected for a similar microbial community regardless of sampling location. GeoChip analysis indicated that the functional community was more affected by ethanol and nitrate than reoxidation. Ethanol addition increased the diversity and abundance of functional genes, while nitrate had the opposite effect. All functional gene groups were detected during starvation and reoxidation conditions, indicating a resiliency in the community. The level of hydraulic connectivity of sampling wells to the injection well was readily tracked by microbial community analysis. Overall, the bacterial community was dominated by denitrifying *Betaproteobacteria*, iron-reducing *Acidobacteria* of the genus *Geothrix*, and sulfate-reducing bacteria from the genera *Desulfosporosinus* and *Desulfocapsa*. We demonstrate quantitatively that specific populations, especially *Desulfosporosinus*, are heavily influenced by geochemical conditions and positively correlate with the immobilization of uranium. Following nitrate reoxidation, populations of Fe(II)-oxidizing, nitrate-reducing organisms (*Thiobacillus*) showed an increase in relative abundance.



## Subsurface pH Manipulation and Geochemical Modeling for the Immobilization of Uranium and Technetium

*Session: Oak Ridge IFRC (Principal Investigator: Philip Jardine)*

Baohua Gu, Oak Ridge National Laboratory, Fan Zhang, Oak Ridge National Laboratory, Wensui Luo, Oak Ridge National Laboratory, Ken Kemner, Argonne National Laboratory, Shelly Kelly, Argonne National Laboratory, Wei-min Wu, Stanford University, C. Schadt, Oak Ridge National Laboratory, Joel Kostka, Florida State University, Jizhong Zhou, University of Oklahoma, Brian Spalding, Oak Ridge National Laboratory, David Watson, Oak Ridge National Laboratory, Jack Parker, University of Tennessee, Philip Jardine, Oak Ridge National Laboratory

This research is part of the Integrated Field Challenge (IFRC) Science Focus Area at Oak Ridge National Laboratory. The source zone soil and groundwater (Area 3) at the IFRC site are characterized by a low pH (~3.5) but extremely high contents of U (~60 mg/L), Tc (~5 µg/L), Al (~500 mg/L), nitrate (up to 10,000 mg/L), and toxic metals (e.g., Ni, Cr, Co). Few treatment options exist to effectively remove or immobilize these contaminant metals and radionuclides *in situ*. In this study, we evaluated the feasibility of controlled based addition (or pH manipulation) for immobilizing uranium (as uranyl,  $\text{UO}_2^{2+}$ ) and technetium (as pertechnetate,  $\text{TcO}_4^-$ ) *in situ* through the precipitation or co-precipitation with aluminum and other metal ions in a highly contaminated sediment. In addition, a generic geochemical model based on aqueous complexation, precipitation, sorption and soil buffering with pH-dependent ion exchange was developed to predict aqueous and solid phase concentrations of metals and anions during the batch titration and column flow experiments. Results indicate that the addition of a strong base provided a rapid yet effective means of sequestering U(VI), Tc(VII), and other toxic metals such as Ni(II) and Co(II) in the soil and groundwater. Greater than 94% of soluble U(VI) and >83% of Tc(VII) could be immobilized at pH above 4.5 by the co-precipitation and/or adsorption with Al-oxyhydroxides. The presence of sediment minerals appeared to facilitate the immobilization of these contaminants at lower pH values, possibly through the specific sorption or inner-sphere surface complexation between uranyl/pertechnetate and Al-oxyhydroxides. The immobilized U(VI) and Tc(VII) were found to be stable against leaching by relatively high concentrations of  $\text{Ca}(\text{NO}_3)_2$  solution (up to 50 mM). However, significant amounts of dissolution or desorption of U(VI) and Tc(VII) can occur in the presence of carbonates (50 mM  $\text{KHCO}_3$ ), because of the formation of uranyl-carbonate species and the surface complexation between carbonate and Al-oxyhydroxides. The geochemical model using HydroGeoChem v5.0 did well in predicting the acid-base behavior of the sediment-solution system under variable pH conditions and by treatment of sediment solids as a polyprotic acid or alkali. Comparison of model results with experimental data of major ionic species (e.g., U, Tc, Al, Ni, Co, Ca, Mg,  $\text{SO}_4^-$ ) indicated close agreement. The present study demonstrates the feasibility and a potential remedial option for immobilizing U(VI) and Tc(VII) through the subsurface pH manipulation, particularly for soils and groundwater contaminated with high levels of Al, U, and Tc at a low pH.

## Hydrogeophysical Quantification of Plume-Scale Flow Architecture and Recharge Processes at the Oak Ridge IFRC

*Session: Oak Ridge IFRC (Principal Investigator: Philip Jardine)*

Susan S. Hubbard, Lawrence Berkeley National Laboratory; Gregory S. Baker, University of Tennessee; David Watson, Oak Ridge National Laboratory; Jinsong Chen, Lawrence Berkeley National Laboratory; Michael Kowalsky, Lawrence Berkeley National Laboratory; Erika Gasperikova, Lawrence Berkeley National Laboratory; David Gaines, University of Tennessee; Aubrey Modi, University of Tennessee; Yuxin Wu, Lawrence Berkeley National Laboratory; Philip Jardine, Oak Ridge National Laboratory

We present two joint inversion approaches for integrating multiscale hydrogeological, geochemical, and geophysical datasets to: (1) quantify subsurface architecture that may influence flow at the plume scale, and (2) monitor subsurface processes potentially induced by recharge. We test the approaches using datasets collected across the ORNL DOE Integrated Field Research Center at the ORNL in Tennessee, where large precipitation events may impact contaminant concentration and transport.

Quantification of aquifer architecture was met through development of a Bayesian framework that permits simultaneous inversion of surface geophysical and wellbore datasets, thereby jointly honoring all available (multiscale) datasets and minimizing errors associated with conventional inversion of surface-based geophysical datasets. The developed framework was tested using surface seismic refraction travel time and wellbore-based lithofacies datasets collected at the Oak Ridge site. The analysis indicated that the incorporation of local-scale depth constraints, provided by wellbore datasets in the geophysical inversion procedure, significantly reduced the uncertainty in the estimates of subsurface architecture obtained using surface seismic-refraction datasets. The analysis also delineated the location of a seismic low velocity zone that may serve as a preferential subsurface flowpath along the plume axis.

To meet the second objective, we are using coupled numerical models to explore the sensitivity of time-lapse electrical tomographic (ERT) methods for monitoring recharge-related processes, such as changes in moisture due to infiltration or contaminant dilution. We have developed a coupled numerical framework that co-simulates hydrological and geophysical phenomena, and that permits incorporation of hydrological, geochemical, and geophysical measurements collected at or between wellbores. The modeling is allowing us to distinguish the impact of different recharge-related processes on the geophysical signature and to gain confidence in the use of surface-based ERT measurements for monitoring recharge-related processes across the plume.

Ultimately, we will superimpose our seismic-based architecture quantification with the electrical-based recharge monitoring estimates to gain insights about processes associated with natural episodic, seasonal, and annual recharge along the plume extent.

## Site-Wide Oak Ridge FRC Watershed Monitoring of Contaminant Distribution and Attenuation Processes

*Session: Oak Ridge IFRC (Principal Investigator: Philip Jardine)*

Brian Spalding, Oak Ridge National Laboratory, David Watson, Oak Ridge National Laboratory, Phil Jardine, Oak Ridge National Laboratory, Fan Zhang, Oak Ridge National Laboratory, Guoping Tang, Oak Ridge National Laboratory, Jack Parker, University of Tennessee, Joel E. Kostka, Florida State University, Stefan J. Green, Florida State University, Om Prakash, Florida State University, Wei-Min Wu, Stanford University, Gengxin Zhang, Oak Ridge National Laboratory, Shelly Kelly, Argonne National Laboratory, Tonia Mehlhorn, Oak Ridge National Laboratory, Kenneth Lowe, Oak Ridge National Laboratory, Kenneth Kemner, Argonne National Laboratory, Craig Criddle, Stanford University, Christopher Schadt, Oak Ridge National Laboratory, Wensui Luo, Oak Ridge National Laboratory, Baohua Gu, Oak Ridge National Laboratory, Juske Horita, Oak Ridge National Laboratory, Scott Brooks, Oak Ridge National Laboratory

As part of the ERSP IFRC at the Oak Ridge National Laboratory, an intensive groundwater and stream water sampling campaign was initiated throughout Bear Creek watershed in November 2008, to obtain a spatial distribution of contaminants (uranium, technetium, nitrate, and acidity), aqueous geochemical characteristics, dissolved gases, isotopic abundances of key species, and microbiological characteristics. The objective of this characterization effort was to obtain a current spatial and temporal picture of contaminant distribution as an aid to site-wide modeling tasks, and to provide key correlations with ongoing natural attenuation processes for uranium, nitrate, and acidity originating from the former S3 wastewater impoundments at the head boundary of the watershed. Maps showing current distribution of contaminants and pertinent chemical characteristics, including dissolved gases, are presented. The distribution of denitrifying microbial activities was assessed using molecular biological techniques. Isotopic abundances were measured on dissolved nitrogen and nitrous oxide and on dissolved nitrate. The distribution of groundwater characteristics was used to bound site-wide model and to identify promising locations delineated by geophysical characterization. Selected soil samples were analyzed for speciation of their uranium contamination using x-ray adsorption spectroscopy. In a supporting task to measure the magnitude of natural attenuation of uranium, samples of uranium-contaminated soil were encapsulated in hydrogels and weathered *in situ* in minimally impacted groundwater for over two years. The kinetic “half-life” of uranium retention in six naturally attenuated soils ranged between 0.5 and 1.5 years. The *in situ* hydrogel technique is being employed to evaluate several ongoing manipulation tests to improve uranium immobilization using pH-adjustment, *in situ* bioreduction with slow-release substrate, and solubility control with phosphate amendment.

## Laboratory and *In Situ* Biostimulation of Uranium Reduction and Immobilization Using Long Chain Fatty Acids Containing Organics as Sustained Release Electron Donors at the Oak Ridge IFRC Site

Session: Oak Ridge IFRC (Principal Investigator: Philip Jardine)

W-M Wu and C.S. Criddle, Stanford University; D.B. Watson, T. Mehlhorn, K. Lowe G. Zhang, B. Spalding, S. Brooks, Ch. Schadt, F. Zhang and P. Jardine, Oak Ridge National Laboratory; S. Kelly and K. Kemner, Argonne National Laboratory; Y. Wu and S. Hubbard, Lawrence Berkeley National Laboratory; G. Baker, University of Tennessee

Bioreduction of U(VI) to U(IV) with ethanol as an electron donor was tested over a 4 year period at two locations on the ORIFRC site. Low U concentration (below US EPA MCL of 0.03 mg/L) were achieved by frequent injection of electron donor. To reduce the costs and improve the sustainability for remediation and site maintenance, we are exploring the effectiveness of slowly degrading substrates, such as a Ca-oleate, and a commercial emulsified vegetable oil (SRS) as alternative electron donor sources.

Microcosm bottle tests using site subsurface media (U= 650 mg/kg) and groundwater (pH 6.9; Ca, 2.6 mM; nitrate, 0.5 mM; sulfate, 1.1 mM; bicarbonate, 6.8 mM) were used for the performance evaluation under laboratory conditions. In order to examine the effect of sulfate, one set of microcosms was amended with varying levels of sulfate. Both oleate (a long-chain fatty acid with 18 carbons) and the emulsified vegetable oil (60% oil with 6% biodegradable surfactant) supported nitrate reduction, sulfate reduction, Fe(III) reduction and U(VI) removal from aqueous phase. U concentrations fell from >3.0 mg/L to around 0.1 mg/L within 3 months. The reduction of U(VI) to U(IV) in sediments was confirmed by XANES analysis where after 3 months, the fraction of U(IV) in total U increased to 56, 35, and 56% with ethanol, oleate, and oil, respectively. The Fe(II) and acetate content in sediment also increased after reduction. Acetate was the major intermediate of the degradation of ethanol, oleate, and oil. Results from the various microcosm tests suggested that bioreduction of U with oil can be sustained longer.

Microbial community analysis using 16S rRNA gene clone library analysis indicated that the microcosms amended with ethanol, versus those amended with long chain fatty acids such as Ca-oleate and vegetable oil mixtures (SRS), have strong differences. While a predominance of Fe(III)-reducing and sulfate-reducing Deltaproteobacteria were found all amended sediments compared to controls, genera level composition and diversity patterns varied dramatically. Diversity was greatest in the complex oil substrate (SRS) amendment, followed by Ca-oleate, and finally ethanol. Ethanol stimulated a community dominated by *Clostridia* and *Desulfovibrio* (with *Desulphorhopalus* and *Geobacter* also important). Contrastedly oleate and SRS were dominated by *Desulforegula*, *Syntrophomonas* and *Desulfocapsa* (with *Geobacter* and *Desulphorhopalus* also important). Varying sulfate concentrations tended to shift the relative importance of dominant genera within a substrate type, but to a much lesser extent than substrate type (e.g., ethanol versus fatty acids). Archaeal libraries also showed methanogens tended to decrease with sulfate level. The increased complexity observed with fatty acid substrate types (SRS and oleate) combined with our knowledge of the physiology of cultured representatives of dominant genera, such as *Syntrophomonas* and *Desulforegula*, suggest that more complex syntrophically based relationships may be important in determining community structure and reduction outcomes than previous efforts with ethanol and other simple donor substrates.

Based on the results of microcosm tests, we identified the emulsified vegetable oil (SRS) for our first *in situ* bioreduction experiments. Laboratory batch and flow-through column experiments were carried out to investigate the sensitivity of geophysical method to SRS injection. Prior to the injection of SRS, groundwater samples were taken from more 50 wells for background geochemistry survey, microbial samples of groundwater and sediment were collected from selected wells, and site hydrology was characterized by bromide tracer test and surface electrical methods. The field stimulation test has been started at the ORIFRC site On February 9, 2009, diluted SRS solution (20% concentration, 900 gal volume) was injected into three injection wells within 1.5 hours. The distribution of SRS injected and biogeochemical process has been monitored since the SRS injection through analysis of well fluid samples and time-lapse surface electrical methods. Primary results showed that bioactivities of denitrification, Fe(III) reduction and sulfate-reduction occurred in several monitoring wells one week after the injection, much faster than microcosm tests. Long-term monitoring is in place to track the continuing dynamics and reduction duration in this test area.

## Multiprocess and Multiscale Modeling and Data Analysis at IFRC Site, Oak Ridge, Tennessee

*Session: Oak Ridge IFRC (Principal Investigator: Philip Jardine)*

Fan Zhang, Guoping Tang—Oak Ridge National Laboratory (ORNL); Jack Parker, University of Tennessee; Jian Luo, Georgia Institute of Technology; Wei-Min Wu, Stanford University; Gengxin Zhang, Oak Ridge National Laboratory; Shelly Kelly, Argonne National Laboratory; Tonia Mehlhorn, Jack Carley—ORNL; Kenneth Kemner, Argonne National Laboratory; Craig Criddle, Stanford University; Christopher Schadt, Wensui Luo, Baohua Gu, Brian Spalding, Scott Brooks, David Watson, Philip Jardine—ORNL

Multiprocess and multiscale modeling and data analysis of the former S-3 Ponds waste disposal site on the Oak Ridge Reservation have been performed to develop a practical model, to simulate the biogeochemical processes that control contaminant uranium mobility and (ultimately) to predict remediation of the geochemically complex site. The computer code HydroGeoChem v5.0 is a comprehensive model for fluid flow, thermal, and reactive transport. HydroGeoChem was coupled with the nonlinear inversion code PEST and used as our primary modeling tool.

Uranium containing precipitates have been observed in a dolomitic gravel fill as a result of exposure to acidic groundwater from the underlying saprolite contaminated with U, Al,  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$ . Over 80 sampled regions show that uranyl carbonates are present with amorphous basaluminite as coatings on the gravel and as individual precipitates in the fine material. An equilibrium precipitation/dissolution reaction model was developed to simulate the groundwater geochemical composition and U removal resulting from exposure to high pH carbonate gravel. To investigate the feasibility of U(VI) reduction with a slowly degrading electron donor, microcosm tests using uranium (VI) contaminated sediments and groundwater amended with calcium oleate were performed under anaerobic, ambient temperature conditions in the presence of sulfate. Oleate was degraded by indigenous microorganisms with acetate as a major product. A kinetic bioreaction model was developed to simulate the rapid removal of U(VI) from the aqueous phase associated with oleate degradation, acetate production, and sulfate reduction. Local-scale models were developed for Area 2 and Area 3 to facilitate interpretation of flow and tracer studies and to investigate the impacts of aquifer heterogeneities and nonequilibrium mass transfer on degradation rates of contaminants. Reaction models developed through simulation of batch, column, and field experiments will be incorporated into the local-scale models to help interpret future on site investigation.

Long computational times and high spatial and temporal variability have been constraining our ability to predict site-wide contaminant transport in the field. We have made significant progress in reducing computational time using parallel computers and are addressing observation variability in calibrating the site-wide transport model for the OR IFRC site. Parallelization of HydroGeoChem using OpenMP, use of a parallel version of PEST, minimization of the model domain to focus on the dissolved plume, and use of 10 to 20 computer nodes with two or four processors on each node for model calibration has reduced computational time from months to days. With an equivalent single porosity model, we are able to capture major spatial and temporal trends in historical nitrate concentration observations. We are further improving the efficiency of HydroGeoChem and Parallel PEST with MPI, using a dual porosity model to better describe the nonequilibrium mass-transfer processes, and increasing the resolution to incorporate variability in the smaller-scale processes. We are moving toward development of a multiscale, multiprocess numerical model for the prediction of contaminant migration in complex field-scale environments.

## ***In Situ* Bioreduction of Uranium in an Alluvial Aquifer: Overview of Results from the Integrated Field-Scale Subsurface Research Challenge Site (IFRC) at Rifle, Colorado**

*Session: Rifle IFRC (Principal Investigator: Philip Long)*

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The U.S. Department of Energy's IFRC at Rifle, Colorado, conducts multiscale, multidisciplinary research on uranium bioremediation in naturally complex subsurface environments. The overarching goal of the project is to develop a mechanistic understanding of the physical, chemical, and biological processes and properties controlling uranium mobility in these settings. Key areas of study include (a) spatially variable material properties that can evolve during biostimulation, (b) geochemical and microbial controls on stimulated U(VI) bioreduction by iron-reducing microorganisms, (c) U(VI) sorption under iron- and sulfate-reducing conditions, (d) post-biostimulation uranium stability and removal from groundwater, and (e) rates of natural bioreduction of U(VI). The Rifle IFRC has completed three of seven planned field experiments, including two that used novel proteogenomic and stable isotope probing techniques to track the metabolic status of microorganisms stimulated by acetate addition and involved in the removal of U(VI) from groundwater. Results from the acetate amendment field experiments demonstrate the ability to rapidly remove U(VI) from groundwater to levels at or below the maximum contaminant level (0.13  $\mu\text{M}$ ) under high alkalinity conditions (8 meq/L) and in the presence of elevated calcium (6 mM). Enzymatic reduction of U(VI) initially occurs when Fe(III) is the principal terminal electron acceptor and the subsurface is dominated by *Geobacter* sp. However, results from 2008 confirmed the importance of achieving sulfate reduction for continued removal of U(VI) from influent groundwater after acetate amendment is stopped. A third field experiment conducted in 2008 under conditions of increased alkalinity in the absence of biostimulation resulted in desorption of U(VI) from the sediments, enabling us to enhance our predictions of sorption behavior under ambient field conditions at the Rifle site. In 2009, we will assess the effect of abiotic reducing conditions (Fe(II) amendment) on U(VI) sorption and, in a separate experiment, determine if limited additional acetate can be correlated with enhanced U(VI) removal. The project will then be in a position to combine bioreduction and desorption experiments in the field under Fe(III)-reducing and then sulfate-reducing conditions. Results should enable us to accurately combine surface complexation models (SCM) and bioreduction in reactive transport models across a range of subsurface hydrological, geochemical, and microbiological conditions.

## Uranium Desorption from Contaminated Sediments at the IFRC Site in Rifle, CO: From Batch to Field

*Session: Rifle IFRC (Principal Investigator: Philip Long)*

Patricia M. Fox, U.S. Geological Survey, Sung Pil Hyun, University of Michigan, James A. Davis, U.S. Geological Survey, Kate Campbell, U.S. Geological Survey, Kim F. Hayes, University of Michigan, Richard Dayvault, SM Stoller, Aaron Peacock, Haley Aldrich, Mark Conrad, Lawrence Berkeley National Laboratory, Kenneth H. Williams, Lawrence Berkeley National Laboratory, Philip E. Long, Pacific Northwest National Laboratory

Uranium contamination in the subsurface is a part of the legacy of nuclear weapons and energy production, resulting from both mining activities and nuclear waste disposal. Integrating biological, geochemical, and hydrological studies, the Rifle IFRC project focuses on gaining a better mechanistic understanding of U mobility in the subsurface and the use of bioremediation to achieve groundwater U concentrations below the MCL at a former U mill tailings site. As a part of this project, we are initially investigating U(VI) sorption-desorption and transport behavior under oxidizing conditions at this site.

To obtain parameters needed for field-scale modeling, a series of U(VI) adsorption and desorption experiments with aquifer sediments were conducted in batch and column reactors. Using the batch adsorption and desorption data, a surface-complexation model was developed to describe U(VI) desorption and transport behavior under high alkalinity conditions in a column experiment with the same sediments. Results from the column experiment indicate that U(VI) desorption is kinetically limited at flow rates corresponding to typical groundwater velocities in the field, and that calcite precipitation has a large effect on the predicted U(VI) desorption.

An array of multilevel samplers (MLS) was installed at the Rifle field site to monitor U(VI) transport over 3D spatial and temporal scales. Sediment core sample analyses, nonreactive tracer tests, and biogeochemical groundwater sampling indicate a high degree of geochemical, hydrological, and biological heterogeneity. To assess uranium desorption behavior at the field scale, a U(VI) desorption tracer test was performed in which 1000 L of water, amended with 40 mM NaHCO<sub>3</sub> along with Br and D<sub>2</sub>O as conservative tracers, was injected into two adjacent wells at three different elevation intervals. Breakthrough data collected from downgradient MLS demonstrated the existence of a slow-moving zone (0.15 m/day) in the shallowest region of the aquifer (21 ft bgs) and a faster-moving zone (0.5 m/day) located deeper in the aquifer (25 ft bgs). These zones correlated with finer-grained sediments and a sandy layer, respectively, observed in core samples. U(VI) desorption from the sediments was greater in the shallow zone of the aquifer and increased with transport distance in both the fast and slow-moving zones. These results are being used to assess the impact of field-scale heterogeneities on U reactive transport in contaminated aquifers. Experiments investigating U(VI) sorption, abiotic reduction, and transport under reducing conditions are under way in order to understand U(VI) behavior over a wide range of geochemical conditions.

## Microbial Growth, Activity, and Succession in Response to Electron Donor Amendment at the Rifle IFRC: II. Results from Column and Batch Experiments

Session: Rifle IFRC (Principal Investigator: Philip Long)

Peter Jaffe, Princeton University, Lee Kerkhof, Rutgers University, Ravi Kukkadapu, Pacific Northwest National Laboratory, Lora McGuinness, Rutgers University, Hee Sun Moon, Princeton University, Lucie N'Guessan, Pacific Northwest National Laboratory, Aaron Peacock, Microbial Insights, Nikolla Qafoku, Pacific Northwest National Laboratory, Mike Wilkins, Lawrence Berkeley National Laboratory, Kenneth Williams, Lawrence Berkeley National Laboratory, Philip Long, Pacific Northwest National Laboratory

Column and batch experiments were conducted with Rifle sediments and groundwater to help interpret results of ongoing field experiments and design new ones. Experiments have focused on: (1) the effect of iron addition and limiting concentrations of acetate on the transition to sulfate reduction and efficiency of uranium reduction; (2) the effect of microbial composition on long-term uranium removal; and (3) post-stimulation dynamics of the microbial community.

(1) Column experiments were conducted to study the link between bioavailable Fe(III) and the switch from iron-dominated to sulfate-dominated reducing conditions, and its effect on the bioreduction of uranium. Fe-57 goethite (0.1 wt%) was amended to half of the columns to track goethite biotransformation via Mössbauer spectroscopy and to study the effect of Fe(III) amendment on the onset of sulfate-reducing conditions and the microbial population dynamics. Columns were sacrificed at regular intervals and analyzed for their geochemistry and biomass composition. Results showed that U(VI) reduction was not negatively affected by the shift to sulfate reduction, and that the addition of Fe(III) did not affect timing for the onset of sulfate reduction, but suppressed the amount of sulfate reduced and affected the microbial community composition. Microbial population dynamics were tracked by TRFLP profiling of 16S rRNA genes. Active microbes were determined by RT-PCR/TRFLP. *Geobacter*-like species comprised 0-60%, *Dechloromonas* and *Rhodoferrax*-like species accounted for 2-25% of the total profile. Comparison of the RT-PCR profiles from inflow and outflow indicated the *Dechloromonas* and *Rhodoferrax*-like groups were more active during days 10-80, while the *Geobacter*-like group was more active during days 80-100. Column experiments with acetate added at limiting conditions (0.5  $\mu$ M) found a significant delay in the transition from iron-dominated to sulfate-dominated reducing conditions as compared to columns run at higher (3 to 5 mM) acetate concentration, with the transition occurring after 40 and 200 days, respectively. While these results suggest that some fraction of the total pool of Fe(III) minerals is bioavailable over extended time scales, suppression of low redox conditions in the low-acetate column inhibited the overall extent of uranium removal in this column as compared to the high-acetate column.

(2) The contribution of microbial community to the continued removal of uranium was investigated in two types of reduced sediment. The first sediment sample (S1) was collected within a plot that had been biostimulated for several months in 2007 and 2008. The second sediment sample (S2) was collected outside of any previously treated area. 16S-rRNA based microbial community analysis, phospholipid fatty acid analysis, and estimates of the microbial population using quantitative PCR showed that the microbial community composition of the sediment samples differed greatly. For example, the percentage the *Geobacter*-like group was greater in S1 than in S2. Batch incubations revealed that the sediment collected from S1 was able to remove uranium from groundwater, while S2 did not. Columns with S1 and S2 sediment suggested that the activity of iron-reducing bacteria is longer lasting than previously anticipated. Results from this investigation suggest that the presence and/or activity of an optimal microbial population might be essential to the continued removal of uranium from the groundwater.

(3) Rifle sediment was reduced via acetate injection in laboratory columns for 100 days, after which acetate amendment was discontinued and columns were sacrificed at regular intervals over 70 days to track post-stimulation changes in the geochemistry and biomass. Results showed a small remobilization of uranium during the first 30 days. The qPCR data showed that the total biomass (including *Geobacter* and *dsr* gene) remained stable for the first 50 days, after which the total biomass declined rapidly, and no *dsr* genes or *Geobacter* were detected in the sediment after that time. This was also observed for the effluent, where only total bacteria were detected (from  $10^7$  to  $10^5$  cells/ml), but no *Geobacter* and *dsr* gene.



## Sedimentology and Fe-Mineralogy of the Rifle IFRC Alluvial Sediments: Implications for *In situ* Bioremediation and Long-term Behavior of U(VI)

*Session: Rifle IFRC (Principal Investigator: Philip Long)*

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Characterization of the sedimentology and reactive transport properties of sediments, especially their Fe-mineralogy, is critical for understanding the processes that will control bioreduction of contaminant uranium in alluvial aquifers. The Rifle IFRC provides an excellent opportunity to examine the interaction between uranium and sediments that are heterogeneous in grain size, mineralogy, and redox status.

*Sedimentology:* We analyzed geologic borehole log data from the Winchester/Big Rusty gallery, classifying the descriptions into 3 sedimentary facies: Sandy gravel, muddy gravel, and fines. More than 95% of the sediment in the gallery were gravel, with twice as much muddy gravel as sandy gravel. Geologic log data were also used to identify portions of the gallery where reduced sediments were present. Geostatistical methods were used to analyze the spatial continuity of the sediment types, and to produce block diagrams and maps of the sedimentary facies distribution.

To provide field-scale estimates of the physical and geochemical properties associated with the lithofacies, we are developing property-transfer models between grain-size distributions and properties important for uranium reactive transport. To develop those models, we have separated Rifle sediment into size fractions for analysis of porosity, hydraulic conductivity, particle shape and density, cation exchange capacity, surface area, gamma energy analysis, and extractable iron geochemistry. Functions developed using gamma energy analysis data will enable prediction of grain size from spectral gamma borehole logs. Once grain-size distributions can be estimated from borehole log data, the property transfer models will be used to predict physical and geochemical properties from the estimated grain-size distributions.

*Fe-mineralogy:* We analyzed samples obtained under various biogeochemical conditions to gain insights into Fe-mineral (bio)transformation and its implications on U(VI) removal from groundwater: (a) back-ground/prebiostimulated, (b) biostimulated, (c) naturally bioreduced, and (d) column samples. In all the samples, Fe-oxides (Al-goethite, hematite, and magnetite) and Fe-clays (clinochlore and smectite) were dominant Fe-minerals. Pyrite (FeS<sub>2</sub>) was evident in XRD of some naturally bioreduced sediments.

In some naturally bioreduced sediments (Type A; Winchester gallery), U was primarily associated with a polycrystalline magnetite-like mineral (up to 200 ppm) and framboidal pyrite (up to 120 ppm). Almost the entire reduced sulfur (TRIS) was associated with Fe. Uranium amounts on framboidal pyrite, however, were inhomogeneous, apparently related to weathered state and microcrystal aggregation in the framboid. U existed both as U(VI) and U(IV), based on U-XANES of Fe-rich regions of a polished thin-section. The mixed U speciation may be due to the mineral phase (magnetite versus pyrite), the framboidal characteristics, or the age of the mineral-U aggregate.

Bioavailable Fe(III), TRIS, and bicarbonate extractable U contents of the biostimulated (to sulfate-reducing conditions Big Rusty gallery) and a different type of naturally bioreduced sediment (Type B; La Quinta sediment) were similar. In these samples, Fe-sulfides were not as dominant as in the Type A sediment. Also, unlike in the Type A sediments, only a fraction of the TRIS was associated with Fe; pyrites and other Fe-sulfides were rare. The amounts of TRIS were an order of magnitude less than in the Type A sediments. A new, yet-to-be-identified Fe-phase was evident from Mössbauer. The nature and role of this Fe-mineral phase in U sorption are under investigation.

## Microbial Growth, Activity, and Succession in Response to Electron Donor Amendment at the Rifle IFRC: I. Results from Field Experiments

Session: Rifle IFRC (Principal Investigator: Philip Long)

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Research activities at the Integrated Field Research Challenge site in Rifle, CO, have demonstrated that uranium can successfully be removed from groundwater to levels far below the maximum contaminant level set by the U.S. Environmental Protection Agency. During two successive summer experiments, Winchester (2007) and Big Rusty (2008), 5 to 25 mM acetate were added to the subsurface over a total period of ~160 days to stimulate the activity of dissimilatory metal-reducing bacteria able to reductively precipitate uranium and/or create conditions favorable for the sequestration of the radionuclide. As observed and reported in previous field experiments, the addition of acetate to the subsurface resulted in a succession of bio-geochemical changes broadly characterized by an iron-reduction phase, a transitional period, and a sulfate-reduction phase. The Winchester experiment was designed to elucidate the mechanisms of uranium sequestration under iron-reducing conditions, while microbial processes contributing to the removal of uranium from groundwater under the transitional and sulfate-reduction phases were the focus of the Big Rusty experiment. Several molecular-analysis approaches were used to distinguish between the different phases of biostimulation initiated by the addition of acetate to the subsurface.

Phospholipid fatty acid analysis (PLFA) as well as quantitative-PCR using primers specific to different groups of bacteria showed that the overall groundwater microbial population increased over the first 15 days of biostimulation, but remained stable thereafter. Terminal restriction fragment length polymorphism (TRFLP) of 16S rRNA genes, used to fingerprint the groundwater bacterial populations during the Winchester experiment, demonstrated that *Geobacter* sp., as well as *Geobacter*-like strains, dominated the overall community profile in groundwater under iron-reducing conditions. Furthermore, a stable isotope probing (SIP) experiment was initiated and ascertained that the groundwater *Geobacter* and *Geobacter*-like organisms were actively utilizing acetate to replicate their genome. Similarly, SIP incubations with sediment collected within the biostimulation plot suggested that there was a shift of bacteria from the sediment to the aqueous phase during biostimulation.

During the Big Rusty experiment, a microarray-based environmental diagnostic system was developed to monitor microbial community structure and dynamics in the groundwater on the field. The v2.0 Bioremediation TruArray™ containing four replicates of 150 thermodynamically balanced probes targeting 35 genera of metal-, sulfate- and nitrate-reducers, dechlorinators and fermenters was used to analyze samples in the field. Probes targeting *Geobacter* were the most reactive during the Fe-reduction phase, while those targeting *Desulfobacter* and *Desulfitobacterium* were the most reactive during the transitional phase and into sulfate reduction, with a consistent *Desulfotomaculum* signature throughout the field experiment and a general decrease in *Geobacter* S/N ratios. These corroborated in-field screening of SRB that utilized 16S-based primers specific for different groups of SRB and demonstrated that the microbial community composition of the groundwater during sulfate-reduction is much more diverse than during iron-reduction, and the community of SRB is complex.

Additionally, gene transcripts levels were quantified for *Geobacter*-specific citrate synthase (*gltA*). Results from this study suggested that *Geobacter* sp. were still active during sulfate reduction when the system was dominated by SRB. This finding implies that during the sulfate-reduction phase of biostimulation, dissimilatory metal-reducing bacteria such as *Geobacter* continue to contribute to the reductive precipitation of uranium.

## Estimating the *In Situ* Rates of Natural Bioreduction of U(VI) at the Rifle Integrated Field-Scale Subsurface Research Challenge (IFRC) Site

Session: Rifle IFRC (Principal Investigator: Philip Long)

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Understanding where to use active remedial technologies, and where passive or natural systems can be relied upon for remediation of uranium plumes, requires knowledge of the subsurface natural attenuation capacity. An important component of understanding the natural attenuation capacity of a site is to quantify the extent and rate of natural uranium bioreduction occurring indigenously.

One feature of the Rifle IFRC site is naturally occurring zones of bioreduction. The effect of these zones on the fate and transport of uranium in the subsurface is likely key to accurate prediction of natural attenuation of U in alluvial aquifers with high organic matter. During the summer of 2008, geophysical surveys were conducted to locate zones of natural bioreduction at Rifle. In the fall of 2008, exploratory boreholes were drilled in three of the most promising areas identified by the geophysical surveys, and a total of five wells were installed. The location of the wells was largely successful, in that each of the areas did contain bioreduced zones. Sediment from well LQ-107 was chosen for initial characterization due to apparent sulfide precipitates. Geochemical results showed the total Fe content of sediment from the 20 ft depth was 10.5  $\mu\text{mol/g}$ , Fe(II) was 8.28  $\mu\text{mol/g}$  and total U was 3.36  $\mu\text{grams/g}$ . The amount of total reduced inorganic sulfur (TRIS) extracted from LQ-107 at the 20 ft depth was 6.85  $\mu\text{mol/g}$  and was similar to that extracted from biostimulated sediments after prolonged (100+ day) acetate amendment. We infer that the TRIS from LQ-107 is the result of natural microbial activity, which was at least partially responsible for the observed geophysical anomalies that led to selection of the well location. Microbial quantitative polymerase chain reaction (qPCR) analysis targeting total *Eubacteria* provided an estimate of  $10^6$  cells/g at the 20 ft depth and  $10^4$  cells/g of  $\Delta$ -proteobacteria. Previous results from another bioreduced zone at Rifle contained a significant amount of solid phase carbon, even relative to the amounts utilized during active biosimulation of iron and sulfate reducing bacteria. The high carbon fractions in the natural bioreduced zones may have the potential to maintain long-term reducing conditions and thus uranium stability. Analysis of sediments recovered from other such locations is currently under way.

An immediate question about natural bioreduction zones is their possible role as net sources or sinks for uranium and other compounds. A thorough understanding of how these zones impact the overall uranium flux at a site will be critical to the decision-making process and management of these plumes. As a first step in this process, we are deploying passive flux meters (PFMs) in the natural bioreduction zones. The PFMs will provide both the water and uranium flux *in situ*. We also plan other reactive and nonreactive tracer tests to ascertain the actual rates of bioreduction in these zones. Another related part of our science effort includes the detailed characterization of background microbial communities at the Rifle site. During this year's field experiment, changes in the microbial community profile of background wells were studied. Results suggested that the microbial community composition may be affected by seasonal fluctuations in the groundwater table typified by changes in organic carbon content, dissolved oxygen, and other parameters. We have also identified several types of bacteria in the background samples, including those known for nitrate reduction (*Dechloromonas* spp.), Fe reduction (*Geobacter* spp. and *Rhodoferax* spp.), and sulfate reduction (*Desulfotomaculum* spp. and *Desulfovibrio* spp.). Understanding the role these bacteria may play in the natural bioreduction of U at Rifle is a focus of future research.

## Evolving Geochemical Conditions Drive Changes in *Geobacter* Physiology during Stimulated Early-Stage Uranium Bioremediation

Session: Rifle IFRC (Principal Investigator: Philip Long)

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Uranium bioremediation within the acetate-stimulated Rifle aquifer is sustained throughout a transition from Fe(III)- to sulfate-reducing conditions. Analyses to date of the Fe(III)-reducing proteome demonstrated metabolic shifts as geochemical conditions evolve during the ongoing reduction of U(VI) to insoluble U(IV). Three planktonic *Geobacter*-dominated communities were recovered from the U-contaminated aquifer during the 2007 *in situ* acetate amendment experiment. The samples were taken over a period of stimulated Fe(III) reduction. Over 2,500 proteins were identified per sample by matching LC MS/MS spectra to peptides predicted from seven isolate *Geobacter* genomes. Genome-specific peptides indicate early proliferation of multiple M21 and *G. bemidjiensis*-like strains and later dominance by M21 and *G. bemidjiensis*-like strains more closely related to *G. lovleyi*. Throughout biostimulation, the proteome is dominated by enzymes that convert acetate to acetyl-CoA and pyruvate for central metabolism. Later in bioreduction, decreasing abundances of ribosomal proteins, ATP synthase, and phosphate and nitrogen acquisition proteins indicate slowing biomass production, whereas increases in TCA cycle proteins and flagellin suggest a transition toward attachment, possibly driven by dependence on less readily reducible iron oxides. Changes in U(VI) groundwater concentrations may be associated with both the community and physiological shifts that occur over the period of microbial Fe(III) reduction. Results demonstrate that metagenomics-independent proteomics can resolve population physiology and strain composition of subsurface communities. Research currently under way aims to employ proteomics coupled directly with metagenomics (in the absence of sufficient pure-culture genomic data) to explore the dynamic functions of sulfate-reducing consortia dominating during later stage bioremediation.

## Induced Polarization and Biosensor Studies at the IFRC Site in Rifle, CO

Session: Rifle IFRC (Principal Investigator: Philip Long)

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Research activities at the IFRC site in Rifle, Colorado, are designed to integrate biological, geochemical, and hydrological studies to enhance our understanding of subsurface uranium mobility associated with both natural and stimulated biogeochemical processes. As part of this effort, we have developed and field-tested a variety of novel sensor-based analytical approaches. First, we have used the surface spectral induced polarization (SSIP) geophysical technique to characterize the distribution of zones of natural bioreduction across a portion of the Rifle floodplain and to estimate variations in hydraulic conductivity. This effort extends ongoing work using SSIP measurements to monitor biogeochemical changes accompanying acetate amendment performed under the LBNL SFA research program. Spatial variations in the phase response between imposed electric current and resultant electric field were found to correlate with changes in mineralogy and pore fluid composition associated with the accumulation of reduced chemical species (e.g., Fe(II) and bisulfide) and mineral precipitates (e.g., FeS) accompanying natural microbial activity. By altering the flow of electrical charge, the precipitates and electroactive ions generated sufficiently large phase anomalies ( $<15$  mrad) to allow for the identification of drilling targets subsequently found to contain elevated levels of microbial biomass, reduced inorganic sulfur, and uranium. The SSIP technique was also used to estimate values of saturated hydraulic conductivity via existing empirical relationships between complex conductivity and permeability. Although non-site specific, these relationships yielded permeability estimates in excellent agreement with values obtained using both slug tests and borehole flow meter data. Second, we have developed sensor platforms capable of (a) monitoring the *in situ* activity of *Geobacter* strains during stimulated bioremediation and (b) rapidly quantifying levels of uranium removal. For the former, anodic graphite electrodes were installed at multiple depths in boreholes downgradient from a region of acetate injection; cathodes consisted of graphite electrodes embedded at the ground surface. Increases in current density ( $\leq 50$  mA/m<sup>2</sup>) resulting from electron transfer by anodophilic microbes tracked the delivery of acetate and the period of optimal uranium removal, with vertical variations in current flow reflecting aquifer heterogeneity. Changes in current density were not observed for an upgradient control. Cessation of acetate addition resulted in a rapid decrease in current density; however, current flow was maintained for extended periods of time (100+ days) thereafter. Confocal microscopy of an electrode revealed a firmly affixed biofilm, with community analysis detecting sequences dominated by *Geobacter* strains (ca. 70%). Field-testing of two uranium-selective sensors was also completed along with refinement of the methodological approach. The sensors utilized a fluorescent monoclonal antibody capable of binding a  $\text{UO}_2^{2+}$ -dicarboxyphenanthroline complex in a kinetic exclusion immunoassay format. After calibration, changes in fluorescence resulting from binding of the antibody to the uranium-chelate complex successfully quantified decreases in uranium concentration accompanying acetate amendment. Decreases calculated using the immunosensors (ca. 1  $\mu\text{M}$  to  $<0.13$   $\mu\text{M}$ ) were corroborated by kinetic phosphorescence analysis of duplicate samples, with the two methods exhibiting excellent agreement ( $R^2=0.88$ ).

## Reactive Transport Modeling of *In Situ* Biostimulation Experiments at the Rifle IFRC: Progress to Date and Remaining Knowledge Gaps

Session: Rifle IFRC (Principal Investigator: Philip Long)

Steve Yabusaki, Pacific Northwest National Laboratory, Yilin Fang, Pacific Northwest National Laboratory, Jim Davis, U.S. Geological Survey, Li Li, Lawrence Berkeley National Laboratory, Carl Steefel, Lawrence Berkeley National Laboratory, Philip Long, Pacific Northwest National Laboratory

The reliable engineering of microbially mediated uranium immobilization under site-specific subsurface conditions will require a predictive and quantitative understanding of the principal processes and properties controlling uranium biogeochemistry. Here, we describe the conceptualization of reactive transport process models for the Rifle IFRC, evolution to the current modeling capability, and principal knowledge gaps that are being addressed by ongoing field and laboratory studies.

The initial conceptualization of the biogeochemistry of uranium bioremediation was founded on laboratory studies published in the early 1990s that identified acetate-oxidizing Fe(III)-reducing microorganisms that could concomitantly reduce aqueous U(VI) to insoluble U(IV). Biostimulation experiments in 2002 and 2003 at Rifle confirmed that the growth of acetate-oxidizing Fe(III)-reducers dominated by *Geobacter* sp., was accompanied by significant uranium removal from groundwater. An important feature of these two field experiments was the eventual onset of sulfate reduction, which was characterized by a decrease in aqueous sulfate, near complete consumption of acetate, and less efficient U(VI) removal from groundwater. Thus, the earliest biogeochemical reactive transport simulations were based on Fe(III)-reducing microorganisms stimulated by acetate that simultaneously used Fe(III) mineral and aqueous U(VI) as terminal electron acceptors, followed by the onset of acetate-oxidizing sulfate-reducing microorganisms that were not generally associated with significant U(VI) reduction. A reaction network based on reactions and rate laws from the literature was successfully calibrated to simulate the observed changes in the Fe(III), U(VI) and sulfate terminal electron acceptors during acetate biostimulation.

A key observation from field experiments at the Rifle IFRC was that longer-term, post-biostimulation U(VI) removal from groundwater was associated with longer periods of sulfate reduction. This paradox led to the inclusion of abiotic chemistry for the bioreduced U(IV), Fe(II), and sulfide products of the principal TEAPs, to address their potential roles in long-term uranium immobilization. Under unamended field conditions, surface complexation is considered the dominant abiotic uranium attenuation mechanism because the U(VI) mineral phases are generally undersaturated with respect to the groundwater chemistry at the Rifle site. During and after biostimulation, however, there are significant changes to uranium and the major ions, pH, alkalinity, redox potential, and biofilms that could have a significant impact on uranium surface complexation. Thus, the fate of sorbed U(VI) during and after acetate biostimulation is an open issue that continues to be investigated.

Recent Rifle IFRC laboratory and field experiments in 2007 to 2008 are providing improved conceptualizations of the processes controlling uranium behavior, as well as confirming or refuting process modeling assumptions. For example: (1) we now include phyllosilicate Fe(III) as a distinct terminal electron acceptor pool for the acetate-oxidizing iron-reducing microorganisms; (2) additional evidence from gene expression and proteomics support the assumption of Fe(III)-reducers remaining active during sulfate reduction; and (3) we confirm that lower acetate concentrations delay the transition from dominantly Fe(III)-reducing to dominantly sulfate-reducing conditions. Other ongoing experimental studies are focusing on post-biostimulation conceptual process models, including the long-term stability of biogenic uraninite in reduced sediments and the effect of biomass and secondary mineral formation on long-term removal of uranium from groundwater.

## Scientific Focus Areas (SFA)





## The Argonne Subsurface Science Program Scientific Focus Area

*Session: ANL SFA (Laboratory Research Manager: Carol S. Giometti)*

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The Argonne Subsurface Scientific Focus Area (SFA) integrates synchrotron-based biogeochemistry with microbiology, molecular biology, and protein biochemistry to pursue the long-term scientific goal of elucidating the interplay, at the molecular level, between specific microbial metabolic activities, solution chemistry, and mineralogy contributing to the transformations of contaminants at DOE field sites. Hypotheses directed toward achieving this goal are tested by experiments that capitalize on unique Argonne capabilities, together with key collaborative efforts at Oak Ridge National Laboratory (ORNL), Pacific Northwest National Laboratory (PNNL), Michigan State University, University of Iowa, University of Illinois Urbana Champaign, Stanford University, Hamilton College, Virginia Commonwealth University, and the Illinois Institute of Technology. The objective of the ANL SFA is to characterize coupled biotic and abiotic, molecular-scale contaminant transformations, integrated over different length scales, to provide knowledge that is necessary for predicting contaminant reactivity and transport processes. This objective drives the development and optimization of synchrotron methods for measurements at the molecular level pertinent to understanding contaminant transformations in subsurface environments. Two scientific themes drive the development of hypotheses. These themes are: (1) microbial metabolic activity— together with changes in solution chemistry, mineralogy, and solid-phase surface reactivity, and the presence of electron donors, acceptors, and shuttles—affects the formation and distribution of (bio)mineral phases, as well as the rate, extent, and mechanisms of biotic and abiotic molecular-scale contaminant transformation; and (2) multiple coupled biological, chemical, and physical processes can affect the molecular-scale transformation of contaminants in the vicinity of mineral-microbe microenvironments. Research is focused on the transformation of the contaminants uranium and mercury at different spatial scales and in the context of iron and sulfate reduction. Research strives to (1) determine which aspects of microbial metabolism, solution chemistry, and mineralogy are key to the distribution of mineral species and to contaminant transformations; (2) evaluate the reactivity of reactive biogenic mineral phases with respect to the chemical transformations of UVI and HgII; (3) provide fundamental knowledge concerning the molecular-scale transformations of contaminants at the mineral-microbe microenvironment; and (4) evaluate the effect of diffusion and advective water flow on contaminant transformations. Research emphasizes laboratory-based experimentation with single-crystalline-phase iron oxide (inclusive of oxides, oxyhydroxides, and hydroxides) powders, fabricated iron-rich mineral assemblies designed to mimic mineralogical conditions in the ERSP Integrated Field Centers (IFRCs), iron oxide thin films, and geomaterial collected from all three of the ERSP IFRC sites (Oak Ridge, Hanford, and Rifle). Inocula for promoting iron- and sulfate-reducing conditions include (1) monocultures of dissimilatory iron reducing bacteria (e.g., *Geobacter* spp., *Anaeromyxobacter* spp.) and dissimilatory sulfate reducing bacteria (e.g., *Desulfovibrio* spp.) representative of organisms identified at contaminated sites and (2) microbial consortia collected from all three of the ERSP IFRC sites. Experimental work of the Argonne SFA drives technological development at Advanced Photon Source beam lines to increase the availability of x ray beam lines with the characteristics required for the proposed work. This research is part of the ERSP SFA at Argonne National Laboratory.

## Respiration by Remote Control: Role of Electron Shuttles in the Biogeochemical Transformations of Fe, S, and U

*Session: ANL SFA (Laboratory Research Manager: Carol S. Giometti)*

Edward O'Loughlin, Argonne National Laboratory (Key Investigator), Michael McCormick, Hamilton College, Maxim Boyanov, Argonne National Laboratory, Deirdre Sholto-Douglas, Argonne National Laboratory, Kelly Skinner-Nemec, Argonne National Laboratory, Man Jae Kwon, Argonne National Laboratory, Carol Giometti, Argonne National Laboratory, Michelle Scherer, University of Iowa, Robert Sanford, University of Illinois Urbana-Champaign, Terrence Marsh, Michigan State University, Kenneth Kemner, Argonne National Laboratory (Program Coordinator)

The use of Fe(III) as a terminal electron acceptor for anaerobic respiration by dissimilatory Fe(III)-reducing microorganisms is a major driver of the biogeochemistry of many subsurface environments. However, effective transfer of electrons from microbes to sparingly soluble extracellular electron acceptors such as Fe(III) oxides requires either (1) direct microbial contact with the mineral surface; (2) enhanced dissolution of the mineral by exogenous or endogenous ligands and subsequent reduction of the dissolved Fe(III) complex; or (3) facilitated electron transfer involving endogenous or exogenous electron transfer mediators, also commonly referred to as electron shuttles. Recent studies suggest that exogenous electron shuttles, such as low molecular mass quinones (e.g., juglone and lawsone) and humic substances, may play a role in many redox reactions involved in contaminant transformations and the biogeochemical cycling of redox active elements in aquatic and terrestrial environments. In addition, many microorganisms excrete soluble electron shuttles, and several of these (e.g., melanin, phenazine derivatives, flavins, and uncharacterized quinones) have been shown to enhance the microbial reduction of Fe(III) oxides. Moreover, soluble electron shuttles may increase the effectiveness of the remediation of contaminated environments by facilitating microbial transformation of contaminants that are physically/ spatially unavailable to microbes (e.g., contaminants located in pore spaces too small to be accessed by bacteria, but large enough to accommodate low molecular mass electron shuttles).

In an effort to better define the role(s) of electron shuttles in biogeochemical processes in natural and engineered subsurface environments, we are investigating the effects of endogenous and exogenous electron shuttles on transformations of Fe, S, and U under conditions favorable for dissimilatory iron and sulfate reduction. Specifically, we are focusing on three areas: (1) the identification and characterization of microbially produced electron shuttles; (2) the effects of electron shuttles on the rate and extent of the reduction of sparingly soluble Fe(III) phases and the formation of secondary mineralization products; (3) the effects of electron shuttles on the community dynamics of Fe(III) and sulfate-reducing microbial populations; and (4) the effects of electron shuttles on the reduction of U(VI) and the oxidation of reduced U species.

This research is part of the ERSP SFA at Argonne National Laboratory.

## The Interplay Between Sulfate- and Iron-Reducing Conditions: Effect on Uranium Speciation Studied in Static and Flow-Through Columns

*Session: ANL SFA (Laboratory Research Manager: Carol S. Giometti)*

Maxim Boyanov, Argonne National Laboratory, Argonne, IL; Edward O'Loughlin, Argonne National Laboratory; Shelly Kelly, EXAFS Analysis, Bolingbrook, IL; Kelly Skinner-Nemec, Argonne National Laboratory; Man Jae Kwon, Argonne National Laboratory; Wei-Min Wu, Stanford University, CA, Craig Criddle, Stanford University; Terry Marsh, Michigan State University, East Lansing, MI; Frank Loeffler, Georgia Institute of Technology, Atlanta, GA; Robert Sanford, University of Illinois at Urbana-Champaign, Urbana, IL; Carol Giometti, Argonne National Laboratory; Michelle Scherer, University of Iowa, Iowa City, IA; Ken Kemner, Argonne National Laboratory (Program Coordinator)

Extensive knowledge has been obtained in the last decade on the factors controlling uranium speciation in biotic and abiotic systems, mostly from well-mixed reactor studies. Transferring this knowledge to the field is complicated by the interplay among all factors, the unknown effect of hydrologic flow, and the difficulty in probing the system as its complexity increases. We are using a variety of approaches (synchrotron x-ray spectroscopy and microscopy, wet-chemistry techniques, microbial community analysis) to study the spatial and chemical evolution of uranium in systems of intermediate complexity, namely static and flow-through columns filled with material and groundwater from Integrated Field Research sites.

The initial stage of these studies involved observing the evolution of static columns filled with material and groundwater from the Oak Ridge IFRC FW26 well of Area 3. The development of visually heterogeneous areas in the sample was observed over the 11-month course of the experiment. Uranium concentration in the top aqueous phase decreased from 10 mM to <0.13 mM after 5 months. Sulfate concentration decreased from 1 mM to 0.005 mM after 7 months. X-ray absorption spectroscopy showed initial homogeneous distribution of U(VI) in the sample, followed by reduction to U(IV) in the top 30% of the solid phase after 3 months, and reduction throughout the entire solid phase after 5 months. Reduction remained stable and incomplete (about 80%U4) after 11 months. This work also completed the synchrotron technique development stage of the experiment.

We are continuing this work in natural material columns with elevated sulfate concentrations (1 mM and 5 mM), both with and without biostimulation. Within one month, the formation of two well-defined dark color bands is observed below the aqueous-solid interface. X-ray absorption spectroscopy indicates reduction of the initial U(VI) to U(IV) in both color bands, but no reduction in the bottom tan part. Interestingly, the spatial distribution of U(VI) in the bottom tan part evolves from uniform to concentrated in regions <200 micron in size, uniformly dispersed, without any visual indication of this change. In contrast, the distribution of U(IV) in the color bands is uniform. The system has remained in this stable state for 8 months.

Future work will test the hypothesis that the nature of U(IV) bioreduction products depends on the prevalence of iron- or sulfate-reducing conditions. Initial results in batch experiments indicate that U(IV) products can be bulk uraninite, nanoparticulate U(IV), or monomeric U(IV), depending on the method of U(VI) reduction. We speculate that these products will have different mobility, and we intend to test this hypothesis in suitably designed flow-through columns. This research is part of the ERSF SFA at Argonne National Laboratory.

## Factors Controlling Uranium Immobilization by Magnetite

Session: ANL SFA (Laboratory Research Manager: Carol S. Giometti)

Christopher A. Gorski, University of Iowa, Drew E. Latta, University of Iowa, Maxim I. Boyanov, Argonne National Laboratory, Edward J. O'Loughlin, Argonne National Laboratory, Kenneth M. Kemner, Argonne National Laboratory (Program Coordinator), Michelle M. Scherer, University of Iowa (PI)

Magnetite is a common iron oxide in the subsurface environment and is often observed as a primary product of dissimilatory iron reduction. Thermodynamically, magnetite is capable of reducing and immobilizing U(VI) under environmentally relevant conditions. Reports of U(VI) reduction by magnetite, however, are inconsistent. For example, partial reduction of U(VI) by magnetite has been observed in some studies (1,2), whereas negligible reduction has been reported by others (3). We have observed that the Fe(II) content of magnetite dramatically influences the redox behavior of magnetite (4), and we hypothesize that the Fe(II) content of the magnetite samples used in previously reported studies may explain some of the discrepancies regarding U(VI) reduction by magnetite.

To test this hypothesis, we exposed magnetite with various Fe(II):Fe(III) ratios (denoted by  $x$ ) to U(VI) and measured uranium speciation with x-ray absorption spectroscopy. Our results suggest that uranium reduction is indeed dependent upon the Fe(II) content of magnetite. Near-stoichiometric magnetite ( $x = 0.48$ ) with a high Fe(II) content reduces a significant amount of aqueous U(VI) to nanoparticulate- $\text{UO}_2$ , whereas less stoichiometric magnetite (i.e.,  $x < 0.48$ ) results in markedly less U(VI) reduction over a similar time period. In light of these results, we propose that the reactivity of magnetite may have been underestimated in previous reports, since biogenic magnetite is expected to have an Fe(II) content near stoichiometry (5), a result which we are currently pursuing with additional experiments.

This research is part of the ERSP SFA at Argonne National Laboratory.

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## Subsurface Immobilization of Metal Contaminants by Amendment-Driven Mineral Precipitation—Scientific Focus and Overview

Session: INL SFA (Laboratory Research Manager: Mark Ankeny)

George D. Redden (PI), Yoshiko Fujita, Hai Huang, Roelof Versteeg, Karen Wright, Mark Ankeny—  
Idaho National Laboratory, Idaho, Falls, ID

The Idaho National Laboratory (INL) Scientific Focus Area (SFA), sponsored by DOE's Environmental Remediation Science Program, is a program of scientific research on amendment-stimulated mineral precipitation in porous, fractured, and heterogeneous subsurface environments that can result in *in situ* immobilization of metal contaminants. Our goal is to advance the scientific knowledge base related to (1) strategies for delivering and mixing two or more reactants in subsurface environments, (2) predicting the rates and physical distributions of physical, chemical and biological processes that affect mineral precipitation, (3) predicting subsequent changes in solute transport and flow, and (4) geophysical methods for monitoring the progress and spatial distribution of the reactions.

The SFA is organized around experimental research campaigns, each of which represents a distinct geometry (or engineering strategy) for mixing two or more soluble reactants in porous media systems. The mixing geometries are dubbed "Pre-mix," "Parallel-flow," "Sequential Injection," "*In situ* Reactant Generation," and "Gas-phase Delivery." "Pre-mix" involves creating supersaturated conditions for a mineral phase (calcium carbonate and calcium phosphates have been selected as model systems) prior to injection. "Parallel-flow" involves creating a lateral mixing zone between two solutions flowing in parallel, each containing one reactant, where the position of the mixing/reaction zone can be displaced by varying the relative flow rates of the injected solutions. "Sequential Injection" involves injecting one solution (containing one reactant) followed by the injection of a second solution (with a second reactant), which results in the formation of a mixing/reaction zone perpendicular to flow that migrates and spreads as a function of flow rate, reaction kinetics and media heterogeneity structure. "*In situ* Reactant Generation" involves producing at least one of the reactants *in situ* through the addition of a precursor amendment, which then creates mixing zones at the pore scale. "Gas-phase delivery" involves the injection of a gas-phase reactant into an unsaturated system where the mixing/reaction zone is generated at the interface between gas and liquid phases.

Successful execution of each experimental research campaign involves the coordinated integration of four basic Research Elements (REs). The Research Elements are: Transport/Precipitation Coupling in Porous Media (RE1), Basic Processes and Parameterization (RE2), Model Application, Validation and Improvement (RE3), and Geophysical Sensing (RE4).

## Subsurface Immobilization of Metal Contaminants by Amendment-Driven Mineral Precipitation—Urea Hydrolysis and Calcite Precipitation

Session: INL SFA (Laboratory Research Manager: Mark Ankeny)

George D. Redden, INL (PI), Don Fox, INL, Yoshiko Fujita, INL, Luanjing Guo, INL, Hai Huang INL, Tim Johnson, INL, Roelof Versteeg, INL, Karen Wright, INL, Mikala Beig, University of Idaho, Robert W. Smith, University of Idaho, Mark Ankeny, INL

Ureolytically driven calcium carbonate precipitation for the purpose of immobilizing  $^{90}\text{Sr}$  is the subject of a field-scale project at the INL that is supported by ERSP (R.W. Smith), and is also being used in other applications such as geotechnical soil stabilization. We have therefore chosen “*In situ* Reactant Generation” as the first of the five planned SFA research campaigns. In general, each research campaign involves the coordinated integration of four basic Research Elements (REs). RE1 consists of multiscale flow cell experiments for the study of: mixing zones and deposition of mineral precipitates, the effect of physical and chemical heterogeneities on mixing and precipitation, the coupling between precipitation reactions and fluid and solute transport, and the effect of precipitation reactions on the mobility of a metal contaminant. Basic governing equations and parameterization for the geochemical or biological processes, such as urea hydrolysis, are the focus of research in RE2. Pre-experiment reactive transport simulations with comprehensive chemistry networks are developed and applied in RE3 for the design of the flow-cell experiments and to generate initial hypotheses regarding the evolution of chemical and physical changes in the flow cell experiments. Comparisons between pre-experiment model predictions and data from flow cell experiments are the basis for model development, which can include integration of pore-scale and continuum-scale modeling. Geophysical electrical sensing of chemical and physical changes in the flow cell experiments occurs under RE4 with the goal of developing a noninvasive monitoring method for application in field-scale remediation projects.

A 1D column experiment design that includes a zone of immobilized urease enzyme (an abiotic analog for the enzyme-driven biological process) has been constructed to study physical and chemical changes caused by urea hydrolysis and precipitation of calcium carbonate. Enzyme-driven hydrolysis of urea generates ammonium and carbonate ions. These reactants are then involved in a network of solution phase, solid-solution (ion exchange), and solid-phase reactions that can induce the precipitation of calcium carbonate, depending also on the local calcium ion activity. Pre-experiment modeling using the code STOMP [Subsurface Transport Over Multiple Phases, Mark White et al., Pacific Northwest National Laboratory] with solution phase thermodynamic models and kinetic models for urea hydrolysis and calcite precipitation has revealed complex behavior for 1D experiments with respect to the spatial evolution of changes in solution composition and calcium carbonate precipitation. In addition to serving as the basis for subsequent 2D flow cell experiments, the 1D experiments are being used to determine whether electrical methods can be used to track the progress of urea hydrolysis and calcium carbonate precipitation via correlations between changes in solution phase ion composition and conductivity. Changes in conductivity are measured using electrodes emplaced along the length of the column. Time-lapse geochemical and geophysical data will be used in coupled continuum-scale electrical resistivity and transport models, with the intention of correlating geophysical information to *in situ* geochemical processes.

## LBNL Sustainable Systems SFA

*Session: LBNL SFA (Laboratory Research Manager: Susan Hubbard)*

Susan Hubbard, LBNL (lead PI), Jonathan Ajo-Franklin, LBNL, Harry Beller, LBNL, Eoin Brodie, LBNL, Jinsong Chen, LBNL, John Christensen, LBNL, Mark Conrad, LBNL, Miles Denham, SRNL, Donald DePaolo, LBNL, Boris Faybishenko, LBNL, Stefan Finsterle, LBNL, Yoshiko Fujita, INL, Terry Hazen, LBNL, Michael Kowalsky, LBNL, Li LI, LBNL, Philip Long, PNNL, Peter Nico, LBNL, Eric Sonnenthal, LBNL, Nic Spycher, LBNL, Carl Steefel, LBNL, Tetsu Tokunaga, LBNL, Jiamin Wan, LBNL, Kenneth H. Williams, LBNL, Yuxin Wu, LBNL

The LBNL Sustainable Systems SFA addresses critical knowledge gaps associated with environmental stewardship of metals and radionuclides in the subsurface. Our research is motivated by the recognition that active remediation and natural attenuation strategies for persistent radionuclides and metals (a) are governed by complex, scale-dependent, and often coupled hydrological, geochemical and microbiological subsurface processes and (b) must be compatible with hydrobiogeochemical conditions that prevail in their immediate environment to be sustainable over stewardship time frames. The overarching premise of our Sustainable Systems SFA is that improvements in the mechanistic connections between fundamental hydrobiogeochemical processes and their macroscopic manifestations are needed to guide sustainable environmental stewardship in the complex subsurface. Our research is being performed within a Systems Framework, where complex subsurface systems hypotheses are tested through consideration of the nature and interaction of key hydrological, microbiological, and/or geochemical components. We have defined the following three Challenges that are allowing us to test the benefits of the Systems Framework across multiple scales.

The “Unraveling Biogeochemical Pathways” Challenge is motivated by the recognition that sustainable bioremediation requires a predictive understanding of the governing complex biogeochemical pathways. This challenge focuses on characterizing critical and interrelated microbial metabolic and geochemical mechanisms associated with chromium *in situ* reductive immobilization and reoxidation from the molecular to the local (push-pull) field scale, as is needed to assess long-term sustainability of chromium bioremediation. The three hypotheses associated with this Challenge are being carried out via interrelated tasks using biomolecular, spectroscopic, isotopic, and reactive transport modeling approaches that are being tested at the chromium-contaminated Hanford 100 Site.

The “Evolution of Pore Structures and Flowpaths” Challenge focuses on development of a predictive understanding of how remediation-induced biogeochemical transformations modify the connectivity of the pore structure and how those changes are manifested at the field scale, as is needed to design, execute, and interpret sustainable *in situ* treatments. The three hypotheses associated with this Challenge are being carried out via integrated tasks using synchrotron, isotopic, geophysical, and reactive transport modeling approaches, which are being tested at the uranium-contaminated Rifle, Colorado, site in collaboration with the IFRC team.

The “Predicting Contaminant Mobility at the Plume Scale” Challenge is motivated by the recognition that predictions of contaminant evolution, migration, and remediation efficacy at the plume scale often fail because of the great simplifications that are typically made in the representation of subsurface heterogeneity and coupled hydrobiogeochemical processes. This Challenge explores a “reactive facies” concept as an organizing principle to integrate laboratory-obtained information about rates and mechanisms with field-based hydrogeological characterization, as is needed to make reliable and computationally tractable predictions of plume evolution. The Challenge includes a formal evaluation of the benefit of increasing complexity on successful predictions of contaminant mobility over stewardship time frames. Research is carried out through integrated laboratory geochemical experiments; synchrotron, isotopic, and geophysical characterization, and reactive transport modeling. The project is being performed in collaboration with EM-supported SRNL scientists, and the insights and tools will be used assess the natural attenuation capacity of the acidic, uranium, and strontium plumes at the F-Area of the Savannah River Site.

## Quantifying Hydrogeological Controls and Induced Biogeophysical Transformations Associated with *In Situ* Bioremediation Using Geophysical Methods

Session: LBNL SFA (Laboratory Research Manager: Susan Hubbard)

Susan S. Hubbard, Lawrence Berkeley National Laboratory (LBNL) (lead PI), Jonathan Ajo-Franklin, LBNL, Jinsong Chen, LBNL, Andreas Englert, LBNL, Adrian Flores Orozco, University of Bonn, Andreas Kemna, University of Bonn, Michael Kowalsky, LBNL, Li Li, LBNL, Darrell Newcomer, Pacific Northwest Laboratory, Dimitris Ntarlagiannis, Rutgers University, Lee Slater, Rutgers University, Frank Spane, Pacific Northwest Laboratory, Kenneth H. Williams, LBNL, Yuxin Wu, LBNL

As part of the LBNL SFA “Evolution of Pore Structure and Flowpath Challenge,” we are exploring the utility of geophysical methods for quantifying hydrological controls and induced biogeochemical transformations associated with *in situ* bioremediation at the column to local field scales. Our research will help to address a key Evolution Challenge hypothesis that the cumulative impact of *in situ* remediation-induced transformations will be significant enough to impact flowpaths at the field scale. Our efforts are focused on the Rifle IFRC site, where bioremediation approaches are being tested within a shallow, unconfined aquifer to immobilize uranium in tailings-contaminated groundwater. Our primary geophysical objectives associated with the Evolution Challenge include: (1) exploring the geophysical signatures of remediation-induced biogeochemical transformations; (2) developing and testing frameworks that can integrate time-lapse geophysical and geochemical datasets for the estimation of remediation end-products; and (3) quantifying hydrogeological controls and induced biogeochemical transformations at the field scale using our petrophysical insights, estimation frameworks, and time-lapse Rifle datasets.

For the first objective, we are conducting flow-through laboratory column experiments using sediments from the Rifle IFRC to explore the impact of remediation-induced transformations on seismic, complex resistivity, and radar signatures. Through co-collection of geophysical, biogeochemical, and hydrological measurements, we consider how the evolution of electrically conductive (e.g., FeS) and nonconductive precipitates (e.g., CaCO<sub>3</sub> and S<sup>0</sup>), as well as changes in electroactive ion composition (e.g., Fe(II) and bisulfide), impact these signatures. These experiments will refine our ability to geophysically differentiate between multiple and often competing processes that occur during biostimulation at Rifle.

To meet the second objective, we have developed a state-space Bayesian framework that permits integration of time-lapse geophysical, geochemical, and other types of datasets with petrophysical relationships in the estimation of biogeochemical parameters. To demonstrate the use of the approach, we have applied it to complex resistivity data collected during column experiments designed to precipitate FeS and ZnS during sulfate reduction. As a case study, we used the framework and time-lapse datasets to estimate the volume fraction of the dispersed, sulfide-encrusted cells; mean radius of aggregated clusters, and permeability reduction that occurred over the course of the experiment due to pore clogging by the precipitates. Our results suggest that the developed state-space approach permits the use of geophysical datasets for providing quantitative estimates of the evolution of end-products and permeability reduction associated with biogeochemical transformations at the column scale.

In collaboration with the Rifle IFRC Big Rusty biostimulation experiment and to address our third objective, we have collected a rich time-lapse field geophysical dataset, including: surface spectral induced polarization data, tomographic radar and seismic data, and wellbore logs. These data are being used together with time-lapse Rifle hydrogeological and geochemical datasets to estimate *in situ* baseline hydrogeological heterogeneity. Preliminary analysis of the spectral induced polarization data indicates sensitivity to spatiotemporal changes in electroactive ion concentration and the accumulation of semi-conductive mineral precipitates. The time-lapse datasets will eventually be used with our developed petrophysical insights and estimation framework to quantify biogeochemical transformations at the local field scale. The aquifer-scale, geophysically obtained characterization and monitoring estimates will be used to parameterize and validate Rifle-based reactive transport models, respectively.



## Use of Biomolecular Signatures To Unravel Biogeochemical Reaction Networks Underlying Chromium Reduction at the Hanford 100H Site

Session: LBNL SFA (Laboratory Research Manager: Susan Hubbard)

Harry R. Beller, Lawrence Berkeley National Laboratory (lead PI), Eoin L. Brodie, Lawrence Berkeley National Laboratory, Katherine C. Goldfarb, Lawrence Berkeley National Laboratory, Ruyang Han, Lawrence Berkeley National Laboratory, Clark A. Santee, Lawrence Berkeley National Laboratory

We are developing the use of biomolecular signatures as part of the LBNL SFA challenge entitled “Unraveling Biogeochemical Reaction Networks Mediating Sustained Chromium Reduction” which focuses on *in situ* reductive immobilization of Cr at DOE's Hanford 100H site. There are two primary research components of biomolecular signatures in this challenge: (a) biomolecular signature discovery, which involves development and testing of meta-transcriptome-based gene expression microarrays and (b) quantifying biomolecular signatures, which involves assessing relationships between microbial metabolic activity (e.g., nitrate reduction) and specific biomolecular signatures (e.g., *narG* gene or transcript copy number). The biomolecular signature work described here is being integrated into an interdisciplinary effort involving spectroscopic, isotopic, and reactive transport modeling approaches. Among the research hypotheses to be addressed in this challenge are the following: (1) Microbial processes mediate both direct (enzymatic) and indirect Cr(VI) reduction at Hanford 100H, but indirect pathways dominate sustained reduction; furthermore, sulfate reduction is the electron-accepting process ultimately driving sustained Cr(VI) reduction at Hanford 100H, and (2) Fermentative/acetogenic versus respiratory metabolism will promote retention of organic carbon in the aquifer.

*Meta-transcriptome-based gene expression microarrays:* We are developing a novel high-throughput approach that uses the meta-transcriptome (cDNA representing the collective mRNA transcripts from the entire microbial community) to design high-density oligonucleotide microarrays, which can be used to identify highly expressed genes in a specific community under conditions of interest, without requiring any *a priori* hypotheses about which genes the community might be expressing or prior sequence information from data repositories. This approach represents an alternative to functional gene arrays, which entail *a priori* assumptions about key gene sequences, and has the potential to reveal novel and important molecular signatures for a specific community based on differential expression of genes represented in a customized array. To generate meta-transcriptome samples, we inoculated anaerobic microcosms with groundwater from the Cr-contaminated Hanford 100H site and supplemented them with lactate and electron acceptors present at the site, namely, nitrate, sulfate, and Fe(III). The microcosms progressed successively through nitrate-reducing, sulfate-reducing, and Fe(III)-reducing conditions, and after a second addition of nitrate, nitrate-dependent Fe(II)-oxidizing conditions. Cr(VI) was rapidly reduced initially and again upon further Cr(VI) amendments. Community DNA and RNA were harvested during each major biogeochemical phase and were subjected to PhyloChip analysis to characterize community composition under various electron-accepting conditions. Community mRNA was amplified and reverse transcribed using methods described elsewhere, which include microbial RNA polyadenylation and linear amplification with a T7 RNA polymerase (MessageAmp II-Bacteria Kit, Ambion). This cDNA preparation method was designed to reduce the amount of cDNAs representing rRNA. Samples representing several different biogeochemical regimes were submitted for pyrosequencing using the 454 Titanium technology. Ultimately, arrays derived from the meta-transcriptomic sequence data will be used to interrogate 100H groundwater samples.

*Microbial Activity-Signature Relationships:* Initially, we are using well-characterized cultures isolated from the Hanford 100H site to establish whether quantitative relationships can be determined between relevant metabolic activity and (a) gene copy number (qPCR) and (b) transcript copy number (RT-qPCR). If consistent activity-signature relationships can be established, these should provide quantitative information (i.e., estimates of key *in situ* metabolic activities) that can be incorporated into the reactive transport modeling effort at Hanford 100H. Gene sequences have been determined for key functional genes (e.g., chromate reductase genes *chrR* and cytochrome *c3*, denitrification genes *narG* and *nirS*, and sulfate reduction genes *dsrA* and *aprB*) in two bacterial species isolated from Hanford 100H groundwater: strain RCH1 (a sulfate-reducing bacterium similar to *Desulfovibrio vulgaris* Hildenborough) and strain RCH2 (a denitrifying strain similar to *Pseudomonas stutzeri*). For Hanford bacterial strain RCH2, we have investigated some activity-signature relationships and Cr(VI) reduction kinetics in batch systems; this work is being transitioned to continuous culture.

## Enhanced Immobilization of Metals and Radionuclides in the Vadose Zone

Session: LBNL SFA (Laboratory Research Manager: Susan Hubbard)

Mark E. Conrad (PI), Lawrence Berkeley National Laboratory (LBNL), Yoshiko Fujita, INL, Cindy H. Wu, LBNL, Markus Bill, LBNL, Karen E. Wright, Idaho National Laboratory, Samuel D. Chamberlain, LBNL, Bonita R. Lam, LBNL, Eoin L. Brodie, LBNL, Peter S. Nico, LBNL, Nicolas Spycher, LBNL, Terry C. Hazen, LBNL

Significant quantities of metals and radionuclides are contained in unsaturated rocks at several DOE sites in the western U.S. In many cases, this contamination has migrated to groundwater, sometimes decades after being released into the subsurface. Immobilizing these contaminants in the vadose zone could greatly reduce the threat they pose to groundwater and decrease the costs of closing these sites. This exploratory project is part of the LBNL SFA and is focused on stimulation of biologically enhanced phosphate mineralization under unsaturated conditions through injection of gas-phase organophosphate compounds. Phosphates will incorporate contaminants such as U and  $^{90}\text{Sr}$  into their mineral structure. To induce precipitation of phosphate minerals in unsaturated sediments, we are testing the use of gas-phase compounds for delivery of the necessary chemicals to the vadose zone. Our initial studies are focused on tri-ethyl phosphate (TEP), which has a moderate vapor pressure ( $\sim 385$  ppmv at  $20^\circ\text{C}$ ), is miscible with water, and has low toxicity. To accelerate release of phosphate from TEP, we are testing methods of stimulating microbial degradation of TEP.

Our current research is focused on determining the chemical and biological properties of TEP and phosphate in materials from Hanford and the INL. To determine the concentrations of phosphate in solution necessary to induce mineral precipitation, inorganic phosphate was added to simulated INL groundwater. Despite a predicted solubility of  $5 \times 10^{-6}$  mM for hydroxyapatite, precipitation was not observed until aqueous concentrations of  $\sim 2$  mM phosphate were reached. When these precipitation experiments were repeated with live bacterial cells, the apparent degree of oversaturation required to induce precipitation of phosphate minerals decreased slightly, possibly due to the availability of cell surfaces as heterogeneous nucleation sites. Experiments to determine the degree of adsorption of phosphate onto sediments from the Hanford site were also conducted. Within 10 days, 80–90% of phosphate added to slurries was adsorbed (0.1 to 0.15  $\mu\text{mol PO}_4$  per gram of sediment). TEP addition did not have a significant effect on phosphate adsorption, but did lead to increased desorption of nitrate.

Two sets of long-term biodegradation experiments with TEP have been performed. Significant biodegradation of TEP was observed during experiments with INL simulated groundwater amended with 5 mM TEP and an enrichment culture derived from INL vadose zone sediments. After 9 months, despite no significant increase in cell counts, phosphate concentrations in live cultures have steadily increased to  $>0.2$  mM (versus no detectable phosphate generation in killed cultures). This indicates significant biodegradation of TEP is occurring; however, these phosphate concentrations are still much lower than the levels at which the onset of precipitation was observed during our abiotic experiments. The second set of biodegradation experiments was conducted with slurries of Hanford sediment containing 5 mM aqueous TEP. No significant accumulation of phosphate has been observed during these experiments (not unexpected, given the results of the adsorption experiments), but there have been measurable decreases in TEP concentrations in the slurries. Although the total cell concentrations in the slurries have decreased, *Arthrobacter* sp. have become the dominant cultivable microorganisms present. A culture-independent method using a high-density microarray (Phylochip) will be used to characterize the microbial communities in both the groundwater and slurry experiments.

The results of these experiments indicate that TEP can be biodegraded by microorganisms found in unsaturated environments, but the rates may be very slow and the degree of oversaturation of phosphate necessary for precipitation of phosphate minerals is large. However, given the slow rates of contaminant migration within the vadose zone, this may still represent a viable mechanism for *in situ* immobilization of contaminants. Future research will include investigation of approaches to accelerate TEP degradation, for example by the provision of supplemental nutrients.

## Field-Scale Investigations of Cryptic Growth and Memory Response Hypotheses at the Chromium Contaminated Hanford 100-H Site

*Session: LBNL SFA (Laboratory Research Manager: Susan Hubbard)*

Terry Hazen, LBNL (lead PI), Boris Faybishenko, LBNL, Harry Beller, LBNL, Eoin Brodie, LBNL, Susan Hubbard, LBNL, John Peterson, LBNL, Eric Sonnenthal, LBNL, Carl Steefel, LBNL, Li Yang, LBNL, Joern Larsen, LBNL, Mark Conrad, LBNL, John Christensen, LBNL, Shaun Brown, LBNL, Dominique Joyner, LBNL, Sharon Borglin, LBNL, Jil Geller, LBNL, Romy Chakraborty, LBNL, Peter Nico, LBNL, Tetsu Tokunaga, LBNL, Jiamin Wan, LBNL, Mary Firestone, LBNL, Phil Long, PNNL, Darrell Newcomer, PNNL, Lucie N'Guessan, PNNL

This presentation relates to the SFA Challenge “Unraveling Biogeochemical Pathways Mediating Sustained Chromium Reduction.”

The 2004–2008 field experiment at the Hanford 100-H Site showed that a single injection of the hydrogen release compound (HRC)—a slow release glycerol polylactate into groundwater—stimulated an increase in biomass and a depletion of terminal electron acceptors, resulting in a significant decrease in soluble Cr(VI). The Cr(VI) concentration has remained below the background concentration in the down-gradient pumping/monitoring well, and below the detection limit in the injection well, for more than 3 years after the HRC injection. Based on the results of this experiment, we hypothesize that one of the main mechanisms contributing to the sustainability of Cr(VI) bioremediation in groundwater is the presence of a resilient biomass that optimally recycles cells as they die, a strategy referred to as cryptic growth. We also hypothesize the presence of the bacterial memory effect, which is supposed to accelerate the biomass production after the next HRC injection. However, these mechanisms have not been verified at the field scale. The overall objective of current field-scale investigations is to characterize critical and interrelated microbial metabolic and geochemical mechanisms associated with *in situ* chromium reductive immobilization and reoxidation, from the molecular to the local field scale. On November 13, 2008, LBNL and PNNL researchers injected a small amount (10 lbs) of HRC into the injection well at the Hanford 100-H site to revive the microbial community activity. To assess the biogeochemical processes affecting the pre- and post-HRC injection biomass generation, we have implemented a comprehensive plan of groundwater sampling for 11 types of different microbial and geochemical analyses. To directly assess the microbial generation, specially designed biomass traps were placed in the monitoring wells (the work is conducted jointly with the Center for Biofilm Engineering, Montana State University). The post-HRC injection monitoring and analytical analyses to study the mechanisms of the microbial memory effect will be conducted until March–April 2009. Then, we plan to perform a push-pull test using a KNO<sub>3</sub> injection into the Hanford aquifer.

This project is conducted in collaboration with Genomics: GTL.

## Dynamic Synchrotron Imaging of Pore-Structure Evolution

*Session: LBNL SFA (Laboratory Research Manager: Susan Hubbard)*

Peter S. Nico, Lawrence Berkeley National Laboratory (lead PI), Jonathan B. Ajo-Franklin, Lawrence Berkeley National Laboratory

As part of the *Evolution of Pore Structures and Flowpaths* challenge area within the *LBNL Sustainable Systems SFA*, we are developing the capabilities necessary to image alterations in pore structure resulting from subsurface manipulations. *In situ* remediation strategies are frequently considered for cleanup of DOE sites. However, the impact of feedbacks between multiscale heterogeneity and induced biogeochemical transformations on remediation efficacy is not well understood. Critical to the design, execution, and interpretation of *in situ* remediation approaches is an understanding of how reaction processes modify the properties of the porous medium, or the “*Evolution of Pore Structure to Flowpaths*.”

The *Evolution of Pore Structures and Flowpaths* challenge is driven by four specific hypotheses:

1. Remediation treatments can induce significant enough biogeochemical perturbations to alter pore structures.
2. The cumulative impact of remediation-induced biogeochemical transformations will be significant enough to impact flowpaths at the field scale.
3. The dynamic nature of field-scale flowpaths will impact the efficacy and sustainability of remediation treatments.
4. Geophysical and isotopic methods can be used to track biogeochemical changes at the field scale.

The *Dynamic Synchrotron Imaging of Pore Structure Evolution* task is designed to address the first of these four hypotheses and has two important subtasks: time-lapse imaging to image the evolution of pore structures in response to external perturbations, and chemically resolved micro-tomography to help identify the minerals whose precipitation or dissolution are causing the changes in pore structure.

This year's progress towards accomplishing these two goals includes: the design, construction, and testing of a time-lapse imaging micro-column reactor; calibration experiments determining optimal achievable spatial and density resolution; improvements in image registration necessary to conduct difference tomography, an essential task for both time lapse and chemical imaging; and tomographic images of sediments from the DOE Rifle Colorado Site taken before and after treatments. Plans for the upcoming year will also be presented and include an *in situ* column study using Rifle sediments and the new micro-column reactor, further refinement of data processing and registration approaches, and expansion of the chemical imaging capabilities.

## Predicting Plume-Scale U and <sup>90</sup>Sr Mobility at the Savannah River Site

*Session: LBNL SFA (Laboratory Research Manager: Susan Hubbard)*

Nicolas Spycher, Lawrence Berkeley National Laboratory (lead PI), John N. Christensen, Lawrence Berkeley National Laboratory, Sudipta Rakshit, Lawrence Berkeley National Laboratory, Jiamin Wan, Lawrence Berkeley National Laboratory, Susan S. Hubbard, Lawrence Berkeley National Laboratory, Arthur Wiedmer, U.C. Berkeley, Miles Denham, Savannah River National Laboratory

Low-level radioactive, acid waste solutions were disposed into seepage basins at the F-Area of the Savannah River Site from 1955 to 1989. As a result, an acidic plume has developed in groundwater beneath the basins. The plume contains many contaminants, including various U isotopes, Sr-90, I-129, Tc-99, tritium, and nitrate, with pH values as low as 3.2. After a decade of active remediation, natural attenuation is now being considered as a long-term remediation alternative at this site.

The objective here is to develop efficient predictive capabilities to assess contaminant behavior at the plume scale, and in particular to evaluate natural attenuation over stewardship time frames. Uranium and <sup>90</sup>Sr are given priority over other contaminants, because these radionuclides become strongly associated with sediments upon pH rise to background values and are long-term risk drivers. A three-prong approach is taken, which includes (1) investigating the hierarchy of mechanisms controlling natural attenuation of U and <sup>90</sup>Sr at the F-Area, (2) using this information to parameterize mechanistic reactive transport models, and (3) through these models, assessing the level of complexity that is sufficient and necessary for successful long-term prediction of contaminant fate and mobility, including natural attenuation.

Our approach is to identify specific “reactive facies,” representing unique reactive transport properties (e.g., sorption, surface area, permeability, mineralogy) for specific types of subsurface geologic materials. The identification of these “reactive facies” is currently ongoing, using a variety of laboratory and field-scale geochemical, geophysical, and isotopic approaches. Sediment cores from new boreholes drilled in both contaminated and uncontaminated regions of the F Area are being analyzed for various constituents. Analyses of pore waters extracted from the core materials have been conducted to determine site-specific U and <sup>90</sup>Sr sorption characteristics and U isotopic composition. Sorption studies are being conducted on clean sediments as a function of pH and facies to develop site-specific surface-complexation models. In parallel with the core characterization effort, U isotopic analyses of a series of groundwater samples have been conducted to assess variability and dynamics at the plume scale and discern contaminant sources and transport pathways. Iterative co-analysis of tomographic geophysical field data, wellbore hydrological data, and laboratory analysis results is under way to establish if a linkage between physical and geochemical properties exists at the site, as needed, to exploit the reactive facies concept. Geochemical and reactive transport analyses are being applied to evaluate the acidic (advanced argillic) alteration of subsurface sediments and the role of elevated dissolved and exchangeable Al concentrations in buffering pH to low values in the main part of the plume. The historical contaminant inventory and mass balance in the F Area are also being assessed using existing and recently collected data.

These efforts will be used to parameterize reactive transport models of the site at various scales and with various levels of complexity. This will ultimately include a plume-scale model specifically intended to assess the natural attenuation capacity of the F Area.

## Reactive Transport Modeling of Microbially-Mediated Processes at the Old Rifle and Hanford 100H Sites

*Session: LBNL SFA (Laboratory Research Manager: Susan Hubbard)*

Carl I Steefel, Lawrence Berkeley National Laboratory (LBNL) (lead PI), Eric Sonnenthal, LBNL, Li Li, LBNL, Sumit Mukhopadhyay, LBNL, Michael Kowalsky, LBNL

*Evolution Hypothesis (Old Rifle Site):* We have focused on the development of thermodynamic/kinetic models for U(VI), V(V), and Fe(III) bioreduction, with application to a set of column experiments using natural sediments from the uranium bioremediation site at Old Rifle, Colorado. In the experiments, uranium-contaminated groundwater from the site is amended with variable concentrations of acetate to investigate explicitly the thermodynamic controls on the bioreduction of U, V, and Fe, along with consideration of sulfate reduction that with time comes to dominate the reaction network. At 3 mM acetate, U and V are nearly completely removed from solution. However, sulfate reduction begins well before the breakthrough of sulfide from the column, and based on an analysis of the electron balance, most of the acetate (>90%) is used in sulfate reduction. This implies that the sulfide produced must be consumed through abiotic reduction of iron hydroxide in the column, rather than through direct reaction with Fe(II) from bioreduction of Fe(III) phases. Breakthrough of sulfide occurs when the “bioavailable” Fe(III), as defined by a 0.5 N hydroxylamine extraction, is exhausted. In contrast, U decreases only about 40% when 0.5 mM acetate is injected, with Fe reduction initially dominating the reaction network without completely suppressing sulfate reduction.

A second effort involves assessing the role of physical and chemical heterogeneities and pore clogging on the efficacy of uranium bioreduction. We examined the effects of physical and geochemical heterogeneities on the spatial distributions of mineral precipitates (principally calcite, FeS, and siderite) and biomass accumulated during a uranium bioremediation experiment near Rifle, CO. Field data were used to infer heterogeneous distributions of permeability and Fe(III) content through inverse transport modeling. After validating with field aqueous geochemical data, we used reactive transport modeling to estimate the spatial distribution of precipitates and biomass. Results show that the maximum mineral precipitation and biomass accumulation occurs at the vicinity of the injection wells and is dominated by sulfate-reducing reaction products. Reduction of as much as 5.4% of the pore space is predicted to have occurred locally. Biomass accumulation near the injection wells is not affected by heterogeneity. However, accumulation in the downgradient regions is observed, and is dominated by the iron-reducing reaction products, with spatial distributions strongly controlled by both physical and geochemical heterogeneities.

*Unraveling Hypothesis (Hanford 100H site):* A reaction-transport model was developed to investigate the lactate- and acetate-induced bioreduction of Cr(VI) in the groundwater of the Hanford 100H aquifer. Bioreduction of Cr(VI) with these electron donors is captured using a mixed thermodynamic-kinetic approach, with the modified Gibbs free energies of the reactions based in part on Liu et al. (2002). These calculations also consider other organic acid metal aqueous complexes affecting the thermodynamic driving force. The principal kinetic reactions include HRC dissociation to lactate, direct reductive precipitation of Cr(OH)<sub>3</sub>, bioreduction of goethite to produce Fe(II) leading to abiotic reduction of Cr(VI); calcite dissolution in the acidic plume and precipitation elsewhere, with accounting for the influence of bicarbonate concentrations on the extent of Cr(VI) reduction. A 3D reaction-transport model of the 100H site was developed including discretized wells, a heterogeneous permeability field conditioned on geophysical data, and hydrological parameters and flow velocities tuned on multiple Br tracer tests. It was found that the time-dependent drop in total Cr concentrations observed in pumping wells could be captured quite closely, assuming that the viscous HRC plume was relatively immobile with its distribution indicated by geophysical imaging. The model was further extended to include the effects of Cr isotopic (<sup>53</sup>Cr/<sup>52</sup>Cr) fractionation by reduction, which has been shown in laboratory experiments to result in a shift in d<sup>53</sup>Cr of ~3.5‰ (Ellis et al. 2002), compared to Cr(VI) sorption showing a small fractionation of ~0.04‰. Modeling results for 100H showed Cr fractionation of ~2–3‰ (heavier) in fluids during Cr reduction, consistent with measured data and providing an independent method for determining reaction pathways and their individual rates.

## Uranium Immobilization in Oxidizing Environments: Potassium Uranyl Vanadate Precipitation

*Session: LBNL SFA (Laboratory Research Manager: Susan Hubbard)*

Tetsu K. Tokunaga, Lawrence Berkeley National Laboratory (lead PI), Yongman Kim, Lawrence Berkeley National Laboratory, Jiamin Wan, Lawrence Berkeley National Laboratory

We propose that a primary criterion for any sustainable approach for remediating groundwaters contaminated with radionuclides and metals is that it maintain contaminant concentrations below regulatory levels without requiring long-term intervention, even after regional groundwater conditions return. Using this criterion, bioreduction-based methods for remediation of U-contaminated sediments and groundwaters are unsustainable because oxidizing conditions that remobilize U return soon after the supply of electron donor is discontinued. Therefore, it is becoming apparent that remediation methods are needed to immobilize U in oxidizing environments. *In situ* remediation approaches based on precipitation of uranyl phosphate minerals (autunites) are in this category and may moderate U concentrations. However, our calculations indicated that autunite-based remediation will also be unsustainable, because U concentrations will exceed the Maximum Contaminant Level (MCL = 0.13  $\mu\text{M}$  for U) unless phosphate concentrations are maintained at much higher levels than are typically found in groundwaters, or dissolution rates are low enough to maintain strong disequilibrium. The objective of this project is to identify truly effective and sustainable methods for immobilizing uranium (U) such that its concentrations remain below regulatory levels in oxidizing environments. Our thermodynamic calculations predict that carnotite,  $\text{K}_2(\text{UO}_2)_2\text{V}_2\text{O}_8$ , and tyuyamunite,  $\text{Ca}(\text{UO}_2)_2\text{V}_2\text{O}_8$ , can keep U(VI) concentrations below regulatory levels in oxidizing, slightly-acidic-to-neutral solutions under environmentally reasonable conditions. Batch experiments were conducted to test the thermodynamic predictions and determine the kinetics of the precipitation reactions. These experiments on U removal from oxidizing solutions exhibited complex dependence on vanadate (V(V)) and  $\text{K}^+$  concentrations. Nevertheless, conditions were identified where U concentrations dropped below its MCL within 1 to 5 days of contact with oxidizing solutions containing  $\text{K}^+$  and V(V). These calculations and experiments on U removal from aqueous solutions indicate that precipitation of a carnotite-like phase is a potentially viable strategy for treating some U-contaminated groundwaters. This method can also be employed to economically extract (mine) U and V from groundwaters where both elements occur at elevated concentrations. Uranium-contaminated sediments are being tested in batch and column experiments in our next stages of exploring the potential for vanadate-based *in situ* U remediation.

## ORNL SFA: Biogeochemical and Molecular Mechanisms Controlling Mercury Transformation at a Contaminated Site in Oak Ridge, Tennessee, USA

*Session: ORNL SFA (Laboratory Research Manager: Liyuan Liang)*

Liyuan Liang, Oak Ridge National Laboratory (lead PI), Scott C. Brooks, Oak Ridge National Laboratory, Anthony V. Palumbo, Oak Ridge National Laboratory, Baohua Gu, Oak Ridge National Laboratory, Jeremy C. Smith, UT/ORNL Center for Molecular Biophysics. *ORNL participants:* Liyuan Liang, Craig Brandt, Scott Brooks, Steve Brown, Wenming Dong, Baohua Gu, Hao-Bo Guo, Alexander Johs, Carrie Miller, Anthony Palumbo, Jerry Parks, Mircea Podar, Jeremy Smith, George Southworth, Thomas Thundat. *Current Collaborators:* C. Gilmour (Smithsonian Institute), H. Guo (Univ. Tenn., Knoxville), S. Miller (Univ. Calif., San Francisco), L. Shi (Pacific Northwest Natl. Lab.), A. Summers (Univ. Georgia), J. Wall (Univ. Missouri, Columbia), H. Zhang (Tennessee Tech. University).

Biogeochemical factors controlling mercury transformation and methylmercury production present a serious knowledge gap at the U.S. Department of Energy Oak Ridge Reservation and many other contaminated sites globally. ORR field data show that reduction in waterborne Hg levels does not necessarily lead to a decrease of methylmercury in water or in fish. This observation suggests that at high Hg levels (often present at highly contaminated source areas), either methylmercury production is inhibited or demethylation significantly exceeds methylation. This presentation will provide an overview of the Science Focus Area (SFA) program at the Oak Ridge National Laboratory (ORNL) that combines molecular to field-scale chemical and microbiological studies, to understand various biogeochemical factors that control the net production of methylmercury. The main objectives are to elucidate the rates, mechanisms, and controls of abiotic and microbial processes affecting Hg speciation and transformation, to resolve the critical Hg precursors that are produced and subsequently methylated, and to develop and validate subcellular models to understand the biochemical and biophysical mechanisms of transformation between Hg species and methylmercury.

Historical data for Upper East Fork Poplar Creek at the Oak Ridge Reservation will be presented, and new data and geochemical modeling results from laboratory to field investigations will be summarized. This is the first year of a multidisciplinary research program in which laboratory and microcosm experiments will focus on the fundamental processes that control Hg speciation, reactivity and methylation using chemical, biomolecular, spectroscopic and stable isotope techniques. Preliminary data from field and laboratory studies will be presented, which examine microbial processes and geochemical controls such as dissolved organic matter, ionic species and photochemical reactions on Hg redox transformation, mechanisms, speciation, and bioavailability for Hg methylation.

Future effort will continue to employ functional genomics to determine key microbial groups that influence methylmercury production under varying geochemical conditions. Investigation of structure and dynamics at the molecular level will reveal mechanisms of regulation and the role of interactions between subcellular components. Molecular simulation will be applied to known demethylation processes to provide an atomic level detailed understanding of key reaction pathways, and will be extended to elucidate microbial methylation mechanisms identified by advanced genomic techniques. These studies will form the basis of understanding of the oxidation-reduction and methylation-demethylation transformations that determine the fate of Hg in sediment-water environments.



## Comparison of Two Mercury-Contaminated Surface Water Bodies

*Session: ORNL SFA (Laboratory Research Manager: Liyuan Liang)*

Scott C. Brooks, Oak Ridge National Laboratory (lead PI), Geroge R. Southworth, Oak Ridge National Laboratory, Ralph R. Turner, RT Geosciences, Inc., Richard Jensen, Unique Environmental Services

As part of the Science Focus Area (SFA) project at ORNL, Task 1 will use complementary field observations and laboratory microcosm experiments to delineate key biogeochemical parameters influencing chemical and microbiological mercury (Hg) transformations in Upper East Fork Poplar Creek (UEFPC), Tennessee. During the initial seven months of the SFA project, task activities have focused on characterization of mercury (Hg) sources to the creek and general field sampling to gather chemical, physical, and microbial (in collaboration with Task 3 led by Palumbo) data. Additionally, we established a collaborative relationship with members of the South River Science Team, which was formed to develop a better understanding of Hg behavior in the South River, VA. This poster compares the hydrogeochemical characteristics of two industrially contaminated water bodies. Due to the methyl mercury (MeHg) burden in fish tissues, the Virginia Department of Health and the Tennessee Department of Environment and Conservation have posted fish advisories for South River, Virginia, and East Fork Poplar Creek, Tennessee (EFPC), respectively. Both streams share broad similarities in terms of their general chemistry and underlying geology. Nevertheless, patterns of waterborne Hg and, importantly, methyl mercury concentration are different. For example, in the South River, both Hg and MeHg concentrations increase with increasing distance downstream from the industrial site of mercury origin, whereas in EFPC, Hg decreases while MeHg increases with increasing distance downstream. During the first five years of monitoring EFPC (1985–1989), Hg in fish tissue decreased with distance downstream (i.e., with dilution of the headwater inputs), suggesting that Hg bioaccumulation would decrease in response to headwater source reduction and removal. A number of actions decreased Hg contributions to the creek headwaters. Nevertheless, the subsequent fifteen years witnessed the emergence of a flat profile of Hg in fish with distance downstream, resulting from a drop in Hg in fish at upstream locations and an increase in Hg in fish at downstream locations. Comparison to similar data from the South River suggests that the situation in EFPC represents a return to a typical condition rather than a deviation. Despite their similarities, the relationship between MeHg and Hg in these two systems is dramatically different. Although both sites are the focus of concerted research efforts to identify effective remediation, the underlying mechanisms that drive the patterns within each system, and therefore account for the differences between them, are poorly understood. We intend for this presentation to provide a context within which attendees can frame their discussion of the challenges inherent to studying the biogeochemical cycling of Hg in general and at contaminated sites in particular where effective remedies can be elusive.

This task will continue field sampling and characterization efforts to understand key parameters governing net MeHg production (methylation versus demethylation) in UEFPC, and explore those parameters via controlled laboratory experimentation. Close integration with the other three SFA tasks is critical to achieving the program goals.

## Understanding Mercury Speciation, Reactivity and Binding Mechanisms with Dissolved Organic Matter

*Session: ORNL SFA (Laboratory Research Manager: Liyuan Liang)*

Baohua Gu, Oak Ridge National Laboratory, Carrie Miller, Oak Ridge National Laboratory, Wenming Dong, Oak Ridge National Laboratory, Yongrong Bian, Oak Ridge National Laboratory, George Southworth, Oak Ridge National Laboratory, Liyuan Liang, Oak Ridge National Laboratory (lead PI)

This research is part of the ORNL Science Focus Area aimed at fundamental understanding of the mechanisms and geochemical controls on the speciation and transformation of mercury (Hg) at the Upper East Fork Poplar Creek (UEFPC) contamination site in Oak Ridge, Tennessee. Our initial focus is on the effect of dissolved organic matter (DOM), which has been shown to play a dominant role in the complexation, reactivity, and transformation of mercury in aquatic systems. To date, two reactive DOM components (hydrophobic and hydrophilic fractions) have been isolated from UEFPC stream water and studied for their complexation with Hg(II), using a modified ion exchange technique. The rate of formation of Hg-DOM complexes was examined using stannous chloride reactive Hg measurements and C<sub>18</sub> solid phase extractions. Results indicate that ionic Hg(II) forms strong complexes with thiol functional groups in DOM. The Hg(II)-DOM complexes were predicted to be the dominant aqueous species in UEFPC, which agrees with most freshwater environments where equilibrium conditions are established. However, further studies indicate that equilibrium conditions at UEFPC cannot be assumed because of a constant input of inorganic Hg(II) to the creek. The formation of Hg-DOM complexes appear to be kinetically limited, with a pseudo first-order rate constant in the range of 0.11–0.29 hr<sup>-1</sup> when DOM isolates were allowed to react with mercury in UEFPC water. The formation rate of Hg-DOM complexes was even slower ( $k = 0.03 \text{ hr}^{-1}$ ) when the UEFPC headwater (from Outfall 200) was mixed with the creek water. These results suggest the UEFPC headwater contains other reactive organic or inorganic ligands, which are retarding the formation of the Hg-DOM complex. The slow formation kinetics of the Hg-DOM complex likely results in a persistent presence of reactive mercury in the upper reaches of UEFPC that cannot be described satisfactorily using equilibrium models. These reactive species need to be considered when investigating the production of methylmercury and the particle reactivity of Hg(II) in this system. Continuing research is being conducted to understand the binding mechanism of Hg(II) with DOM, and how the interactions of mercury and DOM influence the reduction of inorganic Hg(II) and the abiotic demethylation of methylmercury.

## Mercury Methylation: Genes and Communities (Hg SFA at ORNL, Task 3)

Session: ORNL SFA (Laboratory Research Manager: Liyuan Liang)

Anthony V. Palumbo, Oak Ridge National Laboratory (lead PI), Tatiana A. Vishnivetskaya, Oak Ridge National Laboratory, Tingfen Yan, Oak Ridge National Laboratory, Craig C. Brandt, Oak Ridge National Laboratory, Mircea Podar, Oak Ridge National Laboratory, Meghan M. Drake, Oak Ridge National Laboratory, Liyuan Liang, Oak Ridge National Laboratory, Scott C. Brooks, Oak Ridge National Laboratory, Steven D. Brown, Oak Ridge National Laboratory, Cynthia Gilmour, Smithsonian Environmental Research Center, Amy A. Kucken, University of Missouri-Columbia, Dwayne A. Elias, University of Missouri-Columbia, Judy D. Wall, University of Missouri-Columbia, Zamin K. Yang, Oak Ridge National Laboratory

As part of the mercury SFA at ORNL (Task 3), we have been examining the genes involved in mercury methylation and the microbial communities that may be involved in mercury methylation in streams contaminated with mercury as the result of past operations in Oak Ridge. We have used a comparative genomics approach and a comparative gene expression methodology to identify candidate mercury methylation genes in *Desulfovibrio*. We have identified a number of candidate genes using the comparative genomics approach, and several of these have been tested to see if they confer mercury methylation abilities in *Desulfovibrio* unable to methylate mercury. To date, none has proved to have this ability. The comparative gene expressions methods have indicated that the genes involved in methylation are likely constitutive, as the addition of mercury at levels even beyond what would be expected in the environment does not induce notable changes in gene expression in either the methylators or nonmethylators tested under sulfate-respiring conditions. We are currently testing this response under other growth conditions. Using both a functional (FGA) and phylogenetic (454 sequencing of 16s genes) approach, we examined the community characteristics of streambed microbial communities across a range of mercury contamination. We hypothesized that there is a greater diversity of genes related to pollutants at the contaminated sites, but a lower phylogenetic diversity. We also hypothesized that the number of deltaproteobacteria (the group involved in methylation) would be positively correlated with methyl mercury concentrations. In a preliminary analysis of the data, we found pronounced phylogenetic and functional differences that appear to be related to seasonal trends. Also, we have found that representatives of the deltaproteobacteria are present at all sites, though at relatively low levels. The predominant groups found in these aerobic surface sediments were *cyanobacteria*, *betaproteobacter*, and *verrucomicrobia*. More detailed analysis of the data will be done to test our specific hypotheses. Future research will focus on comparative genomics and transposon mutagenesis approaches for identifying the genes involved in mercury methylation in *Desulfovibrio* and *Geobacter*, and in quantifying the presence of those genes in the environment.

## Biomolecular Aspects of Mercury Transformations

Session: ORNL SFA (Laboratory Research Manager: Liyuan Liang)

Jeremy C. Smith, University of Tennessee (UT)/Oak Ridge National Laboratory (ORNL) (lead PI), Liyuan Liang, Oak Ridge National Laboratory, Alexander Johs, ORNL, Hao-Bo Guo, UT/ORNL, Jerry M. Parks, UT/ORNL, Hong Guo<sup>1,3</sup>, UT/ORNL, Liang Shi, PNNL, Susan M. Miller, UC San Francisco, Anne O. Summers, University of Georgia, Liyuan Liang, ORNL.

Bacteria participate significantly in mercury transformation in natural and contaminated industrial environments. Bacterial mercury resistance is mediated by the *mer* operon, typically located on transposons or plasmids. It encodes specific genes that facilitate uptake of mercury species, cleavage of organomercurials (MerB), and reduction of Hg(II) to Hg(0). Bacterial reduction of Hg(II) can also occur at concentrations too low to induce *mer* operon function by a dissimilatory metal-reduction mechanism.

Expression of *mer* genes is mediated by MerR, a metal-responsive transcriptional regulator. *In vitro* studies have shown that MerR forms a nontranscribing pre-initiation complex with RNA polymerase and the promoter DNA. Binding of Hg(II) induces conformational changes in MerR and other components of the complex, resulting in the transcription of *mer* operon genes. We have used small-angle scattering techniques to study the regulatory mechanism of MerR in the presence and absence of Hg(II). Our results show that in the presence of Hg(II) the MerR dimer undergoes a significant reorientation from a compact state to a conformation revealing two distinct domains. Molecular dynamics simulations on MerR homology models can be combined with molecular envelope shapes obtained from small-angle scattering experiments to yield detailed structural information. Molecular mechanics (Charmm22) force-field parameters have been developed for simulating the trigonal planar Hg(II) coordination geometry observed in Hg(II)-bound MerR.

The bacterial organomercurial lyase, MerB, catalyzes the demethylation of a wide range of organomercurials via Hg-C protonolysis. We have investigated the two major proposed reaction mechanisms of MerB using quantum chemical density functional theory calculations. A model of the active site was constructed from an x-ray crystal structure of the Hg(II)-bound MerB complex. The calculations support a mechanism in which Cys159 forms an initial covalent adduct with MeHg. Cys96 donates a proton to Asp99 and coordinates with Hg(II). Asp99 then protonates the organic leaving group to cleave the Hg-C bond and release the hydrocarbon reaction product. Two other substrates, vinylmercury and *cis*-2-butenyl-2-mercury, were also tested, and the computed activation barriers for the three organomercurial substrates reproduces the trend in experimentally determined reaction rates.

Dissimilatory metal-reducing bacteria, such as *Shewanella* and *Geobacter*, are able to reduce Hg(II) in the presence of mineral oxides. This process has been linked to the activity of outer-membrane multi-heme cytochromes. We have isolated and purified the decaheme outer-membrane cytochrome OmcA from *Shewanella oneidensis* MR-1 and characterized its envelope shape in solution by small-angle X-ray scattering (SAXS). Low-resolution structural features were identified and indicate an elongated shape. X-ray crystallography trials are currently under way in an effort to identify potential molecular interaction sites for Hg(II) reduction.

This Science Focus Area (SFA) Task at ORNL will continue to strive for a fundamental understanding of subcellular processes that profoundly influence mercury speciation and to determine mechanisms at atomic detail concerning mercury transformation, using structural biology and high-performance computer simulation.

## Mercury Speciation Using Piezoresistive Microcantilever Array Sensors

*Session: ORNL SFA (Laboratory Research Manager: Liyuan Liang)*

Adam F. Bange, Steven S. Brown, Tony Palumbo, and Thomas Thundat (lead PI)—  
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Mercury contamination is a significant environmental problem, as mercury and its compounds are implicated in a huge range of both acute and long-term health effects. Alkyl mercury compounds, and methylmercury in particular, are of particular concern, because they are water soluble, acutely toxic, and can be concentrated in predator organisms—they are not readily eliminated from tissue. As part of a multifaceted research program to study the production, transport, and demethylation of methylmercury, this research represents the development of a high-throughput sensing system that can be used to monitor the rise and fall of methylmercury levels in microbial samples. Most existing methods for determining alkylmercury, and methylmercury in particular, require the collection of samples followed by analysis in a laboratory. Some of the most sensitive methods used for determining methylmercury involve a chromatographic separation followed by cold vapor atomic emission spectroscopy, while other established methods include GC, GC-MS, and voltammetric electro-analytical techniques.

A recently proposed sensing system uses a microcantilever functionalized with a selective coating. Microcantilever sensors have been shown to be effective sensors for a wide variety of analytes in the gas and liquid phase. Microcantilever sensors operate on the principle that a chemical interaction confined to one side of the cantilever will result in a differential stress, which can be measured using a variety of methods, including optical and piezoresistive methods. Using piezoresistance as a method to measure the deflection of a microcantilever beam has many advantages; perhaps the most important practical advantage is that it is miniature. This permits the detector to be part of a very compact device and also allows large numbers of cantilevers to be used in arrays for high-throughput applications. Other advantages include simple electronics for readout and low cost.

Work has been done towards developing a high-throughput sensor system for the detection and speciation of alkylmercury compounds. A piezoresistive microcantilever sensor array platform is used, since it can be easily multiplexed for multiple simultaneous analyses. Piezoresistive microcantilevers were functionalized with 1,6 hexanedithiol self-assembled monolayers and used to detect methylmercury in milliliter-volume aqueous samples using flow injection. Adsorbed methylmercury resulted in a stress change on the cantilever beam with a detection limit of 1 ng/mL. Experiments are presently under way to improve the selectivity and sensitivity of cantilever-based detection.

## ERSP SFA for Pacific Northwest National Laboratory: Role of Microenvironments and Transition Zones In Subsurface Reactive Contaminant Transport

*Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)*

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The PNNL Scientific Focus Area (SFA) is resolving critical Hanford and basic subsurface science issues through integrated, multidisciplinary, science-theme focused research on the role of microenvironments and transition zones in the reactive transport of technetium (Tc), uranium (U), and plutonium (Pu). Microenvironments are small domains within larger ones that exert a disproportionate influence on subsurface contaminant migration. Transition zones are field-scale features where chemical, physical, or microbiologic properties change dramatically over meter-scale distances. Microenvironments and transition zones can dominate subsurface contaminant reactivity, with strong effects resulting from the coupling of chemical reaction, physical transport, and microbiologic processes. Past EMSP and NABIR research has documented the importance of these zones at the Hanford site.

The overall goals of the SFA are to develop: (1) an integrated conceptual model for microbial ecology in the Hanford subsurface and its influence on contaminant migration; (2) a fundamental understanding of chemical reaction, biotransformation, and physical transport processes in microenvironments and transition zones; and (3) quantitative biogeochemical reactive transport models for Tc, U, and Pu that integrate multiprocess coupling at different spatial scales for field-scale application. Targeted contaminant chemical reaction and biotransformation processes include heterogeneous/biologic electron transfer, precipitation and dissolution, and surface complexation. The SFA includes lab- and field-based research, coupled computation and experimentation using relevant physical/biologic models, and sediments and microbial isolates from various Hanford settings to explore molecular, microscopic, and macroscopic processes underlying field-scale contaminant migration. It is also refining geophysical techniques and modeling approaches to define, characterize, and map spatial structures and reactive transport properties of microenvironments and transition zones in the field. The SFA is closely coordinated with the Hanford 300A Integrated Field Research Challenge (IFRC) where relevant samples are obtained, and well access exists to subsurface vadose and groundwater zones where microenvironments and transition features exist and are important. The SFA utilizes numerous capabilities within PNNL's Environmental Molecular Sciences Laboratory (EMSL) for materials characterization and evaluation of important mechanisms at different scales.

The projects fall in the following science categories: (1) microbial ecology, (2) molecular-scale mechanisms, (3) pore-scale coupled processes, (4) reactive transport science, (5) multiscale reactive transport models, and (6) *in situ* structures and reactive transport properties. Cohesiveness and integration will be achieved by focus on SFA hypotheses formulated to enhance information upscaling, resolution of broader science issues, and Hanford impact.

## **Impact of the Local Environment and Electron-Transfer Initiated Transformations of the Structure and Chemical Properties of Mineral and Contaminant Microparticulates**

*Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)*

Donald R. Baer, Pacific Northwest National Laboratory (lead PI), Kenneth Krupka, Pacific Northwest National Laboratory, R. Lee Penn, University of Minnesota

The objective of this project is to obtain fundamental information about reactivity, structural, chemical, and compositional transformations of sediment- and biogenic-derived metal oxide microparticles induced by electron-transfer reactions and other interactions with their local environment. The initial phase of this activity is focusing on the isolation, identification, and characterization of Fe(II), Fe(II/III), Mn(III/IV) and Ti containing microparticulates in Hanford sediments of different ages and facies types. This is essentially a census of the relevant materials and includes assessing existing information about these phases and isolating materials for detailed studies. The second phase will involve comparison of the reactivity, the isolated particles, and the performance of mechanistic reactivity studies on isolated microparticles, using probe molecules (such as carbon tetrachloride and Tc(VIII)) to probe electron-transfer reactions and particle transformations. Impacts of surface precipitation and surface passivation on particle reactivity and the time dependence of reactivity will be examined. The nature of the reactive surfaces, the accessibility of reactive sites, and the structures of the surface and interface layers (or whole particles) differ for different particles and can change significantly as particles respond to and react with their local environment (e.g., surface structure alterations, phase changes, and passive layer formation).

Current work is focusing on three activities. First, existing information about the mineralogy, composition, and distribution of Fe, Mn, and Ti particulates in sediment samples from the Hanford Site is being collected and analyzed. Second, work is under way to determine and apply appropriate methods for separating and isolating the microparticulates of interest from Hanford sediments without altering their reactive properties. Initial separation studies conducted at the USGS with Dr. Andrew Grosz, using size fractionation, density and magnetic separation techniques, demonstrate that a wide variety of mineral particle types, surface coatings, and mineral aggregation is present in test sediment samples from the Hanford 200 and 300 Areas. To quickly assess significant differences in chemical reactivity, a rapid screening process is being developed.

Studies in this project directly tie to other PNNL ERSP SFA projects on electron transfer (Rosso), microscopic mass transfer (Liu) and reactive transport (Zachara) studies and link to a BES Geosciences Project, "Reaction Specificity of Nanoparticles in Solution," which seeks a fundamental understanding of the mechanism(s) that control overall reactivity and fate of Fe-based nanoparticles.

This research is part of the ERSP SFA at Pacific Northwest National Laboratory.

## An Integrated Approach to Quantifying the Coupled Biotic and Abiotic Mechanism, Rates, and Long-Term Performance of Phosphate Barriers for *In Situ* U Immobilization

Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)

Jeremy Fein, University of Notre Dame, Dawn Wellman, Pacific Northwest National Laboratory (lead PI), A.L. Miracle, PNNL, E.M. Pierce, PNNL, D.H. Bacon, PNNL

The chemical stability of phosphate minerals (e.g., autunite) is a seminal issue governing the success of phosphate-based remediation for *in situ* immobilization of uranium. There is limited understanding about the thermodynamic stabilities and durabilities of phosphate minerals, and about the effect of microorganisms on the precipitation reactions. We hypothesize that bacteria can exert a strong influence on both the precipitation and dissolution processes of these minerals, thereby affecting the longevity of phosphate remediation technology for *in situ* remediation of uranium. The objectives of this investigation are to: (1) determine the effect of dominant microbial metabolites on the long-term durability of autunite, (2) measure and compare the thermodynamic stabilities of the most likely uranyl phosphate phases to form in phosphate-amended groundwater systems, (3) determine the effects of bacteria on the extent, mineralogy, and morphology of precipitation of uranyl phosphate minerals, and (4) incorporate a kinetic rate equation into current reactive transport codes to account for the biotic contribution to autunite weathering.

*Effect of Hanford Micro-organisms on the Dissolution Kinetics of Autunite and Apatite* A series of single-pass flow through tests were conducted utilizing a natural microbial extract from the Hanford site in the presence of calcium meta-autunite,  $\text{Ca}_2[(\text{UO}_2)(\text{PO}_4)]_2 \times 3\text{H}_2\text{O}$  to quantify the effect of microbial metabolites on the dissolution kinetics of apatite and autunite, under the pH range of 7 to 10 and temperature range of 5 to 90°C. These results are being combined with solubility results to expand the kinetic rate equation and account for the biotic contribution to autunite and apatite weathering in current reactive transport codes, enabling more accurate predictions of the long-term fate of phosphate amendments for the *in situ* immobilization of uranium.

*Abiotic and Biotic Solubility of Autunite and Apatite.* A series of solubility experiments are being conducted in the presence of the bacterial consortia, cultured from 300 Area aquifer sediment samples. The inorganic solubilities of phosphate minerals serve as baselines against which we can compare the effects of microorganisms. We used solubility measurements to determine thermodynamic properties of the uranyl phosphate phases autunite, uranyl hydrogen phosphate, and uranyl orthophosphate. Conducting the solubility measurements from both supersaturated and undersaturated conditions and under different pH conditions rigorously demonstrates attainment of equilibrium and yields well-constrained solubility product values. We used the solubility data, in conjunction with calorimetry data, to calculate standard-state Gibbs free energies of formation and standard-state enthalpies of formation for these uranyl phosphate phases. Combining these results allows us also to calculate the standard-state entropy of formation for each mineral phase. These results are part of a combined effort to develop reliable, internally consistent thermodynamic data for environmentally relevant uranyl minerals. Such data are required to optimize and quantitatively assess the effect of phosphate amendment remediation technologies for uranium contaminated systems.

*Effect of Bacteria on the Precipitation of Uranyl Phosphate Minerals.* The effects of microorganisms on extent of precipitation and the precipitation mechanisms of uranyl phosphates are unknown; these effects can control the ultimate mobility and fate of U in the subsurface. We conducted bacteria-bearing precipitation experiments and abiotic controls under identical chemical conditions in 0.1 M  $\text{NaClO}_4$  at pH 4.5, with extent of supersaturation controlled by varying both dissolved U and P concentrations. Biotic systems contained nonmetabolizing *Bacillus subtilis* cells, a gram-positive aerobic soil bacterial species. The samples were allowed to react for 2 h, and then were centrifuged to separate the solid and aqueous phases. The extent of precipitation was determined by analyzing the supernatant for remaining aqueous U and P. The solid phases were characterized by TEM, XANES, and EXAFS. The results indicate that bacteria can exert a large effect on the extent, mineralogy, and morphology of the precipitates. The abiotic systems precipitated two distinct micron-scale uranyl phosphate phases with similar crystal sizes under all conditions studied. Conversely, the biotic systems exhibited less U removal than the abiotic controls, and precipitated only one dominant uranyl phosphate phase. Under low P concentrations, there was no evidence of a direct impact of cell walls with precipitation. However, with increasing P concentration, precipitation became located within the cell wall, and nanocrystals of the uranyl phosphate were formed. This study offers the first evidence of the cell wall's direct influence on nonmetabolic precipitation, a mechanism likely resulting from interaction between aqueous uranyl cations and cell-wall phosphate sites. Formation of nanoparticulate uranyl phosphates could greatly affect the mobility and fate of U in contaminated systems subject to polyphosphate remediation.



## **Biomolecular Studies of Microbiological Processes Controlling Contaminant Fate and Transport from Hanford 300 Area Sediments**

*Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)*

Alexander Beliaev, Pacific Northwest National Laboratory (lead PI), Matthew Marshall, Pacific Northwest National Laboratory

This project focuses on genetic and physiological characterization of Hanford site-relevant subsurface microbial isolates and consortia. Emphasis is placed on single-organism investigations to understand the molecular mechanisms involved in metal and radionuclide biotransformation. Consistent with the overall scope of the project, initial studies to isolate relevant subsurface microbial isolates are currently in progress using materials derived from the Microbial Ecology project. Sediment samples from three depths of the “deep characterization borehole”, Well 399-2-25 (C6209), were obtained and added to a series of enrichment tubes to isolate relevant microorganisms that are able to reduce Fe(III)- and Mn(IV)-oxides either directly or indirectly. The enrichments were transferred into a defined minimal media containing synthetic groundwater based on the composition of Hanford IFRC 300 Area groundwater and amended with either synthetic ferrihydrite (FH) or MnO<sub>2</sub> as the sole electron acceptor. At each sediment depth, enrichment tubes were provided with one of fourteen different electron donor sources to stimulate growth and activity of subsurface organisms capable of dissimilatory metal reduction. After multiple (1%) transfers, we have determined that at least eight of the electron donors selected are capable of serving as electron donor sources for the reduction of FH. Several tubes reveal the biotransformation of FH to a mineral phase with ferrimagnetic properties, suggestive of biomagnetite. While some electron donors appear to rapidly stimulate FH reduction (<5 days), others require significantly longer (~60 days) to observe biotransformation of the FH. Currently, we are analyzing the consortia in each of the positive enrichments to isolate pure organisms. As isolates are obtained, emphasis will first be placed on characterizing the relative rates of biotransformation for both metal and radionuclides. Subsequent studies will be designed to identify genes, pathways, and subsystems involved in those key reactions, which will ultimately result in developing a comprehensive cross-species model of biological mechanisms involved in contaminant biogeochemical transformations with site-relevant organisms.

## Plutonium Contamination Issues in Hanford Soils and Sediments

*Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)*

Andrew R. Felmy, Pacific Northwest National Laboratory, EMSL (lead PI), Steven Conradson, Los Alamos National Laboratory

This project focuses on the identification of the extent, chemical nature, and depth distribution of plutonium (Pu) contamination at the Hanford site. The overall objective is to provide a baseline of knowledge on Pu contamination issues that can form the basis for future detailed research studies into possible mechanisms of subsurface migration. Specific activities include: (1) a comprehensive and ongoing literature review of Pu contamination in both soils and groundwaters across the entire site, as well as the chemical nature of discharged Pu-containing wastes; (2) obtaining and archiving samples from previous field studies of Pu contamination at Hanford; and (3) wherever possible, screening studies to determine the possible form of Pu in the archived materials. In FY09, the project initiated the comprehensive literature review, obtained key field samples from the surface and deeper regions beneath the Hanford Z-cribs, and performed initial characterization studies of these materials. The Z-cribs received effluent generated from plutonium production activities at the Z-plant (i.e., Plutonium Finishing Plant). During that time, it is estimated that the Z cribs received more than 4.5 billion liters of liquid waste containing more than 168 kg of plutonium. Such discharges to the Z-cribs represent one of the largest known sources of plutonium contamination in the country. A complete summary of the subsurface Pu contamination in the Z-9 and Z-12 cribs will be presented, along with our initial XAS analysis of the subsurface materials (performed by SD Conradson).

## Biogeochemical Redox Transformations in Hanford 300 Area Subsurface Sediments

Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)

James K. Fredrickson, Pacific Northwest National Laboratory (lead PI), Ji-Hoon Lee, Pacific Northwest National Laboratory, David W. Kennedy, Pacific Northwest National Laboratory, Andrew E. Plymale, Pacific Northwest National Laboratory, Allan E. Konopka, Pacific Northwest National Laboratory, John M. Zachara, Pacific Northwest National Laboratory

As part of PNNL's Scientific Focus Area "Role of Microenvironments and Transition Zones in Reactive Contaminant Transport," this project is characterizing biogeochemical processes in Hanford 300 Area subsurface sediments with an emphasis on defining redox reactions at the pore scale projected to influence the subsurface fate and transport of U and Tc. In coordination with the Hanford Integrated Field Research Challenge (IFRC), a series of subsurface core samples were collected during July 2008 from the 300A IFRC site as part of site characterization efforts and installation of the experimental well field. Sediments were collected from the saturated zone within the Hanford formation as well as from a fine-grained unit in the upper Ringold that forms a confining layer at the base of the Hanford formation (~56 ft). Within the upper Ringold, there is a sharp redox boundary at a depth of approximately 60 ft, as evidenced by a distinct color change from light tan (oxidized) to greenish blue (reduced) with a concomitant increase in weak HCl-extractable (0.5 N, 24 h) Fe(II) from 15 to 136  $\mu\text{mol/g}$ , respectively. To investigate potential biogeochemical redox processes, sediment microcosms ( $\text{N}_2$ -headspace) were amended with a mix of organic electron donors (acetate, lactate, and glucose) in a synthetic groundwater (that included 0.6 mM sulfate) to which nitrate (2 mM), ferrihydrite (2 mM), or no exogenous donor were added. Nitrate was reduced over an ~10-day period in the Hanford and Ringold oxidized sediment, but more slowly (>20d) in the Ringold reduced sediment. The concentration of 0.5 N HCl-extractable Fe(II) in the Hanford sediment was 1.9 mM in the live sediment compared to 0.4 mM in the sterile control, following a 32-day incubation period. There was little evidence for microbial Fe reduction in either the Ringold oxidized or reduced sediments over the same time period. Interestingly, the concentration of HCl-extractable Fe(II) was the same in the Hanford sediment regardless of whether the microcosms were amended with ferrihydrite, suggesting that either native Fe(III) was being reduced or that microbial activity was modifying Fe(II)-bearing mineral phases (i.e., phyllosilicates), increasing the extractability of Fe(II) by weak HCl. In addition, upon extended (45 day) incubation in microcosms with no exogenous electron acceptor (other than sulfate associated with the synthetic groundwater), sediments turned dark, suggesting further reduction, and aqueous sulfide was detected at low concentrations (5  $\mu\text{M}$ ). In spite of groundwater from the Hanford formation sampled from the IFRC well field being oxic (~5 mg/L  $\text{O}_2$ ), the biogeochemical potential exists for anaerobic processes using nitrate, sulfate, and potentially Fe(III) as terminal electron acceptors. We hypothesize that anaerobic biogeochemical processes are spatially and/or temporally heterogeneous, occurring in microenvironments and transition zones within the aquifer where advective transport of oxygenated groundwater is limited. Current and near-term investigation will focus on further characterizing the redox reactions and reaction products in laboratory experiments and investigating redox biotransformations via *in situ* experiments at the Hanford 300A IFRC site.

This research is part of the ERSF SFA at Pacific Northwest National Laboratory.

## Microbial Ecology in Subsurface Sediments from Hanford 300A Area

*Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)*

Allan Konopka, Pacific Northwest National Laboratory (lead PI); Xueju Lin, Pacific Northwest National Laboratory; David Kennedy, Pacific Northwest National Laboratory; Jim Fredrickson, Pacific Northwest National Laboratory; Rob Knight, University of Colorado; Mary Lipton, PNNL

One goal of the PNNL Scientific Focus Area is to develop a conceptual model for microbial ecology in Hanford's unconfined aquifer with specific emphasis on implications for long-term contaminant fate and transport. The first essential element is a characterization of microbial biomass, phylogenetic diversity, and biogeochemically-relevant activities. In coordination with the Hanford IFRC, core samples were collected during drilling of the 52 m "deep characterization borehole" (Well C6209) in July 2008. Detailed analyses were made on 17 strata traversing multiple geological formations. The samples included coarse-grained sediments from the oxic-saturated zone of the Hanford formation (0-10 m), the interface (18 m) between the Hanford and the fine-grained upper Ringold Formation, and the transition from oxic to reduced sediments within the Ringold formation. Microbial cell counts ranged from  $1-50 \times 10^7$  per g, with highest counts found in the highly transmissive Hanford sediments. In samples from 0-19 m depth, 8-30% of the cells were metabolically responsive (in a 3 h incubation), with the highest proportion present within 2 m of the Hanford-Ringold interface. From 30-52 m, reactive cells declined to less than 3%. Archaea comprised 0-8% of 16S rRNA gene copy numbers detected in samples. Several physiological groups were quantified via real time PCR. The *nosZ* gene, associated with denitrification, was quite prevalent, with an abundance of 5-17% relative to total 16S gene copy number below 18.3 m and <5% in samples above 18.1 m. Sulfate-reducing bacteria (*dsrA* gene) ranged from below detection to 7% of total 16S rRNA genes in Ringold sediments, and were near the detection limit in Hanford sediments. Detection of metal-reducing *Geobacteraceae*, *Anaeromyxobacter* or *Shewanella* was sporadic, and their abundance was relatively low. The analyses suggest that the highly transmissive Hanford formation and the Ringold Formation near its interface with the Hanford are regions of substantial microbial abundance and potentially high metabolic activity. Archaeal and Bacterial 16S rRNA genes have been amplified from environmental DNA. Clone libraries have been constructed and are in the process of being sequenced at the Joint Genome Institute. In addition, >90 samples of environmental DNA have been isolated from 27 of 34 wells (spaced 10 m apart) in the IFRC well array. These samples will be analyzed by pyrosequencing of 16S rRNA gene amplicons, to interrogate lateral changes in microbial diversity and relative abundance. A significant challenge in applying proteomics to microbial community analysis is their removal from sediment particles. Physical-chemical properties of the proteins and the sediment particles, as well as the physiological state of the microbes, all impact the effective removal of proteins. Individual amino acids have been tested to passivate nonspecific protein binding sites; they are too small to interfere with mass spectrometric analysis. Positively charged amino acids were effective in enhancing protein recovery. In the future, tests will be conducted with salts, chelating agents, surfactants, and chaotropic agents.

This research is part of the ERSF SFA at Pacific Northwest National Laboratory, and has been done in collaboration with the Hanford IFRC project.

## Grain-Scale Mass Transfer of U(VI) in Contaminated Sediments

*Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)*

Chongxuan Liu, Pacific Northwest National Laboratory (lead PI), Zhenqing Shi, Pacific Northwest National Laboratory, Sebastien N. Kerisit, Pacific Northwest National Laboratory, Xiaohong S. Li, Pacific Northwest National Laboratory, John M. Zachara, Pacific Northwest National Laboratory

Uranium in subsurface sediments can concentrate in microenvironments where mass fluxes are dominated by diffusive mass transfer. The objectives of this research are to: (1) investigate grain-scale mass transfer in controlling U(VI) desorption, (2) evaluate a multirate, surface complexation model (SCM) for its applicability in describing the mass-transfer rates under variable geochemical conditions that affect U(VI) aqueous and surface complexation reactions, and (3) provide microscopic and molecular insights for the observed phenomena.

A flow-cell reactor was used to investigate the grain-scale mass transfer during U(VI) desorption from a contaminated sediment collected from Hanford 300A. Three influent solutions of variable pH, Ca, and carbonate concentrations that affected U(VI) aqueous and surface speciation reactions were used under dynamic flow conditions to evaluate the effect of geochemical conditions on the rate of U(VI) desorption. The measured rate of U(VI) desorption varied with solution chemical composition, which evolved as a result of thermodynamic and kinetic interactions between the influent solutions and sediment. The solution composition that led to a lower equilibrium U(VI) sorption to the solid phase yielded a faster desorption rate. Experimental results were used to evaluate a multirate SCM model to describe U(VI) desorption kinetics from the sediment containing complex, sorbed U(VI) species in mass transfer limited domains. The model was modified by including a multirate, ion exchange model to describe solution-sediment interactions. With the same set of model parameters, the modified model described the evolution of major ions and the rates of U(VI) desorption under variable geochemical conditions reasonably well, implying that the multirate SCM is an effective approach to describe U(VI) desorption kinetics in subsurface sediments. The rate constants in the multirate model followed a log-normal distribution, ranging from  $10^{-6}$  to  $10^1$  h<sup>-1</sup>, reflecting a heterogeneous nature of the mass transfer properties for the U(VI) surface complexation.

Pore-size distribution and connectivity in the sediment were measured to provide insights for the multirate behavior of U(VI) adsorption/desorption. Pore-size distribution in the sediment was determined using BET-N<sub>2</sub> sorption isotherms. The derived pore size in the sediment followed a log-normal distribution, consistent with the distribution of the multirate constants for U(VI) adsorption/desorption. Pore connectivity in the sediment was derived by analyzing BET-N<sub>2</sub> sorption/desorption hysteresis data using the percolation theory. The results revealed a complex pore network in controlling the diffusive mass transfer in the sediment.

Molecular dynamics (MD) simulations have been performed to calculate ion diffusion coefficients of U(VI) species to provide insights into diffusive mass transfer in porous media. The diffusion coefficients of  $\text{UO}_2^{2+}$ ,  $\text{CaUO}_2(\text{CO}_3)_3^{2-}$ ,  $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3(\text{aq})$ ,  $\text{UO}_2\text{CO}_3(\text{aq})$ ,  $\text{UO}_2(\text{CO}_3)_2^{2-}$ ,  $\text{UO}_2(\text{CO}_3)_3^{4-}$ , and  $\text{MgUO}_2(\text{CO}_3)_3^{2-}$  have been calculated. The value of the calculated diffusion coefficient linearly decreases with increasing molecular size, while the effect of species charges on individual diffusion coefficient is negligible. Research is ongoing to link molecular diffusion coefficients, pore size, and pore network to provide insights into the multirate behavior of U(VI) adsorption/desorption at different scales.

This research is part of the ERSF SFA at Pacific Northwest National Laboratory.

## Molecular Scale Mechanisms of Biogeochemical Electron Transfer Underlying U/Tc Valence Transformation and Stability at Hanford

*Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)*

Kevin M. Rosso, Pacific Northwest National Laboratory (PNNL) (PI), Odeta Qafoku, PNNL, Juan Liu, PNNL, Nicholas S. Wigginton, Ecole Polytechnique Federale de Lausanne, Sebastien Kerisit, PNNL, Liang Shi, PNNL, Zheming Wang, PNNL, Timothy Droubay, PNNL, Michael Toney, Stanford Synchrotron Radiation Laboratory, Tanya Peretyazhko, PNNL, Jim K. Fredrickson, PNNL, John M. Zachara, PNNL

The disposition of contaminant U and Tc at the Hanford site is dependent on local redox conditions and biogeochemical processes that establish or modify redox gradients in subsurface microenvironments. For example, intragrain fractures and microporosity in lithic fragments in Hanford formation sediments are thought to be one important reducing microenvironment, due to the presence of Fe(II)-bearing mineral phases that can consume oxygen and/or reduce contaminants. The premise is that the parameterization of needed pore-scale and coupled mass-transfer biogeochemical models describing fluxes of ions and charge equivalents through such microenvironments will require that reaction rates have been determined and that mechanisms are well understood. This project has the objective to supply such an understanding for key abiotic and microbial electron-transfer reactions that transform U, Tc, and relevant forms of iron in Hanford-specific microenvironments. Reactions selected for detailed study are coordinated with pore-scale biogeochemistry, diffusive mass transfer, and microbiological projects within the SFA at PNNL. Our initial focus is on determining mechanisms and kinetics for heterogeneous reduction of U by Fe(II)-bearing mineral phases in Hanford sediments, and for microbial reduction of mineral forms of Fe(III).

Fe(II)-bearing mineral phases prospectively capable of reducing Tc(VII) in intragrain regions of basalt lithic fragments include magnetite ( $\text{Fe}_3\text{O}_4$ ) and titanomagnetite ( $\text{Ti}_x\text{Fe}_{3-x}\text{O}_4$ ) of varying Ti content ( $0 < x \leq 1$ ). Natural specimens from the Hanford site will be collected and used in conjunction with model mineral materials for Tc(VII) reduction experiments. A complete solid solution exists between magnetite ( $x = 0$ ) and ulvöspinel ( $x = 1$ ) at crystallization conditions in basalt. Ti(IV) substitutes for Fe(III) in the octahedral sublattice yielding concomitant increases in lattice Fe(II) concentration. Therefore, in principle, reducing equivalents available for Tc(VII) reduction in intragrain locations are dependent on the Ti-content. Use of model mineral materials possessing well-understood structure, composition, and morphologic characteristics provides unique access to molecular-scale mechanisms. We have synthesized titanomagnetite in two complementary morphologic forms, epitaxial thin films and nanoparticles. Films will enable detailed insight into surface structure and compositional changes, whereas nanoparticles will enable batch measurements of Tc(VII) concentration change in solution. Morphologically regular and relatively monodisperse nanoparticles in the 10-20 nm size range, of composition  $x = 0, 0.25, 0.50,$  and  $0.75$ , were successfully synthesized by an aqueous solution protocol. For the first time, an epitaxial thin film of composition  $x = 1.0$  was also successfully grown, using pulsed laser deposition in the Environmental Molecular Sciences Laboratory at PNNL. We will present on the approach, characteristics of these materials, and plans for their use in Tc(VII) reduction experiments.

For the purpose of understanding rates of Fe(II) supply by microbial Fe(III)-oxide reduction, we have also performed measurements and developed molecular models of electron-transfer kinetics between model cytochromes and solid surfaces, including surfaces of  $\alpha\text{-Fe}_2\text{O}_3$  hematite. We will report findings from a multi-method approach, including use of protein film voltammetry, scanning tunneling microscopy, and synchrotron-based x-ray reflectivity, to understand the structure and electron-transfer properties of the periplasmic small tetraheme cytochrome covalently linked to Au electrode surfaces. We will contrast these initial measurements with analogous ones under way using an epitaxial hematite thin film as the electrode. The findings show that molecular orientation, distance of separation, and structural properties of the solid surface are all important factors governing the electron-transfer rate. Rates of Fe(III) reduction by this mechanism are sufficiently slow to hypothesize that supply of reactive Fe(II) from this biogeochemical pathway is limited by electron transfer at the microbe-mineral interface. This research is part of the ERSF SFA at Pacific Northwest National Laboratory.

## Multiscale Reactive Transport Modeling for the PNNL ERSP Science Focus Area

*Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)*

Tim Scheibe, Pacific Northwest National Laboratory (lead PI), Marshall Richmond, Pacific Northwest National Laboratory, Mark Rockhold, Pacific Northwest National Laboratory, Brian Wood, Oregon State University

Reactive transport simulation models (RTMs) serve as a critical integrating element among the various experimental elements of the SFA, in particular integrating information from fundamental process scales (molecular, subpore, and pore) to phenomenological prediction scales (column, field). RTMs provide support for experimental design, are being updated based on new experimental results, and will support postexperimental analysis and interpretation. Our work is focused on the Hanford IFRC, for which we are developing a field-scale model (in cooperation with IFRC researchers) that will systematically incorporate information from SFA experiments at smaller scales. Currently, there remains a gap between scientific understanding at fundamental scales and simulation at field scales that leads to an unsatisfactory degree of model and parameter empiricism in many field-scale simulations. Quantitatively linking across this wide range of physical scales with minimal empirical calibration is an ambitious goal, one that is being enabled by strong coordination of SFA research and ongoing computational advances. Work reported in this poster is focused on three aspects of pore-scale simulation: (1) incorporation of reactive transport processes relevant to uranium transport at the IFRC into our 3D smoothed particle hydrodynamics (SPH) code; (2) incorporation of numerical volume averaging solution methods into our 3D computational fluid dynamics (CFD) code; and (3) testing and cross-validation of the SPH and CFD codes. At the field scale, we are solidifying our conceptual model of uranium transport at the IFRC and developing a preliminary 3D transport model. We will develop a field-scale model of the nature and spatial heterogeneity of categorical sediment types (“hydrofacies”) in conjunction with IFRC researchers and supported by geophysical characterization data. For each hydrofacies, we will define characteristic particle- and pore-size distribution functions, pore-scale geometry and mineralogy by direct observation (e.g., EMSL and synchrotron facilities), and simulation of synthetic pore geometries. Pore-scale simulations based on these defined pore geometries will be rigorously upscaled to define porous-media-scale models and parameters for each hydrofacies. The upscaled models will be validated against column-scale reactive transport experimental data, and will be applied to simulate field-scale reactive transport at the Hanford IFRC. Model uncertainties associated with sparse characterization data will be evaluated using stochastic simulation methods, and field-scale predictions will be compared to IFRC experimental observations (without empirical parameter fitting) to test the models’ predictive power. Our modeling activities will initially focus on physical and chemical mass-transfer processes involved in uranium transport, and will subsequently be expanded to include other target contaminants and biological mass transformations (including metabolic functions of, and mass transfer limitations within, microbial communities) as SFA characterization of Hanford-specific conditions proceeds.

## Functional Characterization of *Shewanella* Proteins Important for Extracellular Electron Transfer

Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)

Liang Shi, Pacific Northwest National Laboratory (lead PI), David J. Richardson, University of East Anglia, Thomas A. Clarke, University of East Anglia, Zheming Wang, Pacific Northwest National Laboratory, Matthew J. Marshall, Pacific Northwest National Laboratory, John M. Zachara, Pacific Northwest National Laboratory, James K. Fredrickson, Pacific Northwest National Laboratory

The overall objective of this research project is to understand the molecular mechanisms by which microbial macromolecules (e.g., redox proteins) engage and react with uranium (U) and technetium (Tc) contaminants and with redox-active metals (e.g., Fe, Mn) with which they interact. The research has initially focused on the proteins used by the dissimilatory metal-reducing bacterium *Shewanella oneidensis* MR-1 for extracellular reduction of U(VI), Tc(VII), and Fe(III) oxides. In the future, the focus of this project will be shifted to characterization of the biomolecules identified from Hanford-relevant microorganisms (Konopka & Beliaev projects). The characterization results will be used in the development and evaluation of molecular model of electron transfer (ET) developed by the Rosso project. In turn, the predictions of the model will help generate new hypotheses and design new characterization experiments.

The proteins directly involved in *S. oneidensis* MR-1-mediated extracellular reduction of U(VI), Tc(VII) and Fe(III) oxides include (i) the inner membrane tetraheme *c*-type cytochrome (*c*-Cyt) CymA, (ii) the periplasmic decaheme *c*-Cyt MtrA, (iii) the outer membrane (OM) protein MtrB, and (iv) the OM decaheme *c*-Cyts (OMCs) MtrC and OmcA. To understand how electrons are translocated across the OM, we characterized a trans-OM icosaheme complex, MtrCAB. The results showed that a stable MtrAB subcomplex could assemble in the absence of MtrC, but that an MtrBC subcomplex was not assembled in the absence of MtrA. Protein film voltammetric analyses revealed the functional capacity of MtrCAB. We propose a model for the modular organization of the MtrCAB complex in which (i) MtrC is an extracellular element that mediates ET to extracellular substrates, and (ii) MtrB is a trans-OM spanning  $\beta$ -barrel protein that serves as a sheath to embed the MtrA subunit in the membrane, where it forms a trans-membrane electron delivery module that services MtrC. To the best of our knowledge, this is the first molecular model of electron translocation across bacterial OM. This basic model could also apply more widely to the mechanism for electron exchange of a number of bacterial genera with extracellular electron sources and sinks. We also crystallized MtrA and MtrF (an MtrC homolog) and the efforts for determining their molecular structures are under way. At present we have full datasets at 3Å and 5Å resolution for MtrA and MtrF, respectively, and are resolving the location of the heme groups in the protein structures. A number of new crystal forms that may diffract to higher resolution are currently being sought. Completing the determination of these structures will lay the foundation for elucidating the ET mechanisms used by these proteins for extracellular reduction U(VI), Tc(VII) and Fe(III) oxides.

We have initiated the characterizations of the *c*-Cyts from 7 *Shewanella* strains isolated from the Hanford Reach of the Columbia River (HRCR). PCR and sequencing results showed that all 7 tested strains possessed *mtrC*-like genes, while 3 strains had *omcA*-like genes and the remaining 4 strains contained *unda/unda1*-like genes, which is consistent with previous findings that MtrC genes are more conserved than OmcA genes. We have sequenced the entire coding region of an *unda/unda1*-like gene from HRCR isolate 6 (HRCR-6). The results showed that this *c*-Cyt (i) contained 843 amino acid residues, (ii) had 11 putative heme-binding sites, and (iii) was 73% and 93% identical to the UndA1 of *S. putrefaciens* CN-32 and the UndA of *S. baltica* OS223, respectively. Based on these results, we named this protein as UndA-HRCR-6. UndA-HRCR-6 could be heterologously expressed as a heme-containing protein in *S. oneidensis* MR-1 cells. We purified UndA-HRCR-6 from the membrane fraction of *S. oneidensis* MR-1 cells after its overexpression, indicating that UndA-HRCR-6 is most likely an OMC. We plan to determine (i) the ability of UndA-HRCR-6 to complement MtrC/OmcA for reducing U(VI) and Fe(III) oxides *in vivo*, and (ii) ET reactivity of UndA-HRCR-6 *in vitro*.

We have determined the ET rates from STC, a *c*-Cyt whose structure is known, to the surface of a gold electrode and will measure its ET rates to a hematite (Fe<sub>2</sub>O<sub>3</sub>) electrode. The results will be used to evaluate the ET model developed by the Rosso project. In collaboration with the Rosso team, we also plan to use an approach that couples experiments and theoretical computation to understand mechanistically the reduction kinetics of electron shuttles, such as FMN and AQDS, by MtrC and OmcA. This research is part of the ERSP SFA at Pacific Northwest National Laboratory.



## **Facies-Based Characterization of Hydrogeologic Structures and Reactive Transport Properties**

*Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)*

Andy Ward, Pacific Northwest National Laboratory (lead PI), Christopher Murray, Pacific Northwest National Laboratory, Alexandre Tartakovsky, Pacific Northwest National Laboratory, Roelof Versteeg, Idaho National Laboratory, Steve Yabusaki, Pacific Northwest National Laboratory

Heterogeneity in flow and reactive transport properties is largely controlled by the distribution of facies and can vary over a wide range of spatial scales. The overall objective of this project is to develop a robust framework for integrating physical, hydrologic, and geophysical data, with transition probability geostatistics to generate quantitative 3D facies models that reflect variability in depositional environment. There are four research lines: (1) establish a basis for using K-40, U-238, and Th-232 logs to quantify grain-size distributions and variations; (2) develop property-transfer models to predict hydraulic properties from grain-size statistics; (3) develop a robust method for merging fine-scale and large-scale heterogeneous structures; and (4) characterize factors controlling resistivity and induced polarization response in unconsolidated sediments. Laboratory gamma-energy analysis of IFRC sediments show a strong relation between natural isotope abundance and grain-size statistics derived from sieving and laser diffraction, suggesting a potential for use of gamma logs as a particle size proxy. Particle-size distribution data from IFRC samples, and information on particle shape, allow generation of realistic packs of unconsolidated sediments for use as input in a pore-scale model for predicting flow and reactive transport properties and processes. Output from these simulations was used as input to a pore-scale model of frequency-dependent electrical properties. Simulations reveal the importance of particle shape, grain surface roughness, intergranular porosity, and clay coatings on the complex resistivity and its anisotropy. This research is part of the ERSP SFA at the Pacific Northwest National Laboratory.

## Understanding the Fate of Uranium at the Hanford Site: The Role of Uranium Mineral Dissolution Kinetics

*Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)*

Dawn Wellman, PNNL (lead PI), Eric Pierce, PNNL, Diana Bacon, PNNL

Physical, hydrodynamic, microbial, and geochemical variations in the Hanford subsurface environment, as well as compositional differences in disposed contaminate waste streams, have resulted in significant variability in the processes that affect uranium migration. Past waste disposal practices within the Hanford 300 Area have resulted in significant subsurface uranium contamination, the speciation of which varies as a function of depth. In the deeper vadose zone sediments, uranium is found predominantly sorbed to phyllosilicates, chlorite, and smectite and as the discrete uranyl mineral uranophane,  $\text{Ca}(\text{UO}_2)_2[\text{SiO}_3(\text{OH})]_2 \cdot x\text{H}_2\text{O}$ . At shallower depths, EXAFS analyses suggest the major uranium-controlling phase is meta-torbernite,  $\text{Cu}(\text{UO}_2)_2(\text{PO}_4)_2 \cdot x\text{H}_2\text{O}$ . The rate and extent of uranium release has typically been investigated in laboratory column investigations using reactive mineral fragments and size fraction isolates. These investigations have observed kinetically controlled release of uranium from contaminated sediments and provide critical information on the release and transport of uranium. The observed kinetic behavior has been attributed to multiscale diffusion, mass transfer, and kinetic processes, including uranium mineral dissolution. However, the complex kinetic processes coupled with intragrain diffusion and solubility behavior, as the uranium concentration fluctuates in these microenvironments, confounds the ability to isolate the rates attributed to individual processes. The complex kinetic relationship that results from the coupling of diffusion and dissolution under dynamic conditions requires independent quantification of the respective rate parameters. The objective of this investigation is to develop parametric rate laws to define the dissolution kinetics of uranophane and meta-torbernite under environmental conditions relevant to the Hanford 300 Area. A series of single-pass flow-through tests were conducted utilizing natural meta-torbernite and synthetic uranophane, respectively, to quantify the effect of pH, temperature, flow rate, and solution saturation state, under the pH range of 7 to 10 and temperature range of 5° to 90°C. Results of this investigation provide the necessary fundamental data to refine bulk kinetic parameters, currently being used to predict the fate of uranium, with definitive values describing the effect of changing environmental conditions on key uranium mineral phases. These results allow for pore-scale dissolution processes to be incorporated into reactive transport codes, in order to accurately predict multiscale, mass-transfer processes on the release and long-term fate of uranium in the subsurface environment.

## Radionuclide Sensors for Subsurface Water Monitoring

*Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)*

Jay W. Grate, Pacific Northwest National Laboratory (lead PI), Matthew J. O'Hara, Pacific Northwest National Laboratory, Timothy A. DeVol, Clemson University

Radionuclides in the subsurface are a persistent and vexing problem for DOE. The detection limits required are extremely challenging, and the detection of alpha and beta emitters in condensed media is difficult due to their short penetration ranges. Our objectives are to develop advanced sensor concepts, materials, and analytical approaches to enable monitoring alpha- and beta-emitting radionuclides in water, so that new analytical methods are available to support DOE missions in contaminant fate and transport, and in environmental monitoring. Our fluidic sensor designs use sorbent materials to separate radionuclides of interest from radiological and matrix interferences, simultaneously preconcentrating and localizing analytes for radiometric detection. These designs can be incorporated into monitoring systems that determine contaminants at much more frequent intervals than is feasible by field sampling and laboratory analysis. We have developed preconcentrating minicolumn sensors for Tc-99 water that can detect to levels that are ten times lower than drinking water standards of 33 Bq/L (mass equivalent concentration 0.05 ppb). Recently, we have investigated potential interferences that may exist in natural waters or as co-contaminants in plumes and have determined their effect on Tc-99 quantification. We have developed an automated standard addition method for accurate monitoring, and new functionalized scintillators by a atom transfer radical polymerization process to overcome these interferences. We have also developed a monitoring approach for Sr-90 in an automated fluidic system. The system isolates Sr-90 from water to create a pure, Y-90 free source on a separation column. After an ingrowth period, new Y-90 from Sr-90 decay is transferred to a flow cell for detection of Cherenkov radiation from the high-energy beta particles from Y-90 decay. This system can analyze for Sr-90 to levels below the drinking water standard of 0.30 Bq/L (mass equivalent concentration  $5.8 \times 10^{-11}$  ppm). We have developed a monitoring approach for tritium utilizing a proton exchange membrane (PEM) electrolysis unit and a gas-flow proportional counter. The electrolyzer uses a Nafion membrane as the electrolyte. The resultant tritiated hydrogen gas is combined with a small amount of propane (6%) put into the gas-flow proportional counter for detection of the tritium. The detection efficiency is 49%, and the minimum detectable concentration is 740 Bq/L (the drinking water standard) for a 4-hour count time. We are investigating the application of time-interval analysis of the radiation signals to increase the signal-to-noise for the detection system. Preliminary results indicate that the application of the sequential probability ratio test (SPRT) to the time-interval data significantly reduces the number of false positives relative to the fixed time-interval analysis of the data. Under the OBER STTR program, we are working with Burge Environmental to develop and deploy automated monitors for Sr-90, Tc-99, and uranium on the Hanford site. Recently, the system for Sr-90 was installed in the field.

### *Selected Recent Publications:*

- (1) Grate, J.W.; Egorov, O.; O'Hara, M.J.; DeVol, T.A., "Radionuclide Sensors for Environmental Monitoring: from Flow Injection Solid Phase Absorptiometry to Equilibration-based Preconcentrating Minicolumn Sensors with Radiometric Detection," *Chem. Rev.*, 108, 543-563 (2008).
- (2) O'Hara, M.J.; Burge, S.R.; Grate, J.W., "Automated Radioanalytical System for the Determination of  $^{90}\text{Sr}$  in Environmental Water Samples by  $^{90}\text{Y}$  Cherenkov Radiation Counting," *Anal. Chem.*, 81, 1228–1237 (2009).
- (3) O'Hara, M.J.; Burge, S.R.; Grate, J.W., "Quantification of Tc-99 in Complex Groundwater Matrixes Using a Radiometric Preconcentrating Minicolumn Sensor," *Anal. Chem.*, 81, 1068–1078 (2009).

## Reactive Transport of U and Tc in Subsurface Sediment Systems

*Session: PNNL SFA (Laboratory Research Manager: Harvey Bolton)*

John Zachara, PNNL (lead PI), Jim Davis, USGS, Jim McKinley, PNNL, Steve Heald, Argonne National Lab., Mart Oostrom, PNNL, Tanya Peretyazhko, PNNL, Phil Jardine, ORNL

As part of the PNNL SFA, this project is investigating biogeochemical reaction processes of U(VI) and Tc(VII) in different subsurface sediments from the Hanford site that are important to contaminant migration. A primary project emphasis is to understand how microscopic reaction and transport processes influence the advective transport velocities of these two solutes, through intact sediments with natural structure and heterogeneities at the macroscopic to mesoscopic scale. A pragmatic, iterative approach using refined characterization, experiment, and modeling is being taken to develop field-relevant reactive transport models involving fundamental descriptions of coupled processes.

Research in FY09–FY10 is focused on two campaigns. The first addresses the surface complexation behavior (desorption and adsorption) of contaminant U(VI) in coarse, field-textured sediments from the Hanford IFRC site that host complex intragrain domains. Uranium adsorption and desorption exhibits kinetic behavior in these materials. Experimentation begins with intact column experiments of contaminated sediments to investigate U desorption/adsorption rate and extent, and nonreactive tracer behavior under advective conditions representative of the field. The columns are deconstructed after x-ray imaging, cobble mapping, and measurement of hydrologic properties—and a rigorous suite of physical and mineralogic characterizations, and geochemical reaction measurements is performed on the <2 mm fraction. Various modeling approaches—advection, diffusion, thermodynamic, and kinetic relationships—are then evaluated for their ability to upscale reaction parameters for description of the intact system.

The second campaign addresses the reduction and oxidation behavior of Tc in a unique series of subsurface sediments collected through and below a redox transition zone in Hanford's unconfined aquifer. These sediments contain Fe(II) in complex mineral forms, including dioctahedral smectites and discrete ferrous precipitates that vary in their heterogeneous kinetic redox reactivity. Experiments use flow-through reactors to study kinetic processes. Transmission Mossbauer spectroscopy, SEM/TEM, and x-ray absorption spectroscopy are being used to characterize the identity of the reactive Fe forms and determine the molecular nature of resulting Tc(IV) redox products. Collaborations are under way with other SFA investigators investigating microbe-facilitated reactions and diffusion regulated processes. A poster will be delivered at the ERSD annual meeting that displays research progress on these campaigns.

This research is part of the ERSP SFA at Pacific Northwest National Laboratory.

## SLAC SFA: SSRL Environmental Remediation Sciences Program

*Session: SLAC SFA (Laboratory Research Manager: J.R. Bargar)*

J.R. Bargar, SLAC National Accelerator Laboratory (lead PI), R. Bernier-Latmani, Ecole Polytechnique Federale de Lausanne, H. Boukhalfa, Los Alamos National Laboratory, G.E. Brown, Jr., Stanford University, K.M. Campbell, U.S. Geological Survey, S.E. Fendorf, Stanford University, C.C. Fuller, U.S. Geological Survey, D.E. Giammar, Washington University St. Louis, E.S. Ilton, Pacific Northwest National Laboratory, A. Mehta, SLAC National Accelerator Laboratory, P. W. Reimus, Los Alamos National Laboratory, D.M. Wellman, Pacific Northwest National Laboratory

The success of *in situ* subsurface contaminant immobilization is governed by contaminant stability relationships, interfacial reactions, and the interplay of rates and hydrological factors. The mission of the SLAC SFA program is to elucidate the fundamental molecular-scale biogeochemical processes that control stability and rates, with the goal of improving subsurface remediation strategies. A key focus of this effort is stimulated uranium reduction. We are using innovative approaches, tightly integrated collaborations, and advanced analytical techniques to couple laboratory and field studies, and address first-order issues, of the DOE subsurface uranium problem. Key questions include:

How do reducing conditions immobilize uranium in the subsurface? Uraninite is the most desirable product of stimulated uranium reduction, because it is orders-of-magnitude less soluble than most other U species. Little is known about the behavior of biogenic uraninite in the subsurface and the fundamental factors that control its stability. Recent studies suggest that bioreduced U(IV) may also occur as noncrystalline products. The SLAC SFA will elucidate and quantify the biogeochemical controls that govern the stability and rates of corrosion of biogenic uraninite in the subsurface. We will also investigate the nature of noncrystalline reaction products and the factors that impede formation of the more desirable uraninite products.

How does Fe redox cycling enhance or hinder stimulated uranium reduction? Reduction of abundant but poorly crystalline ferrihydrite grain coatings and colloids, strong sorbents for U(VI), produces more stable phases such as magnetite ( $\text{Fe}_3\text{O}_4$ ) and goethite ( $\alpha\text{-FeOOH}$ ). Recent studies have shown that surface-bound uranium can become immobilized as an impurity cation within Fe oxide products during ferrihydrite reduction, providing a potentially important route to highly stable sequestered uranium and enhancing stimulated remediation. Numerous key questions remained unanswered about these processes. The SLAC SFA program will probe and quantify the fundamental biogeochemical controls over the stability and rates of ferrihydrite transformations and uranium sequestration/release in the subsurface.

Collaborative site-specific contaminant characterization and remediation: the SLAC SFA team is collaborating on research at the Hanford site, the Old Rifle site, the Fry Canyon site, and the Yucca Mountain potential repository site to help bring synchrotron capabilities to bear on field-scale contaminant migration and remediation problems.

Research conducted by the SLAC SFA program will illuminate our understanding of *in situ* uranium immobilization, improve remediation strategies for accelerated cleanup of contaminated sites, and help gain public and regulatory acceptance for pathways to closure.

## SLAC SFA: Uranium Remediation Research at the Old Rifle, Hanford, and Fry Canyon Sites

*Session: SLAC SFA (Laboratory Research Manager: J.R. Bargar)*

J.R. Bargar, SLAC National Accelerator Laboratory (lead PI), R. Bernier-Latmani, Ecole Polytechnique Federale de Lausanne, K.M. Campbell, U.S. Geological Survey, C.C. Fuller, U.S. Geological Survey, D.E. Giammar, Washington University St. Louis, P.E. Long, Pacific Northwest National Laboratory, J.O. Sharp, Ecole Polytechnique Federale de Lausanne, K.-U. Ulrich, Washington University St. Louis, H. Veeramani, Ecole Polytechnique Federale de Lausanne, S.M. Webb, SLAC National Accelerator Laboratory, and D.M. Wellman, Pacific Northwest National Laboratory

An important goal of the SLAC SFA is to leverage core expertise in synchrotron environmental sciences through site-specific remediation and performance assessment research in the DOE legacy complex and at other sites contaminated from nuclear materials production. In the past year, SLAC SFA team members have participated in research related to the Old Rifle site, the Hanford site, and the Fry Canyon site. This work is advancing the scientific basis for remediation technologies and their optimization.

In collaboration with the Rifle IFRC team, we initiated a new research project to characterize the corrosion of bacteriogenic uraninite in groundwater at the Old Rifle, Colorado, site. This project will focus on the molecular mechanisms of corrosion, the rates of uranium release in the subsurface, and on the chemical properties of the corroded products. This project will definitively address the suitability of biogenic uraninite as a long-term sink phase for uranium.

Phosphate-based remediation strategies may provide a means for remediating the large and tenacious vadose-zone subsurface uranium contamination problem at the Hanford 300 area. We are investigating the mechanisms of uranyl-phosphate formation/transformations, the roles of geochemical micro-environments, and the roles of wet-dry cycling on the formation, identity, and stability of uranium-phosphate phases from laboratory- and field-phosphate-amended sediments. Results from the past year indicate that polyphosphate treatment of U-bearing solids results in the formation of uranyl phosphate minerals. This work is advancing validation and development of this nascent remediation technology.

The collaborative project at the Fry Canyon Site focuses on identifying biogeochemical processes occurring in permeable reactive barriers (PRBs) that govern uranium sequestration and PRB performance at the Fry Canyon, Utah, permeable reactive barrier (PRB) field demonstration project. Questions of central importance are: (1) What reactions result in U sequestration in field-scale PRB deployments? (2) What is the long-term stability of the sequestered U with respect to remobilization? and (3) How do biogeochemical processes occurring within the PRB affect U removal and PRB performance? Initial results indicate that U is sequestered dominantly as U(IV) by the zero valent iron (ZVI) PRB and that secondary Fe(II) minerals have filled ZVI grain pores and cemented grains, which reduce porosity and reactivity to U, limiting PRB longevity and performance. Intriguingly, U removal by the bone char apatite PRB has occurred largely by bioreduction of U(VI) to U(IV) with no evidence of uranyl phosphate precipitation. Bioreduction has enhanced U sequestration by up to 20 times greater than expected for removal by U(VI) surface complexation. This research is providing the scientific basis to evaluate and the long-term behavior of sequestered uranium and to optimize barrier design and operation.

## SLAC-SFA: Fundamental Biogeochemical Factors Governing the Subsurface Stability of Bioreduced Uranium and Plutonium

Session: SLAC SFA (Laboratory Research Manager: J.R. Bargar)

D.E. Giammar, Washington University St. Louis, K. Uwe-Ulrich, Washington University St. Louis, R. Bernier-Latmani, Ecole Polytechnique Federale de Lausanne, J. O. Sharp, Ecole Polytechnique Federale de Lausanne, H. Veeramani, Ecole Polytechnique Federale de Lausanne, E.S. Ilton, Pacific Northwest National Laboratory, H. Boukhalfa, Los Alamos National Laboratory, E.J. Schofield, SLAC National Accelerator Laboratory, A. Mehta, SLAC National Accelerator Laboratory, J.R. Bargar, SLAC National Accelerator Laboratory (lead PI)

The production of biogenic uraninite from the microbially mediated reduction of U(VI) has the potential to immobilize subsurface uranium contamination *in situ*. The success of such a bioremediation strategy will depend on the long-term stability of the biogenic uraninite, which is governed by particle size, molecular-scale structure, and the composition of both the solid phase and contacting groundwater. Similar factors can affect the structure and stability of biogenic PuO<sub>2</sub> from the reduction of more soluble Pu(V) and Pu(VI) species. We are elucidating and quantifying these structure/composition/reactivity relationships as part of the SLAC SFA focus on molecular-scale processes of first-order importance to the DOE subsurface- contaminant remediation.

The molecular-scale and nanoscale structure of biogenic uraninite produced by reduction of U(VI) by *Shewanella oneidensis* and *Desulfovibrio vulgaris* was characterized using EXAFS, synchrotron-based powder diffraction, and TEM. Its lattice parameter is consistent with bulk UO<sub>2</sub>, and there is no evidence for compression of the particle interior, which suggests that the thermodynamic properties of coarser-grained abiotically synthesized UO<sub>2</sub> may be appropriate for modeling the properties of biogenic uraninite nanoparticles. When biogenic uraninite was produced in the presence of the common groundwater cation Mn<sup>2+</sup>, the latter was found to become structurally incorporated in the solid phase, resulting in smaller particle sizes and loss of structural order.

Equilibrium solubility and dissolution rates of biogenic uraninite with and without incorporated Mn<sup>2+</sup> were measured as a function of pH, dissolved oxygen, and dissolved inorganic carbon. Consistent with their structural similarities, synthetic UO<sub>2</sub> and biogenic uraninite had similar equilibrium solubility and surface area-normalized dissolution rates at most conditions. Carbonate promoted uraninite dissolution not only under moderately oxidizing, but also under reducing conditions. As expected, dissolution rates increased with increasing dissolved oxygen. XPS was used to identify oxidized uranium as both U(VI) and U(V) on the uraninite surface, even at anoxic conditions, and the fraction of surface (VI) increased with increasing oxygen concentration. Mn<sup>2+</sup>-substituted biogenic uraninite was substantially less soluble and less susceptible to oxidative dissolution than pure biogenic uraninite.

Uraninite is the most desirable, most common, and until recently, the only known product of biological U(VI) reduction. However, the Gram positive sulfate-reducing bacterium *Desulfotomaculum reducens* was found to form a U(IV) product that lacked the EXAFS U-U shell characteristic of uraninite. The product was concluded to be an uncharacterized molecular complex of U(IV). A U(IV) product similarly lacking in uranium near-neighbors was formed in flow-through systems consisting of Rifle sediments exposed to an electron donor and U(VI) for three months. This product was re-oxidized rapidly upon exposure to oxygen.

Freshly precipitated biogenic plutonium oxides produced by the enzymatic reduction of Pu(VI) and Pu(V) are structurally similar to biogenic uraninite. Based on TEM imaging and EXAFS analysis of biogenic Pu oxides produced by *S. oneidensis*, the Pu precipitates have a crystalline structure and particle size distribution similar to that of uranium precipitates. These results significantly advance our ability to model the subsurface behavior of bioreduced uranium and plutonium.





## LANL ERSP Research



## Los Alamos National Laboratory ERSP Project Summary: Actinides in the Subsurface Environment

*Session: LANL ERSP Research (Laboratory Research Manager: David Hobart)*

David E. Hobart, Los Alamos National Laboratory, Los Alamos, NM (lead PI), Hakim Boukhalfa, Los Alamos National Laboratory, Los Alamos, NM, Don Reed, Los Alamos National Laboratory, Los Alamos, NM, Amr Abdel-Fattah, Los Alamos National Laboratory, Los Alamos, NM, Paul Reimus, Los Alamos National Laboratory, Los Alamos, NM, Robert Roback, Los Alamos National Laboratory, Los Alamos, NM, Peter Lichtner, Los Alamos National Laboratory, Los Alamos, NM, Steve Conradson, Los Alamos National Laboratory, Los Alamos, NM

Plutonium (Pu) environmental contamination poses serious health risks and is therefore a significant public concern. Although Pu is very insoluble, highly sorptive, and generally immobile in the subsurface environment, there are a few examples where small amounts of Pu have been transported significant distances in relatively short times. In most of these cases, the nature of the mobile mass fraction and the mechanisms responsible for transport are poorly understood. This SFA is an integrated program that combines field, laboratory, and modeling studies to address important knowledge gaps that exist in our understanding of the processes controlling the environmental behavior of actinides, especially Pu. This SFA has focused on four research topics: (I) biotic processes that affect Pu speciation; (II) colloid-facilitated transport processes and their scaling; (III) abiotic process that affect Pu speciation and sorption behavior; and (IV) potential controls of Pu source material on transport behavior. A summary of activities and some important conclusions from each of these areas is provided below:

- I. The structure of biogenic  $\text{PuO}_2$  and  $\text{NpO}_2$  species was examined using x-ray absorption spectroscopy and compared to structures of biogenic uraninite. We also examined the behavior of Pu oxides, including aged  $\text{PuO}_2$  under reducing conditions. Modeling activities focused on upgrading the CCBATCH biogeochemical model to include Pu speciation data and applying this model to coupled abiotic and biotic Pu(V) reduction by metal-reducing bacteria.
- II. Laboratory batch and column experiments were conducted to evaluate the transport of Pu(VI) by calcite colloids through saturated alluvial material collected from the central part of the Idaho National Laboratory. Results showed that high concentrations of colloids were continuously generated from the alluvial material and that the colloid generation process is strongly influenced by flow transients. Batch sorption experiments indicated that Pu(VI) sorption onto calcite colloids was strong and almost instantaneous.
- III. We examined the formation of ternary complexes  $\text{Ca}^{2+}/\text{Mg}^{2+}\text{-PuO}_2(\text{CO}_3)_m(\text{OH})_n^{(2m+n-4)-}$ , and characterized the complexes using UV-VIS-NIR and x-ray absorption spectroscopy. We also examined the structure of aged Pu(VI) complexes formed on the surface of manganese oxides using x-ray absorption fine structure (XAFS). In the presence of manganese oxides and from pH 3.5-8.0 under atmospheric  $\text{CO}_2$ , Pu(VI) plutonium remains oxidized, and is present on the mineral phases as sorbed species and not as a discrete Pu phase precipitated on the mineral surface.
- IV. Pu/Ga alloy coupons composed of a mixture of delta and alpha phase Pu were corroded by moist air at ambient temperature for periods ranging from 3 d to 2 months, and the chemical speciation of Pu and Ga were determined by XAFS. We found that a substantial fraction of the Pu remained metallic, because the local environment around the Ga atoms was unchanged over the experiment duration. In addition, the Pu-O and Pu-Pu distances in the oxidized Pu differed significantly from bulk  $\text{PuO}_{(2+x)}$ , even as their distribution changed with reaction time. Thus, transport and other properties of metallic Pu in the environment may depend strongly on their original composition.

## Los Alamos National Laboratory ERSP Progress Report: I. Biogeochemical Processes Governing the Subsurface Speciation of Plutonium

Session: LANL ERSP Research (Laboratory Research Manager: David Hobart)

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Plutonium released to the environment continues to be a major concern at number of the U.S. Department of Energy (DOE) facilities. The fate and transport of Pu in the subsurface is strongly influenced by coupled physical and biogeochemical processes present, and many knowledge gaps remain. Plutonium, as a subsurface contaminant, exists mostly as a  $\text{PuO}_{2+x}$  species with organic complexing agents also present as co-contaminants. The effects of abiotic and microbiological processes on plutonium speciation are a key focus of the Los Alamos SFA, which examines the Fate and Transport of Plutonium in Subsurface Environments.

The initial emphasis of our plutonium biogeochemistry studies has been on the bioreduction of higher-valent plutonium by facultative metal-reducing bacteria: *Shewanella oneidensis* MR1, *Shewanella alga* BrY, and *Geobacter metallireducens* strain GS-15. These microorganisms are important in defining plutonium speciation in the subsurface. We examined the direct and indirect reduction of several plutonium species, and found that the reduction of oxidized Pu(V) and Pu(VI) species proceeds at rates similar to that observed for U(VI) reduction under the same reducing conditions. However, the reduction products are highly affected by the presence and concentration of organic and inorganic chelators. In the absence of chelating agents, our examinations by TEM imaging and EXAFS spectroscopy indicate that the reduction of Pu(V) and Pu(IV) produce crystalline Pu(IV) phases, which are structurally very similar to biogenic uraninite. In the presence of carbonate, the reduction of plutonium is much slower, and all oxidation states Pu(VI), Pu(V), Pu(IV) and Pu(IV) are simultaneously observed in solution. However, strong chelators like EDTA and NTA favor the reduction of plutonium to Pu(III). We have also examined the direct enzymatic reduction of Np(V), a stable surrogate for Pu(V), by *S. oneidensis* MR1 and characterized the structure of the reduced  $\text{NpO}_2$  phase by x-ray absorption spectroscopy.

We also investigated the bioreduction of aged synthetic plutonium oxides and freshly precipitated amorphous Pu oxides  $\text{Pu}(\text{OH})_4(\text{am})$ , with varying synthetic and natural ligands present, such as carbonate, soil humates, and ethylenediaminetetraacetic acid (EDTA). Our results indicate that aged plutonium oxides are more resistant to biological reduction under most conditions examined. Freshly precipitated  $\text{Pu}(\text{OH})_4(\text{am})$  are readily reduced and solubilized to form Pu(III)-organic species. Qualitative Eh-pH titrations of amorphous  $\text{Pu}(\text{OH})_4(\text{am})$ , performed in the presence and absence of EDTA, suggest that EDTA might enhance Pu(IV) oxides reduction by making them chemically easier to reduce.

An important part of this research is the development of a biogeochemical submodel to explain the observed biogeochemical effects on plutonium speciation. Modeling activities were centered on upgrading the CCBATCH biogeochemical model to include Pu speciation data—mainly Pu(III) and Pu(IV) species. CCBATCH also was applied to the modeling of coupled abiotic and biotic Pu(V) reduction by metal-reducing bacteria to establish the relative contribution of each process.

## Los Alamos National Laboratory ERSP Progress Report: II. Plutonium Transport by Calcite Colloids

*Session: LANL ERSP Research (Laboratory Research Manager: David Hobart)*

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The legacy of six decades of nuclear materials production in the United States has resulted in significant releases of plutonium (Pu) to the environment at many U.S. DOE sites. Plutonium contamination is of significant concern because of its extreme toxicity, radioactivity, public sensitivity, and national security aspects. Thus, it is imperative that DOE can demonstrate a defensible understanding of the processes that control Pu contaminant migration. This work is part of Los Alamos National Laboratory's SFA *Actinides in the Subsurface Environment*. Specifically, we present results of laboratory batch and column experiments conducted to evaluate the transport of Pu(VI) by natural calcite colloids through saturated alluvial material collected from the central part of the Idaho National Laboratory. Column experiments investigate the effect of flow transients on the colloid generation process by varying the flow rate from 0 to 24 mL/hr over a period of over 240 days. Effluent natural calcite colloids were collected from the columns and were subsequently used in Pu sorption and transport experiments. Sorption experiments were conducted by adding Pu(VI) to both unfiltered and ultrafiltered colloid suspensions (initially  $10^{-7}$  M Pu and pH 7.7). Baseline colloid transport experiments were conducted in the columns using a Pu-free colloid suspension and a conservative tracer. Additional transport experiments were conducted by reinjecting the concentrated colloid-Pu solution from the sorption experiments into the columns, along with a conservative tracer, to assess the transport of the dissolved and colloidal Pu.

Analyses of the effluent samples collected during flushing of the columns indicated that calcite colloids were continuously generated, with colloid concentrations ranging from 10 to 250 times higher than the average influent concentration. Colloid concentrations remained 20 times the influent concentration after more than 240 days of flushing. The experiments revealed that colloid generation is strongly influenced by flow transients. During the first ~60 days of flushing, the flow rate was increased by a factor of 12 (2 to 24 mL/hr) and the generation rate increased by a factor of ~6. Increasing the flow rate by a factor of 3 (2 to 6 mL/hr) caused the generation rate to increase by a factor of ~2. Colloid concentrations in the effluent before and after stopping the flow for 4 and 12 hours were almost unchanged. These results suggest that colloid generation under fixed geochemical conditions is controlled mainly by flow rate and may be governed by the thickness of the hydrodynamic boundary layer, which is nonlinearly dependent on flow rate. Interestingly, after the flow rate was decreased and maintained at 0.5 mL/hr (starting at ~60 days), the colloid generation rate initially decreased in accordance with trends established prior to this time. However, after a few hundred hours, the generation rate gradually increased by a factor of ~8 and remained at that rate for over 1000 hours, before eventually dropping back down to rates commensurate with the early time period. This delayed increase in colloid generation is tentatively attributed to slow dissolution of bonding agents between the colloids and the alluvium grains. The eventual decrease in generation is attributed to dissipation of the supply of colloids bound to surfaces by this mechanism.

The batch sorption experiments indicated that Pu(VI) sorption onto the calcite colloids is strong and almost instantaneous. These results suggest a significant potential for colloid-facilitated transport of Pu under the hydrogeochemical conditions investigated so far. Experiments examining the transport of the Pu and colloids through the saturated alluvium columns are in progress. Results and model interpretations will be presented.

## Los Alamos National Laboratory ERSP Progress Report: III. Plutonium(VI) Adsorption onto Goethite and Manganese Oxide

*Session: LANL ERSP Research (Laboratory Research Manager: David Hobart)*

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Plutonium released to the environment continues to be a major concern at a number of the U.S. DOE facilities. The fate and transport of plutonium in the subsurface is strongly influenced by coupled physical and biogeochemical processes present at the contaminated site. Numerous knowledge gaps exist in our understanding of these processes at the fundamental level and their coupling. The research presented here is part of the ongoing LANL-SFA, which focuses on improving our understanding of important abiotic processes that affect the fate and transport of plutonium in subsurface environments.

Under common groundwater conditions, Pu(IV) and Pu(V) are the most stable oxidation states, with the Pu(V) fraction generally remaining in solution and Pu(IV) primarily present in the solid phase as sparingly soluble intrinsic oxyhydroxides and surface-sorbed species. The oxidized Pu species, Pu(V) and Pu(VI), are present at low concentrations under oxic environmental conditions, but are sufficiently soluble to play an important role in the overall transport behavior of plutonium. A number of studies have examined the aqueous speciation of Pu(V) and Pu(VI) under environmental conditions and their interactions with mineral surfaces, but our knowledge of the aqueous speciation of plutonium and the structure of the species formed on mineral surfaces still needs significant advances. Plutonium is particularly difficult to study because of its rich chemistry, radiotoxicity and redox reactivity. We have examined the hydrolysis of Pu(VI) under conditions that insure that the species formed are monomeric, and we have characterized the complexes formed using classical UV-VIS-NIR spectroscopy and x-ray absorption spectroscopy. We have also examined the formation of ternary complexes  $M\text{-PuO}_2(\text{CO}_3)_m(\text{OH})_n^{(2m+n-4)-}$  (where  $M = \text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) and characterized the complexes formed using UV-Vis-NIR, and x-ray absorption fine structure (XAFS).

The migration of soluble oxidized Pu species is strongly influenced by their interaction with mineral surfaces, which provide sorption and redox-active sites. Plutonium interaction with various mineral surfaces has been examined in the past; however, there is a lack of a thorough understanding of surface complex speciation and redox distribution of Pu on the surface of reactive minerals such as iron and manganese oxides. Most mineral sorption studies, primarily focused on iron oxyhydroxides, silica, and aluminosilicates, show that surface-adsorbed or precipitated Pu(IV) forms independent of the initial oxidation state. In contrast, recent studies indicate that some redox-active mineral phases, such as birnessite, a high surface area manganese oxide commonly found as a component of soils and sediments, have the potential to oxidize and preferentially adsorb Pu(V/VI). Pu(VI) surface speciation on manganese oxides surfaces at pH 3.5-8.0 under atmospheric  $\text{CO}_2$  was investigated using batch sorption experiments, XAS spectroscopy, and numerical modeling. Samples with estimated overall sorption capacity of 40 to 100% were prepared by varying the pH between pH 3.5 and 8.0. XAS examination were performed on freshly sorbed Pu(VI) and also after the mineral phases were aged for 9 months. The data suggest that plutonium remains oxidized and is present on the mineral phases as a sorbed species and not as a discrete Pu phase precipitated on the mineral surface. The structure of the complexes formed at pH 3.5-8.0 is currently being analyzed.

## Los Alamos National Laboratory ERSP Progress Report: IV. Characterization of Transuranic Oxides Formed by Biotic and Abiotic Processes Using X-ray Absorption Fine Structure (XAFS) Spectroscopy

*Session: LANL ERSP Research (Laboratory Research Manager: David Hobart)*

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The environmental chemistry—the combination of physical, chemical, and biological processes that determine the fate of hazardous elements released into the environment—of transuranics is of great concern to the Department of Energy, because of the number of its (primarily) weapons production and R&D sites that have been contaminated with plutonium and other transuranics. Transuranics will also be a primary concern at repositories that will contain waste from reactors or fuel processing. Plutonium (Pu) is arguably the most fraught of the transuranics, not only because of its extreme toxicity and radioactivity, but especially because of its public perception and its national security aspects. Neptunium contamination is also of significant concern because its longer half life renders it the regulatory driver beyond 100,000 years, when it is assumed that the repository will have broken down and projected geological processes will control its transport. Los Alamos National Laboratory, under the auspices of its SFA *Actinides in the Subsurface Environment*, has been performing research aimed at understanding the subsurface migration of transuranics. A recent extension of this project has been the use of X-ray Absorption Fine Structure (XAFS) spectroscopy to determine the chemical speciation and reactivity of Pu and Np, specifically the corrosion chemistry of Pu alloys that are the most likely source terms for contamination in many instances and the biogenic chemistry of Pu and Np. For metal corrosion, the dogma has been that Pu in a metallic state on contact with H<sub>2</sub>O liquid or vapor completely degrades to the thermodynamically favored PuO<sub>2+x</sub> within a matter of weeks to months. This issue is vital in predicting the behavior of Pu, because all of the models predicting the behavior of the light actinides in the environment assume that, regardless of the actual source term, the sole important species are the tetravalent oxides (U(VI) is an exception) and their variants. In these experiments, 1.6 atom % Ga Pu/Ga alloy coupons composed of a mixture of delta and alpha Pu after uniaxial polishing were corroded by moist air at ambient temperature, for periods ranging from three days to two months, and the chemical speciation of the Pu and Ga determined separately by XAFS measurements at 80 K. In contrast to the dogma that Pu metal oxidizes rapidly and completely to PuO<sub>2+x</sub>, we found that a substantial fraction of the Pu remained metallic, because the local environment around the Ga atoms was unchanged over the duration of the experiment. The Ga apparently prevents the Pu atoms in proximity from reacting with H<sub>2</sub>O. In addition, the Pu-O and Pu-Pu distances in the oxidized Pu that was observed significantly differed from bulk PuO<sub>2+x</sub> even as their distribution changed with reaction time. It would therefore be expected that the transport and other properties of metallic Pu in the environment may not only differ from those of PuO<sub>2+x</sub> but also from each other, depending upon their original composition and their handling. It could also be expected that this type of nanoscale phase separation of metallic and oxidized Pu resulting from the local oxidation would promote the formation of colloids from the metal via a mechanism that would not occur for material that begins in pure oxide form. In addition, we have found via XAFS measurements that, unlike UO<sub>2</sub>, the speciation of Pu and Np resulting from transformation by specific microbes does not produce ordered tetravalent oxide. While biogenic Pu appears to be a highly transformed variant of PuO<sub>2+x</sub>, biogenic Np is actually more similar to a hydroxycarbonate or other molecular complex. The environmental chemistry of these elements is therefore potentially much more complex than has been assumed.

## Los Alamos National Laboratory Science Focus Area Proposal: Fate and Transport of Plutonium in Subsurface Environments

*Session: LANL ERSP Research (Laboratory Research Manager: David Hobart)*

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The legacy of six decades of nuclear materials production in the United States has resulted in significant releases of plutonium to the environment at many U.S. DOE sites. Plutonium contamination is of significant concern because of its extreme toxicity, radioactivity, public sensitivity, and national security aspects. Thus, it is imperative that DOE can demonstrate a defensible understanding of the processes that control plutonium contaminant migration. Due to the complex behavior of plutonium in natural environments and the fact that its environmental behavior has been inadequately studied, such an understanding does not exist. This Science Focus Area (SFA) combines field, laboratory, and modeling studies to address important knowledge gaps that exist in our fundamental understanding of the coupled physical and biogeochemical processes controlling plutonium migration in the subsurface.

Plutonium environmental behavior is extremely complex. At near neutral pH, plutonium (Pu) can exist in solution in four oxidation states simultaneously. In most environmental settings, Pu(IV) is the most stable oxidation state. Pu(IV) can hydrolyze to form polymeric colloids, which may then be transported primarily according to their physical, rather than chemical, properties. Although Pu(IV) is quite insoluble, recent work has shown that Pu(IV) oxides are actually inhomogeneous and nonstoichiometric, and can be described as  $\text{PuO}_{2-x}\text{nH}_2\text{O}$ , with some Pu(V) typically present under oxic conditions. This property enhances the availability of other plutonium valence states which, in the presence of natural organic and inorganic chelators, may greatly enhance the solubility of plutonium. These observations lead to the key hypotheses that will guide this SFA:

1. Plutonium is capable of being transported over significant distances in the subsurface as colloidal species or as strongly complexed species; and,
2. Natural instabilities of  $\text{PuO}_{2-x}\text{nH}_2\text{O}$ , coupled with biogeochemically induced redox cycling, will alter the speciation, nature of the surface-adsorbed species, absolute amounts of mobile plutonium, and relative proportions of forms of mobile plutonium.

This SFA will integrate extensive characterization of plutonium-contaminated field samples, laboratory experiments in systems of varying complexity, and biogeochemical reactive transport modeling. The field samples will help elucidate the natural biogeochemical processes that affect plutonium speciation and mobility, and the laboratory experiments in simple model systems will aid in determining mechanistic processes. Dynamic transport experiments will utilize site-specific materials and will mimic more complex natural systems. Field samples will be collected in conjunction with ongoing collaborations at LANL and at the Nevada Test Site. These locales have plutonium contamination that exists in a variety of environmental settings, and thus our results will be widely applicable to many other DOE sites.



## **Student Presentations**



## Mixing-Induced Precipitation Phenomena: Range of Applicability of Macroscopic Equations

*Session: Student Abstract*

Ilenia Battiato, University of California, San Diego, Alexandre M. Tartakovsky, Pacific Northwest National Laboratory, Daniel M. Tartakovsky, University of California, San Diego, Timothy D. Scheibe, Pacific Northwest National Laboratory (lead PI)

Reactive transport in porous media is a complex nonlinear phenomenon that involves both homogeneous (bio-)chemical reactions between species dissolved in a fluid and heterogeneous reactions that occur on liquid-solid interfaces. It is commonly studied by means of continuum-scale equations, obtained from pore-scale models through upscaling techniques. The use of macroscopic models stems from the practical impossibility of solving reaction-diffusion equations for pore-scale variables in the whole computational domain, owing to both the computational burden and unknown pore-geometry structure. However, several studies have pointed out the serious shortcomings of macroscopic equations in describing nonlinear and strongly coupled physical phenomena.

In the present study, we establish conditions under which macroscopic reaction-diffusion equations (RDEs) provide an adequate averaged description of pore-scale processes. These conditions are represented by a phase diagram in a two-dimensional space, spanned by Damkohler number and a scale-separation parameter. This phase diagram shows that highly localized phenomena in porous media, including precipitation on (and/or dissolution of) a porous matrix, do not lend themselves to macroscopic (upscaled) descriptions. To compute the predictive errors resulting from the use of macroscopic RDEs to describe such phenomena, we upscaled the pore-scale RDEs to the continuum (macroscopic) scale and used pore-scale numerical simulations to verify various upscaling assumptions.

Our results suggest that the localized nature of reaction fronts calls for hybrid simulations, which resolve a pore-scale model in a small reactive region and its continuum counterpart in the rest of a computational domain.

## Can Microbially Generated Hydrogen Sulfide Account for the Rates of U(VI) Reduction by a Sulfate-Reducing Bacteria?

*Session: Student Abstract*

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At the Oak Ridge FRC, *in situ* remediation of uranium contaminated soil and groundwater is brought about by a diverse range of microbial and abiotic processes. Often these processes are linked. Sulfate-reducing bacteria (SRB), for example, enzymatically reduce U(VI) to U(IV), but they also produce hydrogen sulfide that can itself reduce U(VI). In batch microcosms, I evaluated the relative importance of these processes for *Desulfovibrio aerotolerans*, a SRB isolated from a U(VI)-contaminated site using carbonate levels similar to those of the site. The observed rate of SRB-mediated U(VI) reduction was explained by the abiotic reaction of U(VI) with the microbially generated H<sub>2</sub>S. Trace ferrous iron increased rates of hydrogen sulfide-mediated U(VI) reduction at 5 mM bicarbonate, but had no clear effect at 15 mM. During the hydrogen sulfide-mediated reduction of U(VI), a floc formed containing uranium, phosphorus, and sulfur. U(VI) sequestered in the floc was not available for further reduction.

## Isotopic Signatures for Key Mineralogical Reactions Accompanying Biostimulated Uranium Reduction

Session: Student Abstract

Jennifer Druhan, University of California, Berkeley, Mark Conrad, Lawrence Berkeley National Laboratory, Kenneth H. Williams, Lawrence Berkeley National Laboratory, Eric Sonnenthal, Lawrence Berkeley National Laboratory, Donald DePaolo, University of California, Berkeley; Lawrence Berkeley National Laboratory (lead PI)

Stable isotopes provide a valuable means of distinguishing between microbially mediated and abiotic processes during subsurface bioremediation through characteristic fractionations. The isotopic signature associated with a fractionating reaction is preserved in both the residual reactants and products, providing a means of detecting principal contaminant sequestration processes as well as monitoring the long-term stability of precipitates. This project focuses on identification of isotopic systems useful in studying stimulated subsurface bioremediation processes, and incorporation of these isotopes into reactive transport modeling. A reactive transport model that treats isotopes as individual species and includes fractionations associated with microbial reactions will provide valuable insights into the formation and stability of key mineralogical reactions contributing to permeability and flowpath evolution over the course of an *in situ* amendment. This research is part of the LBNL SFA challenge addressing the evolution of hydrologic flow patterns at the Rifle IFRC site. Previous analysis of samples collected during an acetate amended bioremediation experiment at the Rifle site have shown sulfur isotopes to be a highly sensitive indicator of the onset of sulfate reduction and the transition to and from sulfate limiting conditions. These data show enrichment of up to 7‰ in  $\delta^{34}\text{S}$  of sulfate and a corresponding sulfide  $\delta^{34}\text{S}$  of -20‰ in downgradient monitoring wells, followed by a transition to smaller fractionation factors as sulfate became less available. Preservation of the  $\delta^{34}\text{S}$  of sulfide signature within iron sulfide precipitates further suggested a means of monitoring iron sulfide reoxidation through the isotopic composition of sulfate. An extensive dataset of  $\delta^{34}\text{S}$  of sulfate and sulfide, as well as  $\delta^{18}\text{O}$  of sulfate using samples from the 2008 Rifle amendment, is currently being developed. Preliminary data indicate a correlation between decreasing sulfate concentrations and increasing  $\delta^{34}\text{S}$  values similar to previous results, despite a more complex electron donor injection scheme. The  $\delta^{18}\text{O}$  of sulfate in the same samples averages roughly 2‰ and correlates with both  $\delta^{34}\text{S}$  and sulfate concentrations, though the magnitude of variation is less pronounced. Preliminary  $\delta^{44}\text{Ca}$  data analysis shows no variation due to ion exchange, while column samples obtained during high rates of calcite precipitation are expected to show detectable shifts. Variations in previously obtained sulfur isotope data from the Rifle site are not adequately described by simple mass-balance calculations, suggesting these data incorporate information about the reactive transport processes of the system. Using the TOUGHREACT framework, isotopes will be modeled in this system by modifying the thermodynamic parameters for each specific reaction pathway, including both biotic and abiotic processes. For each of these reactions, the isotopes will be treated individually, thus allowing associated fractionation factors for each process to be incorporated into the model. This approach will allow us to test the influence of individual processes on the overall isotopic composition, and track flowpath evolution through the isotopic signatures of precipitate species.

## Application of Culturing and Molecular Techniques to Elucidate the Influence of Cellulosic Waste on Microbial Community Structure at an INL Simulated Waste Site

*Session: Student Abstract*

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Radioactive waste sites, like those found at various DOE sites, frequently contain cellulosic waste in the form of paper towels, cardboard boxes, or wood contaminated with heavy metals and radionuclides such as chromium and uranium. To date, the interaction of cellulose-utilizing bacteria with heavy metals and radionuclides has received little attention, but may hold important information about contaminant fate and transport at DOE sites. To understand how the soil microbial community responds to the presence of cellulosic waste products, we obtained multiple soil samples from a model (i.e., nonradioactive) test pit near a low-level radioactive waste (LLW) site and analyzed using culturing and culture-independent techniques. Soil samples were obtained at four depths within the pit: the Fill (F), the Fill-Waste interface (FW), the Wood Waste (WW), and Waste-Clay interface (WC). Triplicate clone libraries and duplicate PhyloChips, 16S rDNA microarrays, were run for soil samples collected at each depth. Soil samples were also used to inoculate aerobic and anaerobic enrichments containing either methyl cellulose, a soluble cellulose source, or filter paper, a nonsoluble source, as the sole carbon source.

Both the clone libraries and PhyloChip results revealed changes in microbial community structure with depth at the simulated LLW site. The clone libraries showed that the Bacteroidetes and Actinobacteria phyla made up approximately 61% of the total microbial community in the FW layer and the PhyloChip also detected their large prevalence at this depth. Overall, the PhyloChip detected significantly more unique OTUs, and therefore more relative diversity, than the clone libraries in all samples. Shannon and Simpson's diversity indices calculated for clone library and PhyloChip results suggest that diversity is greater in the WW and WC layers and lower in the F and FW layers. Principal coordinate analysis using Unifrac was performed on the clone library and PhyloChip datasets. Results showed the same trend in which samples from the same layer clustered most closely together. Additionally, the WW and WC layers clustered closely together, while the F and FW clustered by themselves, suggesting a stratification with depth at the site. Lineage specific analysis of the clone library data suggested changes in the *Bacteroidetes* and *Actinobacteria* phyla account for many of the differences observed between the layers. Results indicated an increased abundance of reportedly noncellulose degrading *Actinobacteria* families in the F layer, but an increase of previously reported cellulose-degrading families in the FW layer. Closer examination of the *Bacteroidetes* phyla with depth showed a significant decrease in the number of families present in the FW layer and those present contained known cellulose degraders. Overall, these results suggest that the presence of the cellulosic material influences the microbial community structure, especially at the FW interface, which may represent an enrichment environment for aerobic cellulose degrading microorganisms. Current and future studies include characterizing cellulose degradation and metal-reduction capabilities of the seven bacterial isolates obtained from these soil samples and conducting flow-through column studies to assess the influence of metal mobility on the microbial community, as metal-contaminated cellulosic waste is broken down through the use of PhyloChip and GeoChip (a functional gene microarray) analyses.

## Profiling of Microbial Community Structure with Depth and across Physico-chemical Gradients in the Uranium-Contaminated Subsurface

Session: Student Abstract

Puja Jasrotia, Oceanography Department, Florida State University, Stefan J. Green, Oceanography Department, Florida State University, Denise M. Akob, Oceanography Department, Florida State University, Woo Jun Sul, Center for Microbial Ecology, Michigan State University, Jim Tiedje, Center for Microbial Ecology, Michigan State University, Phil Jardine, Oak Ridge National Lab, Oak Ridge, TN, David Watson, Oak Ridge National Lab, Oak Ridge, TN, Joel E. Kostka, Oceanography Department, Florida State University (lead PI)

The subsurface at the Oak Ridge Integrated Field Research Center (OR-IFRC; Oak Ridge, Tennessee) is contaminated with a mixture of organic and inorganic materials as a legacy of cold war era uranium enrichment. The contaminants of greatest concentration and mobility, and therefore concern, are uranium and nitrate. Tracking and modeling the distribution and fate of contaminants in the subsurface has been impeded by the highly complex subsurface hydrogeology, geochemistry and microbiology. To better understand the relationship between subsurface geochemistry and microbiology, we analyzed multiple depth intervals in OR-IFRC sediment cores from the most highly contaminated zone (Area 3) and from a down-gradient and less contaminated location (Area 2). In Area 2, the sediment core was collected from the saturated zone at 5.5-6.5 m below the surface and subsectioned into 6 depths. In Area 3, a larger depth range was analyzed—from depths of 1-15 m (at 1-1.5 meter intervals) corresponding to roughly 9 m of fill (shale/saprolite or gravel/saprolite) and fractured saprolite below. Six to eight subsections of each core were analyzed by geochemical methods for pH, sulfate, nitrate, Fe(II), and total Fe and by molecular analyses of the microbial community. The bacterial community composition was also assayed by terminal restriction fragment length polymorphism (TRFLP). Less variation in geochemical parameters and a higher pH of 5-6 was observed in the Area 2 subsurface sediments sampled farther from the contaminant source zone. Approximately 38,000 short sequences (205 bp) were recovered from six depth intervals using a deep sequencing approach. Analysis of these data revealed the dominant presence of bacteria from the phylum *Acidobacteria*, while *Proteobacteria*, *Verrucomicrobia*, *Firmicutes*, *Chloroflexi*, and *Actinobacteria* were also common. Distinct geochemical and contaminant gradients were observed with depth close to the contaminant source zone in the Area 3 borehole sequence that paralleled with changes in microbial community structure. Nitrate and uranium concentrations increased dramatically with depth, while the pH was highly acidic (<4.0) at all depths except for the top and lowermost depths. Clone libraries of bacterial 16S rRNA genes were generated for 7 depth intervals from Area 3 cores, and diversity and phylogenetic analyses revealed a strong effect of pH on microbial community structure. Acidic samples contained a less diverse microbial community dominated largely by *Rhodanobacter* or *Bradyrhizobium* spp. The limited bacterial diversity in the deeper subsurface of Area 3 selected almost exclusively for key taxa, resulting in limited overlap in microbial community structure between Area 2 and Area 3 sediments. The abundant presence of these unique sequences suggests adaptation of bacterial populations to pH and contaminant (nitrate and uranium) conditions. Conversely, pyrosequencing of Area 2 sediment reveals a highly diverse microbial community with roughly 8,000 putative species (operational taxonomic units, OTU) detected in 1 m of sediment.

## **Bayesian Geostatistical Approach for Integration of Scale-Dependent Hydrogeological Data Under the Integrated Field Research Challenge Project at the Hanford 300 Area**

*Session: Student Abstract*

Haruko Murakami, Department of Nuclear Engineering, University of California, Berkeley, Yoram Rubin, Department of Civil and Environmental Engineering, University of California, Berkeley (lead PI)

The Integrated Field Research Challenge (IFRC) Project at the Hanford 300 Area includes many different types of field measurements and experiments, which are based upon different underlying physics, assumptions, scale and reliability, such as nuclear geophysical logs, core analysis, electromagnetic borehole flowmeters (EBF), constant-injection tests (CIT), and tracer tests.

It is critical for the project to extract as much information as possible and to combine different type of datasets in a manner consistent with the numerical flow and transport models, which are also based on various assumptions and depend on scale issues such as domain and grid sizes. The geostatistical model, being developed by the UC-Berkeley team, lies in between the flow and transport model and the field data, emphasizing on characterizing spatially variable parameters such as hydraulic conductivity and porosity.

In this poster, we focus on the issue of the scale in hydrological experiments (EBF and CIT). It is a great challenge to characterize heterogeneity of the field from those datasets, since the zone-of-influence expands very rapidly due to the highly permeable Hanford formation. To solve this problem, we have developed a geostatistical model to combine the datasets in a different scale from the small-scale core analysis and the EBF to the large-scale CIT, using the geostatistical inversion.

We implement the model and data in our newly developed Markov-chain Monte-Carlo based method, in which the method of anchor distribution is used for the inversion. It is a Bayesian approach, through which we can obtain posterior probability density functions of the model parameters directly from the data. The advantage of this method is that it can provide us multiple sets of the intercorrelated parameters that represent parameter uncertainty and become inputs in the Monte-Carlo simulations for predicting the plume migration.

Our approach intends to maximize utilization of the field data in a consistent manner, in order to characterize the heterogeneous hydraulic conductivity field, including the parameter uncertainty.

This research is part of the ERSP Hanford IFRC at Pacific Northwest National Laboratory.



## **A Geophysical Characterization & Monitoring Strategy for Determining Hydrologic Processes in the Hyporheic Corridor at the Hanford 300-Area**

*Session: Student Abstract*

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My doctoral thesis work is based on building a hydrological framework to understand groundwater-surface water interaction and mixing of U (VI) contaminated groundwater with Columbia River surface water in the hyporheic corridor of the Hanford 300 area. To facilitate a better understanding of the aquifer framework, geophysical measurements including resistivity (Res), induced polarization (IP) and ground penetrating radar (GPR) were carried out. These measurements were used to characterize the hydrological framework of the hyporheic corridor and map lithologic variability in Hanford 300 area. The geophysics included both land-based and waterborne (continuous) surveys, the latter including Res/IP and GPR. Land-based surveys included Res/IP and GPR, which were done close to the shore, while additional land-based Res/IP and GPR were run adjacent to the river. The waterborne Res/IP data were collected using a 10-channel time-domain Res/IP instrument. A team from the U.S. Geological Survey collected waterborne GPR data using 100-MHz Mala shielded antenna, while the land-based GPR data were collected using a Mala unshielded system with antenna frequencies of 25, 50, 100, and 200 MHz. A single channel instrument from MPT technologies was used for Res/IP measurements close to the shore.

Commercially available software (Res2dInv, Reflex 2D) was used for data processing and visualization. I used Arcmap to integrate the data for comparison and visual observation of the spatial distribution of the acquired geophysical data. I performed an ordinary kriging on the point resistivity and IP data to generate a 3D dataset across a surveyed area for resistivity and chargeability subsurface distribution. The spatial visualization was conducted on a satellite image of the 300 area; A 2D analysis of GPR data was conducted to further understand the shallow subsurface.

Waterborne Res/IP proved to be very successful in mapping the contact between the two main formations on the site, the more permeable Hanford formation (high hydraulic conductivity), and less permeable Ringold formation (low hydraulic conductivity). I was also able to extract the information about bed material (fine-grained Ringold versus coarse-grained Hanford formation) from waterborne GPR. Little information was obtained from the GPR survey conducted onshore, due to signal penetration being limited by ground conditions. The GPR survey parallel to the river bank was able to map a suspected paleochannel on the northern side of the profile. This structure is very similar to other suspected paleochannel features mapped by previous workers in 1993, west of the 300 area. The identified paleochannel is significant because it is in direct contact with the river, making it a candidate location for promoting enhanced groundwater-surface water interaction. The Hanford-Ringold contact (H-R contact) and other reflectors associated with lithological differences are also evident in this profile.

Future work will concentrate on further mapping paleochannels across the site, with further work on the resistivity method being performed to generate elevation of the Hanford-Ringold contact across the hyporheic corridor.

## Mechanisms for Sequestration and Release of Strontium and Cesium in Caustic Waste-Reacted Sediments and Precipitated Phases, and Implications for Long-Term Contaminant Retention

*Session: Student Abstract*

Nelson Rivera (UC Merced), and Jon Chorover, University of Arizona (lead PI)

Flow-through column experiments were conducted with Hanford sediment and aluminosilicate phases precipitated homogeneously to ascertain mechanisms that control the fate and mobility of contaminant Sr and Cs in the subsurface at Hanford. Sediment was reacted with a high ionic strength, caustic synthetic tank waste leachate (STWL) solution containing  $10^{-3}$  M Sr and Cs. Reactant STWL was flushed through the column until breakthrough, and then flow was stopped, allowing the solution to react for 1 day to 3 months. After reaction, columns were flushed with a simulated background pore-water (BPW) solution (pH = 7), and effluent Sr, Cs, and dissolved elements were measured for up to 500 pore volumes.

Previous studies showed that reaction of STWL with reference clays and Hanford sediments produced neoformed feldspathoid and zeolite phases that sequester Sr and Cs. To determine the molecular mechanism of sequestration and rates of dissolution in BPW in a model system, homogeneously nucleated samples were aged for 1 month and 1.5 years, and triplicate column dissolution experiments were run on the resultant precipitates. Three sets of precipitates were produced with Sr/Cs, Sr only, and Cs only at  $10^{-3}$  M concentration of each contaminant. The precipitates were then separated, washed, and mixed with quartz to conduct a complementary set of flow-through experiments as above.

In the sediment system, strontium exhibits greater retention over Cs during contaminant uptake, with Cs showing breakthrough two times faster than Sr. During flush-out with BPW, initial Cs release is rapid and reaches a steady state after ~5 pore volumes. Depending on stopped-flow reaction time, 5-15% of total sorbed Cs is eluted after 500 pore volumes. Strontium desorption is retarded until ~25 pore volumes for all reaction times. However, Sr exhibits more rapid desorption than Cs after the initial retardation, and up to 55% total Sr (sorbed and native in sediment minerals) is eluted from the column after 500 pore volumes.

Analysis by synchrotron XRD indicates zeolite-X (faujasite-type zeolite with ideal formula  $(\text{Na}_2, \text{Ca}, \text{Mg})_{3.5}[\text{Al}_7\text{Si}_{17}\text{O}_{48}] \cdot 32(\text{H}_2\text{O})$ ) as the major phase produced after a one-month reaction time, with minor phases including sodalite/cancrinite ( $\text{Na}_8\text{Al}_6\text{Si}_6\text{O}_{24}\text{OH}_2$ ) and strontianite ( $\text{SrCO}_3(\text{s})$ ). After aging for 1.5 years, precipitated phases are dominated by sodalite/cancrinite that form at the expense of Si-rich zeolite phases. Analyses of residual solutions after solid precipitation show more Sr partitioning to solid phases than Cs. In dissolution experiments, effluent data from flow-through columns demonstrate an initial release of both Sr and Cs, and steady-state release after ~200 pore volumes. Only 5% of the total Sr/Cs was released by the end of the experiment (~500 pv). This indicates Sr and Cs are robustly sequestered and cannot be easily removed from host aluminosilicate phases.

Sediment column experiments exhibit greater contaminant release than the homogeneously precipitated analogs. This behavior can be attributed to Sr and Cs release controlled by a combination of sorption/desorption, cation exchange, and dissolution/precipitation processes operating at different rates, with different reactivities for each contaminant during both uptake and release. In circumneutral groundwaters, dissolution of neoformed aluminosilicate phases releases Sr and Cs at a slower rate than ion exchange or desorption, and will control the long-term release rate.

## Uranium(VI)-Phosphate Interactions in the Presence of Goethite

*Session: Student Abstract*

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Phosphate addition to uranium-contaminated soil and groundwater can potentially provide long-term *in situ* U(VI) immobilization by precipitation of low solubility U(VI)-phosphates. Phosphate may enhance or inhibit U(VI) adsorption to iron(III) (oxy)hydroxides. Such surfaces may also facilitate the heterogeneous nucleation of U(VI)-phosphate precipitates. The interactions among phosphate, U(VI), and goethite ( $\alpha$ -FeOOH) were investigated in a year-long batch experimental study. Dissolved U(VI) and phosphate concentrations were interpreted within a reaction-based modeling framework that included dissolution-precipitation reactions and a surface complexation model to account for adsorption. XAFS and XRD characterization were performed on solids obtained from batch experiments to identify dominant mechanisms for uranium immobilization at the goethite-water interface. To relate these results to the macroscopic transport of U(VI), a complementary set of column experiments with goethite-coated sand was performed. Batch study results indicate that a U(VI)-phosphate phase, most likely chernikovite ( $\text{UO}_2\text{HPO}_4 \cdot 4\text{H}_2\text{O}_{(s)}$ ), formed rapidly by homogeneous nucleation for initially supersaturated suspensions both with and without goethite. The U(VI)-phosphate solids persisted over long time scales and appear to transform from chernikovite to another unidentified phase, depending on the TOTU:TOTP ratio in the system. At high phosphate concentrations that are favorable for U(VI) phosphate precipitation, the goethite surface acts as a sink for dissolved phosphate and consequently increases the dissolved U(VI) present at equilibrium. Even though the effect of phosphate on dissolved U(VI) may not be as significant as desired, it may still benefit *in situ* remediation strategies by alleviating the potential problems of decreased hydraulic conductivity from precipitation of phosphate minerals containing other cations present in the soil or sediment. Results from molecular- and column-scale investigations will also be presented.

## Using Ion Exchange Resins to Measure Uranium Groundwater Flux

### *Session: Student Abstract*

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Ion-exchange resins were evaluated for use in passive flux meters (PFM). The PFMs will be installed in uranium-contaminated aquifers to measure groundwater flow by the use of a displaced tracer, as well as the uranium flux through the meter. Resins were tested in the laboratory for maximum adsorption of uranium in various solution compositions, and also in contaminated Rifle, Colorado, groundwater. Recovery of uranium from the resins by acid desorption was also investigated. Adsorption studies involved addition of a fixed amount of resin to solutions of variable uranium concentrations. After a 24-hour equilibration time, the solution and resins were separated. Percent sorption was determined by difference following analysis of the original solution and the solution exposed to the resin. To examine recovery, which is essential in determining uranium flux, resins were extracted with 1% nitric acid solutions. Uranium analyses were performed by inductively coupled plasma-mass spectrometry and by inductively coupled plasma-atomic emission spectroscopy for other solution constituents. At a pH of 7.3 in synthetic waters, Dowex 21K, Lewatit S6328 A, and Purolite A500 anion exchange resins adsorbed over 99%, 95%, and 94% of the uranium, respectively. These resins performed equally at 99% in natural waters. Effect of pH on the aqueous uranium speciation has some influence on the adsorption of uranium, but these effects are complicated by the presence of nitrate in the system. A solution acidified by hydrochloric acid showed little to no sorption by anion exchange, which is expected due to the dominance of  $\text{UO}_2^{2+}$  at the pH examined (3.8). However, pH adjustment with nitric acid showed a similar or reduced amount of sorption when compared to pH 7.3, at similar acidic pH. A 1% acid treatment showed 100% recovery of the adsorbed uranium from both resins. Tracer capacity (used to determine groundwater flow through the PFM) and cost analysis showed the Lewatit resin to be the best choice for the first PFM installation. Initial results from this installation and removal revealed consistent uranium concentrations removed with the current extraction method. Larger samples of resin had higher uranium recovery in the second extraction, which is possibly due to preferential uranium adsorption to the resin and a shortage of exchange anions in the first extraction. These passive flux meters will be a useful tool in determining the effectiveness of the bio-reduction of uranium remediation strategies employed at contaminated sites.

## Resource-Induced Shifts in Bacterial Populations from Uranium-Contaminated Sediment at Oak Ridge FRC Site

*Session: Student Abstract*

Fan Yang, Michigan State University, Joy D. Van Nostrand, University of Oklahoma, James Tiedje, Michigan State University (lead PI), Jizhong Zhou, University of Oklahoma, Terence L. Marsh, Michigan State University

The microbial reduction of heavy metals *in situ* is a cost-effective bioremediation strategy. However, the optimal phylogenetic structure of an actively metal-reducing community remains poorly described. To assess the subsurface community structure and its function in a uranium (VI) contaminated environment, we have investigated sediment samples from Area 3, a site with measured uranium reduction at the Field Research Center at Oak Ridge National Laboratories. Soil samples were obtained from wells FW102 (two depths), FW102-3 and FB107. Each sediment sample was used as an inoculum in anaerobic batch microcosms incubated at 15°C that were sequentially transferred (x3 @ 35-day intervals) in minimal media. Six enrichment conditions were established that varied electron donor (acetate, lactate, and ethanol) and acceptor (ferric citrate and uranyl acetate). Reduction of Fe(III) was observed in 9 to 13 days, and the gross appearance of iron precipitates varied with electron donor. Although microbial growth was observed in U(VI) enrichments, the reduction of uranium was substantially slower. A 10% depletion of U(VI) was detected in the enrichment system after seven months of incubation. Pyrosequencing of amplified 16S rRNA genes was performed to identify the phylogenetic composition of the original sediment samples (FW102-2, FW102-3 and FB107) and the third lactate-U(VI) enrichment communities (FW102-2\_3rdULac and FB107\_3rdULac). Preliminary data revealed that the community profile of FW102-2 and FW102-3 were similar and distinct from FB107. In addition the bacterial communities in FW102-2 and FW102-3 were more even and diverse than FB107. Phyla *Acidobacteria*, *Actinobacteria*, *Chloroflexi* and *Proteobacteria* dominated the original FW102-2 and FW102-3 soil samples. The bacterial community in FB107 was notably less even with the *Proteobacteria* predominating (approximately 92.8%). A substantial amount of unclassified bacteria, as determined with the Classifier feature of the Ribosomal Database Project, was detected in all three sediment samples. However, after serial enrichment, the communities had considerably fewer unclassifiable rRNA sequences. Phyla *Firmicutes* and *Proteobacteria* were consistently detected as the dominant groups in the enriched communities. The abundance of detectable *Firmicutes* increased from approximately 2% to 24% and 93.9% in FW102-2\_3rdULac and FB107\_3rdULac microcosms, respectively. Surprisingly, high abundance (75.7%) of *Proteobacteria* was detected in enriched FW102-2\_3rdULac while the bacterial community in FB107\_3rdULac shifted to one dominated by *Firmicutes* (~4.7% of *Proteobacteria* was detected). Genus level classification revealed that in FW102-2\_3rdULac, approximately 98% of *Proteobacteria* were *Geobacter* and 84% of *Firmicutes* were *Sporomusa*, and neither genus was detected in enriched FB107\_3rdULac. The *Firmicutes* in the enriched FB107\_3rdULac community appeared to be mostly composed of *Anaerostipes* (23.2%) and unclassified *Verrucomicrobiales* (72.5%). The transcriptome of these reducing communities are currently being investigated along with additional characterization of the community structure and isolation of abundant populations. In addition, studies using GeoChip, a functional gene array with probes for thousands of genes involved in the geochemical cycling of C, N, S, and P, metal resistance and reduction, and organic contaminant degradation, are under way to determine the functional diversity of the communities and how the communities change under the different electron acceptors and donors.