3rd Annual DOE-ERSP PI Meeting:

Abstracts

April 7–9, 2008

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

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U.S. Department of Energy—Office of Science

Environmental Remediation Sciences Program

2008 Annual Principal Investigators Meeting

Welcome to the 2008 Annual Environmental Remediation Sciences Program (ERSP) Principal Investigators Meeting. The past year has been exciting and productive for your science and for our program. On behalf of the program managers in the Environmental Remediation Sciences Division, Office of Biological and Environmental Research, we are looking forward to hearing about the progress of each of your projects and your plans for the future.

We sponsor this meeting annually with three major goals: (1) to provide opportunities for interaction and interchange among the ERSP scientists, something that we feel is critical to the overall success of the program; (2) to provide our staff with an opportunity to evaluate the progress of each project; and (3) to showcase our program to interested parties within DOE and in other Federal Agencies.

This year, we have a very full agenda that includes special platform and poster sessions on Wednesday afternoon and evening to highlight the new approach to funding research at the DOE National Laboratories. In FY 2007, our parent office, the Office of Biological and Environmental Research (BER), notified all DOE National Laboratories of the Scientific Focus Area (SFA) approach to lab funding that would be implemented throughout BER in FY 2008 and FY 2009. DOE's National Laboratories exist as unique and specialized resources for our nation, and the SFA approach is designed to better utilize these unique national resources. The Wednesday afternoon and evening sessions are designed to explain the SFA approach to our PI community, and to provide opportunities to ask questions and learn more about the ERSD SFA plans at the DOE National Laboratories.

While we have compressed the agenda somewhat to include time for the SFA sessions, we have also worked to retain the more relaxed schedule that we implemented last year. We have provided opportunities for discussion and interaction among the formal presentations. We have also provided large blocks of time for the evening poster presentations. Each ERSP-funded project will be represented by a poster and at least one of the investigators. We will use these sessions to explore the progress and plans of each project.

On behalf of the ERSD program managers and staff, I thank you for attending this annual meeting. We are excited by your progress and anticipate more important advances in our mission to support DOE's efforts in environmental remediation. We look forward to meeting each of you and to discussing your research plans and results.

Best Regards,

David Lesmes

ERSD Acting Director

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Preliminary Agenda

Monday, April 7, 2008
Afternoon Session

1:00 p.m.   **ERSP Programmatic Overview and Outlook for the Future**
David Lesmes, Acting Division Director, ERSD

1:30 p.m.   **TBA—Invited Presentation**

2:00 p.m.   **A Geologic Framework for Reactive Solute Transport Properties
in Sedimentary Aquifers**
Richelle M. Allen-King, University at Buffalo (SUNY)

2:30 p.m.   **Contrasts in Biogeochemical Processes Affecting Uranium Transport at
Three Field Sites**
James A. Davis, U.S. Geological Survey

3:30 p.m.   **Coupled Biogeochemical Processes in the Soil and Soil-Plant Systems Re-
sponsible for Enhanced Transport of Plutonium in the Vadose Zone**
Fred J. Molz, Clemson University

4:00 p.m.   **Terry Beveridge Memorial Lecture**

4:30 p.m.   *Grant Ferris, University of Toronto*

5:30 p.m.   **DINNER**

6:30 p.m.   **Poster Session 1**

Tuesday, April 8, 2008
Morning Session

Integrated Field-Scale Research Challenge (IFC) Presentations

8:30 a.m.   **Oak Ridge Site**
              Phil Jardine, ORNL, and Co-PI's

9:30 a.m.   **Old Rifle UMTRA Site**
              Phil Long, PNNL, and Co-PI's

10:30 a.m.   **BREAK**

11:00 a.m.   **Hanford 300 Site**
              John Zachara, PNNL, and Co-PI's

12:00 noon   **A Roadmap for Practical Deployment of Models for Microbially Mediated
              Remediation of Metals and Radionuclides at DOE Sites**
              Jack Parker, University of Tennessee

12:15 p.m.   **LUNCH and FREE (Working Lunch with FREC and IFC teams)**

Afternoon Session

3:00 p.m.   **Nitrite Enhanced Chromium Reduction in Three Model Organisms:**
              ***Geobacter metallireducens*, *Sulfurospirillum barnesii*, and *Desulfovibrio desul-
              furicans* 27774**
              John F. Stolz, Duquesne University

3:30 p.m.   **Structure and Function of Subsurface Microbial Communities Affecting
              Radionuclide Transport and Bioimmobilization**
              Joel E. Kostka, Florida State University

4:00 p.m.   **Metagenomics-Enabled Understanding of Metal-Reducing Communities at
              the ORNL-FRC**
              James M. Tiedje, Michigan State University

4:30 p.m.   **From Community Structure to Functions: GeoChip Development and Its
              Applications to Bioremediation**
              Jizhong Zhou, University of Oklahoma

5:30 p.m.   **DINNER**

6:30 p.m.   Poster Session 2

Wednesday, April 9, 2008

Morning Session

8:30 a.m.   **Integrated Hydrogeophysical and Hydrogeologic Driven Parameter Upscaling for Dual-Domain Transport Modeling**
John M. Shafer, University of South Carolina

9:00 a.m.   **Hyphenated Techniques for Determining pH-Dependent Pore-Scale Uranium (VI) Speciation**
James F. Ranville, Colorado School of Mines

9:30 a.m.   **Development of Modeling and Scaling Methods for Predicting Coupled Reactive Transport Processes**
T. Prabhakar Clement, Auburn University

10:00 a.m.   **BREAK**

10:30 a.m.   **The Role of Microbial Phosphates on Uranium Mobility in the Subsurface**
Patty Sobecky/Martial Taillefert. Georgia Institute of Technology

11:00 a.m.   **Effects of Fermentative Activity on Fate and Transport of U and Cr**
Brent Peyton, Montana State University

11:30 a.m.   **Biochemical Mechanisms and Energy Strategies of *Geobacter sulfurreducens***
Ming Tien, Pennsylvania State University

12:00 noon   **LUNCH** (SFA Panel Working Lunch—Orientation and Charge)

Afternoon Session

Scientific Focus Areas Presentations—DOE National Laboratories

1:30 p.m.   **SFA Overview and Background (ERSD)**

2:00 p.m.   **Argonne National Laboratory**
Carol Giometti/Ken Kemner

2:20 p.m.   **Idaho National Laboratory**
Mark Ankeny/George Redden

2:40 p.m.   **Los Alamos National Laboratory**
Michael Ebinger

3:00 p.m.   **Lawrence Berkeley National Laboratory**
                 Susan Hubbard

3:30 p.m.   **BREAK**

3:45 p.m.   **Oak Ridge National Laboratory**
                 Liyuan Liang

4:15 p.m.   **Pacific Northwest National Laboratory**
                 Harvey Bolton

4:45 p.m.   **Stanford Synchrotron Radiation Laboratory**
                 John Bargar

5:30 p.m.   **DINNER**

6:30 p.m.   **SFA Poster Session**

8:00 p.m.   **MEETING ADJOURNED**

Abstracts

General ERSP Abstracts

Subsurface Colloid-Facilitated Transport of Plutonium

Amr I. Abdel-Fattah (PI), Elmer Garcia, Hakim Boukhalfa, Paul W. Reimus, and Robert Roback

Los Alamos National Laboratory, Los Alamos, NM

Colloid transport has been widely recognized as the most likely mechanism for transporting highly sorbing radionuclides, such as plutonium (Pu). Called "colloid-facilitated transport," this mechanism can be of a major concern at several DOE sites. Our team at LANL has been actively involved in research geared towards understanding and quantifying this challenging transport mechanism. Results from past and ongoing experiments will be presented. Past experiments include the transport of natural groundwater colloids loaded with Pu in two columns packed with saturated alluvium, obtained from a well located in the southwest corner of the Nevada Test Site (NTS). Natural colloids and water used in the experiments were collected directly from groundwater pumped from the same well. Pu(V), which was electrochemically prepared and verified by UV-Visible absorbance spectroscopy to be over 99% pure (in the V oxidation state), was adsorbed onto the natural colloids in batch sorption experiments conducted in filtered groundwater prior to the column experiments. After adsorbing about 40% of the total Pu from solution onto natural colloids (60% in solution), the resulting mixture was injected into two separate columns having different porosities, along with tritiated water. Two experiments were conducted in each column, one with a relatively low injection flow rate of 1.2 mL/hr, and the other at 6 mL/hr. In both columns, results showed that natural colloids traveled through the columns essentially unretarded, with almost 100% recovery at the two flow rates employed. The natural colloids arrived slightly earlier than the tritiated water in all experiments, indicating a smaller effective pore volume for the colloids. No soluble Pu was detected in the column effluents, while Pu adsorbed onto natural colloids was almost completely recovered in the higher-flow-rate experiments, and ~80% was recovered in the lower-flow-rate experiments, indicating that natural colloids are indeed capable of facilitating the transport of otherwise immobile Pu(V) in saturated alluvium at the NTS.

Current experiments were designed for long-term testing of the different mechanisms influencing Pu-colloid transport and for resolving some important hypotheses and questions raised by our previous study and other investigations reported in the literature. Three similar columns were packed with natural fluvial material collected from the Big Lost River at Idaho National Laboratory (INL). The 75 to 2000 μm fraction of the fluvial material was separated by dry-sieving, and then autoclaved prior to use in the experiments. *In situ* natural colloids were extracted by rinsing this fraction of the autoclaved fluvial material several times with ultrapure, de-ionized water and collecting the wash water samples, which were then left to settle for ~2 hours. Natural colloids were carefully siphoned from the top layer (away from the meniscus) of the settled wash water and stored in thoroughly cleaned, autoclaved Teflon bottles. The three columns were wet-packed with the rinsed alluvial material. Flushing with ultrapure, de-ionized water at a steady-state flow rate was immediately started and continued until the concentration of colloids in the effluent samples reached that of the inlet samples. Colloid concentration and size distribution in the effluent samples were measured and used to construct the *in situ* colloid-release kinetics curve for each column. All effluent samples were combined and stored for use in subsequent experiments. A pulse of the extracted colloid suspension was then re-injected into the three columns, together with tritiated water as a conservative tracer. A set of batch Pu sorption and desorption experiments onto natural colloids and fluvial material was started. Preliminary results of these experiments will be presented and discussed.

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

R.M. Allen-King[1] (PI), G. Wang[1], K. Lilienthal[1], G. Weissmann[2], C. Murray[3], B. Bjornstad[3], G. Last[3], and T. Scheibe[3]

[1]University at Buffalo, Buffalo, NY; [2]University of New Mexico; [3]Pacific Northwest National Laboratory

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 analogue 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 place local-scale lithofacies successions, and their distinct hydrologic property distributions, into the basinal context, thus allowing us 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 sandbody inclusions that appear to be bar-top facies. Analysis of K estimates derived from grain size data indicate ~2.5 orders of magnitude variability. The relatively small scale (i.e., tens of meters) of the geologic and hydrologic heterogeneities identified thus far indicate that geostatistical aquifer realizations must be finely resolved (on the order of 1-5 m). The scale of heterogeneity also implies that an investigation of the scale dependence of our results is likely to be an important step towards application to the 200 West Area CT plume.

Low CT sorption was observed in preliminary experiments that also produced correspondingly low log K_{oc} (=1.8-2.6, organic carbon normalized sorption distribution coefficient). These values are consistent with the commonly cited literature value for CT in soil. Very low CT sorption combined with transformation created unacceptably high uncertainty in these preliminary experiments, which has prompted us to develop a surrogate compound approach for sorption measurements. Preliminary CT transformation experiments have focused on assessment of the potential for drilling-induced grain breakage to cause anaerobic CT transformation. Experiments compared the reactivity of pulverized to nonpulverized sediment. We observed a significant difference in the pseudo-first-order transformation rate for samples from different depths, which suggests that geochemical heterogeneity in the aquifer affects CT persistence. We anticipate receipt of fresh core samples, collected using anaerobic procedures from a newly drilled well in early 2008, that will be used for future CT transformation studies.

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.

Mobility of Source Zone Heavy Metals and Radionuclides: The Mixed Roles of Fermentative Activity on Fate and Transport of U and Cr

William Apel[1] (PI), Robin Gerlach[2], Brady Lee[1], and Brent Peyton[2]

[1]Idaho National Laboratory (INL), Idaho Falls, ID
[2]Montana State University, Bozeman, MT

Low-level waste (LLW) pits historically contain cellulosic waste in the form of paper towels, cardboard, wood, and other materials that are contaminated with low concentrations of metals and radionuclides, such as hexavalent chromium (Cr(VI)) and hexavalent uranium (U(VI)). The cellulosic waste can potentially inhibit metal mobility through adsorption and serve as a growth substrate for native microbes that are able to hinder metal and radionuclide migration through reduction or bioaccumulation. However, cellulosic breakdown products, including organic acids and alcohols, have the potential to enhance metal mobility through chelation. We are therefore investigating the mixed contribution of cellulose and microbial metabolic processes to overall metal mobility at a surrogate LLW site at the INL.

Our research goals are to characterize the microbial community at the simulated waste pit, to understand how a native soil microbial community responds to the presence of cellulosic waste products, and also to understand the effect of cellulose breakdown products on Cr(VI) and U(VI) mobility in subsurface soils. To date, a number of culture-independent methods, including the construction of clone libraries, T-RFLP, and the application of new 16S DNA microarray technology (16S Phylochip, LBNL), have been used to reveal a diverse microbial community consisting of both aerobic and anaerobic organisms. These techniques have also revealed a strong microbial response to the presence of cellulosic material.

Enrichments established with cellulose as the sole carbon source have led to the isolation of four aerobic and three anaerobic bacterial isolates, as well as one fungal isolate. As a model aerobic community member, a *Pseudomonas* sp. isolate has been used to quantify the effect of growth substrate on U(VI) toxicity and bioaccumulation during aerobic growth in chemically defined liquid media. Growth substrates included expected products of cellulose degradation. Our results showed that U(VI) inhibition varied significantly among the substrates tested (butyrate, glucose, ethanol, and lactate), with U(VI) most toxic when ethanol served as substrate. The choice of growth substrate was also found to influence Cr(VI) toxicity and reduction rates by the *Pseudomonas* sp. isolate under aerobic conditions, with lactate and dextrose leading to faster, more complete growth and Cr(VI) reduction compared to butyrate and ethanol. The tendency of both Cr(VI) and U(VI) to adsorb to a variety of cellulosic materials has also been investigated. Neither Cr(VI) nor U(VI) adsorbed appreciably to any of the materials tested. Research now under way considers the effect of growth substrate microbe-metal interactions under fermenting and anaerobic conditions. Soil column studies will be performed to better understand the effect of cellulosic breakdown on metal mobility under unsaturated flow conditions often found in DOE LLW sites.

Geophysical Signatures of Bacterial Nanowires

Gamal Abdel Aal[1], Estella Atekwana[1] (PI), Dwayne Elias[2], and Yuri A. Gorby[3]

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[3]J. Craig Venter Institute, La Jolla, CA

The primary goal of our funded research program is to establish a fundamental and mechanistic understanding of the relationship between microbial activity, including nanowires, and the geophysical/geoelectrical responses of subsurface environments. Based on previous studies by Gorby et al. (2006), we conducted preliminary experiments to investigate the biogeoelectrical response of nanowires. We hypothesized that S. *oneidensis* MR-1 and other microorganisms that produce bacterial nanowires should directly impact electrical properties of porous saturated media. We performed controlled laboratory sand-column experiments using wild type S. oneidensis strain MR-1 and a mutant strain that produced nonconductive nanowires as our model organisms. Lactate served as the electron donor and O_2 serving as the sole electron acceptor. Voltage gradients measuring upwards of 600 mV were observed in the MR-1 column and persisted until the end of the experiment. No such self potential (SP) response was detected in the mutant column. Scanning electron microscopy of samples taken from the top, middle and bottom portions of the column revealed that wild type MR-1 cells appeared healthy and produced an intricate network of branched extracellular appendages. In contrast, nanowires of the mutant strain appeared thin and frail with no oriented arrangement, and cells were visibly deteriorated by the end of the experiment. We hypothesize that the presence of structured and oriented nanowires served as the electrical connections that linked the oxidizing and reducing zones, thus permitting electron transfer between the two regions.

Obviously, many questions remain concerning extracellular electron transfer and the development of SP in saturated, nonsulfidogenic porous media. This presentation will update our progress on ongoing experiments designed to answer these questions.

The Reaction Specificity of Nanoparticles in Solution

Donald Baer

Pacific Northwest National Laboratory, Richland, WA

Iron-based metallic and oxide nanoparticles have been shown to have enhanced reactivity towards a variety of chemical species, including chlorinated hydrocarbons and reducible oxyanions, which frequently contaminate groundwater at DOE and other government and industrial sites. Completed work has demonstrated that some types of nanoparticles have the desirable ability to reduce carbon tetrachloride (CT) in contaminated water, while producing a lower yield of chloroform (CF) than other types of iron. The objective of this project is to develop a fundamental understanding of the mechanism(s) responsible for the overall reactivity, reaction selectivity and life cycle of iron-based metal, bimetal, and oxide nanoparticles with the intent of optimizing particle size, formulation, and structure for reduction of environmental contaminants. This combined BER/BES project includes studies specifically addressing issues critical for scaling the laboratory results to field conditions, including the impact of different environmental conditions and particle aging.

Recent progress has been made on three fronts: (1) the aging of zerovalent iron (ZVI) nanoparticles in a water solution and the impact of this aging on reduction of CT; (2) the impact of metal additions to ZVI nanoparticles on the overall reaction rates and pathways and (3) experimental measurements and theoretical modeling of the reaction of simple Fe clusters and individual atoms with CT. A significant issue in the use of ZVI to remediate CT involves controlling the branching ratio, so that benign products such as formic acid (HCOOH) are formed rather than toxic byproducts such as CF. Measurement of particle structures and reactivities at different stages of reaction show that contaminant degradation pathways evolve with time. The ZVI nanoparticles are initially covered with an adherent iron-oxide coating, and the initial reactivity is low. As the oxide is exposed to water, the oxide becomes less coherent, hydrogen evolves from the sample, and the reactivity increases. After five days, the metal content in the nanoparticles decreases as more material is oxidized and the reaction rate slows. Nonetheless, a reasonably high reaction rate and good branching ratio persists for more than thirty days. The addition of metal dopants to the nanoparticles at various stages of growth or processing demonstrates the importance of both dopant type and location on reaction rates and pathways.

Details of CT reaction with Fe are being examined for individual atoms and small clusters. Ultra high vacuum experiments have shown that chlorinated hydrocarbons are inert to the oxygen terminated FeO(111) surface, whereas the addition of small amounts of ZVI leads to formation of reactive intermediates capable of attacking FeO(111) and removing O to form phosgene ($OCCl_2$) and CO. *Ab initio* electronic structure methods are being used to elucidate thermochemistry and reaction pathways, including reaction intermediates. The results suggest that the reactions proceed via competing chlorine atom transfer and Fe atom insertion mechanisms to give stable Fe-C-Cl containing intermediates.
Several theoretical approaches have been developed and used to identify the most important reaction pathways relevant in groundwater. Recently, we have performed multiscale simulations to predict the activation barriers for the reductive dechlorination of CT, using a multiscale dynamical framework for finite temperature that embeds high-level *ab initio* methods into coarse-grained models. Very good agreement was seen between these simulations and experimental data for oxide-free iron electrodes (-0.6 V to -1.2V reduction potentials).

Coupled Biogeochemical Processes Governing the Stability of Bacteriogenic UO_2: Molecular to Meter Scales

John R. Bargar[1] (PI), Rizlan Bernier-Latmani[2], Daniel Giammar[3], and Bradley M. Tebo[4]

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[2]Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland
[3]Washington University St. Louis, St. Louis, MO
[4]Oregon Health & Science University, Beaverton, OR

The structure and composition of biogenic UO_2 may profoundly impact its success as a long-term sink for uranium in the subsurface. Nanobiogenic UO_2 is expected to be strained at the molecular scale. While this factor may significantly destabilize the material, the solubility of nanobiogenic UO_2 is unknown, and there is concern that nano-size may ultimately render it unsuitable as a long-term sink for uranium, even under reducing conditions. Moreover, sedimentary UO_2 commonly contains impurity cations such as Ca^{2+}. Hyperstoichiomety (UO_{2+x}, $0 \leq x \leq 0.25$) is common; stoichiometric UO_2 is extremely rare in nature. Of relevance to ERSP, hyperstoichiometry and cation substitution have been shown to increase the stability of sedimentary uraninites with respect to oxidation/corrosion. Lastly, one of the strongest environmental oxidants, bacteriogenic Mn oxide, is of concern for its potential ability to catalytically oxidize subsurface biogenic UO_2.

Objectives: Our four-institution team is conducting a systematic study to address the basic question, *how do these factors moderate biogenic UO_2 stability?* Specific goals are to characterize: (1) molecular- and nanoscale structures of biogenic UO_2 in the presence and absence of important groundwater cations; (2) intrinsic solubility and rate constants of biogenic UO_2; (3) coupling of biologically mediated Mn(II) oxidation and UO_2 oxidation; and (4) the influence of these molecular-scale processes on meter-scale release of U(VI) in sediments.

Atomic- and nano-scale structure of biogenic UO_2: Biogenic nano-UO_2 was found to be *unstrained*, structurally homologous to stoichiometric UO_2, and to exhibit a disordered 1 nm thick outer rim. UO_2 produced by *Shewanella oneidensis* MR-1, *Desulfovibrio vulgaris*, and *Geobacter sulfurreducens* all exhibit this general structure. Co-solute Mn(II) was found to become structurally incorporated into bio-UO_2.

Dissolution and solubility of biogenic UO_2: The intrinsic solubility of nanoparticulate biogenic UO_2 was found to be *similar to that of bulk synthetic UO_2*. Even at anoxic conditions, the presence of carbonate accelerated the dissolution of both biogenic and abiotic UO_2.

Mn cycling and UO_2 oxidation: Mn-oxidizing bacteria were found to drive the rapid oxidation of biogenic UO_2 in the presence of dilute Mn(II). However, U(VI) inhibits the Mn(II)-oxidizing bacteria at relatively low concentrations, leading to a complex interplay of processes.

Meter-scale dynamics: Column studies are under way, using sediment from the Old Rifle site seeded with a *Shewanella oneidensis* MR-1 culture, to investigate the impact of impurity cations and biological Mn(II) oxidation on the meter-scale response of UO_2 to oxidizing conditions.

Impact: This work significantly improves the confidence in nano-bio UO_2 as a long-term sink for U, particularly under reducing conditions. Furthermore, constants derived from bulk synthetic UO_2 should be appropriate for modeling the behavior of bio UO_2 in groundwater. Biological Mn(II) oxidation may play important roles in accelerating UO_2 oxidation in the presence of O_2.

Toxicity of Mercuric Chloride to Nitrate Reducing Enrichment Cultures Established from Sediments at the Field Research Center in Oak Ridge, TN

Heather Wiatrowski[1], Ria John[1], Kritee[1], Lily Y. Young[2], Nathan Yee[2], and Tamar Barkay[1] (PI)

[1]Department of Biochemistry and Microbiology, and
[2]Department of Environmental Sciences, Rutgers University, New Brunswick, NJ

Microbial transformations of mercury have been extensively studied in lacustrine ecosystems, where microbial methylation and subsequent biomagnification of methylmercury (MeHg) in the food chain is a public health concern. Microbial reduction of ionic mercury, Hg(II), to elemental mercury, Hg(0), which is commonly mediated by the mercury resistance (*mer*) operon in surface waters, can increase the mobility and reduce the toxicity of mercury. However, little is known about microbe-mercury interactions in groundwater aquifers, where both microbial life and the geochemistry of mercury may be very different than in surface waters. Our research examines how microbial transformations may affect the mobility and toxicity of mercury in groundwater aquifers. Redox potential, which is typically lower in groundwater than in surface waters, affects mercury speciation. Geochemical models predict that below an Eh of +450 mV and circumneutral pH, mercury is most stable as the aqueous species of Hg(0).

To determine how mercury toxicity impacts denitrification by subsurface microbial communities, we derived a nitrate-reducing enrichment culture by incubating sediment from the background area at the Field Research Center (FRC) in an artificial ground water medium (AGW) supplemented with 10 mM acetate and 5 mM nitrate. When nitrate reduction was established, we used this enrichment to inoculate serum bottles containing AGW, 10% (weight:volume) autoclaved FRC sediment, 10 mM acetate, 5 mM nitrate, and increasing concentrations of mercury, provided as $HgCl_2$. Nitrate reduction was monitored over the course of two months, and the time taken for the enrichment to initiate nitrate reduction was used as an indication of mercury toxicity. Unexposed microcosms reduced all the added nitrate in a period of 13 days. Inhibition of nitrate reduction was observed at mercury concentrations as low as 50 nM (10 ppb), which delayed complete reduction of the added nitrate by 7 days. Enrichments containing concentrations of mercury at > 1.1 µM $HgCl_2$ were totally inhibited in their ability to reduce nitrate. Molecular analysis of these enrichments, currently in progress, examines what microbes are selected under various mercury concentrations, and what pathways they employ for the detoxification of mercury under dentirfying conditions. These studies form the basis for the evaluation of the role of microorganisms in the mobility and speciation of mercury in the subsurface.

Biomolecular Mechanisms Controlling Metal and Radionuclide Transformations in *Anaeromyxobacter dehalogenans*

Matthew J. Marshall[1], Frank E. Löffler[2], Robert A. Sanford[3], David W. Kennedy[1], Andrew E. Plymale[1], Alice Dohnalkova[1], Jim K. Fredrickson[1], and Alexander S. Beliaev[1] (PI)

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The overall scientific goal of the project is to elucidate the molecular mechanisms of radionuclide biotransformation by *Anaeromyxobacter dehalogenans*, a predominant member of indigenous microorganisms commonly found in contaminated subsurface environments, and to assess the effects of relevant environmental factors affecting these transformation reactions. Bacteria of the genus *Anaeromyxobacter* are facultative anaerobic dechlorinating organisms capable of enzymatically reducing a variety of metal and radionuclide contaminants. *Anaeromyxobacter dehalogenans* strain 2CP-C rapidly reduces U(VI) and Tc(VII) to $U(IV)O_2(s)$ and $Tc(IV)O_2(S)$, respectively. Kinetic studies with resting cells revealed that U(VI) or Tc(VII) reduction rates using H_2 as electron donor exceeded those observed during acetate amendments. However, the reduction of Tc(VII) could not definitively be linked with acetate-oxidation. The localization of reduced UO_2 is extracellular, while TcO_2 nanoparticles are both periplasmic and extracellular. While the electron donor did not affect UO_2 nanoparticle size or the association of UO_2 nanoparticles with an extracellular polymeric substance (EPS), donor-specific differences in UO_2 nanoparticle aggregation were observed. In addition, to direct Tc(VII) reduction, *A. dehalogenans* 2CP-C also reduced Tc(VII) using an Fe(II)-mediated mechanism; as biogenic Fe(II) produced during the reduction of Hanford Site sediments or synthetic Fe oxides rapidly removed soluble $^{99}Tc(VII)O_4$- from solution. These finding expand our knowledge of the radionuclide reduction processes and influence the fate and transport of radionuclide contaminants in subsurface waters.

Similar to other metal-reducing species, the metal reduction in *Anaeromyxobacter* is thought to be linked to a diversified electron transport chain. Based on the whole-genome sequence analysis, *A. dehalogenans* strain 2CP-C encodes a large number of redox-active proteins, including over fifty putative *c*-type cytochromes. Of particular interest is a gene cluster that includes a number of high-molecular-weight multiheme *c*-type cytochromes, two putative lipoproteins; and proteins putatively involved in transport and/or assembly of electron transport machinery. Global transcriptome profiling indicated that 2CP-C expresses a cadre of high molecular multiheme *c*-type cytochromes in response to solid metal electron acceptors. Notably, genes encoding the putative *c*-type cytochromes with 16 and 26 heme binding sites, were specifically induced in the presence of insoluble Fe(III) oxide. To determine the role of these genes in metal reduction, we are developing and utilizing several genome level approaches, including utilization of site-directed and random mutagenesis approaches to isolate metal reduction deficient mutants of 2CP-C. The results from efforts to develop a mutagenesis protocols a will also be presented. This study represents an important step towards the goal of characterizing the metal respiratory system of *A. dehalogenans* 2CP-C on a genomic scale.

Adsorption of Water on Magnesium Oxide and Iron Oxide Surfaces under Ambient Conditions

D.E. Starr[1], Ch.D. Weiss[1], S. Yamamoto[2], S. Kaya[2], E. Mysak[1], T. Kendelewicz[3],
A. Nilsson[2], M. Salmeron[1], G.E. Brown[3], and H. Bluhm (PI)[1]

[1]Lawrence Berkeley National Laboratory, Berkeley, CA
[2]Stanford Synchrotron Radiation Laboratory, Menlo Park, CA
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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, in particular MgO(100), Fe_2O_3(0001) (hematite) and Fe_3O_4(0001) (magnetite). Our experiments focus on the basic mechanism for water adsorption and bonding on oxide surfaces, in particular the role of hydroxils 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. These hydroxide groups seem to act as anchors for subsequent water adsorption. Our future plans are to investigate cooperative effects when water is coadsorbed with other environmentally relevant gases, such as carbon dioxide.

Anaerobic Biotransformation and Mobility of Pu and of Pu-EDTA

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The complexation of radionuclides by co-disposed ethylenediaminetetraacetate (EDTA) has enhanced their transport in sediments at DOE sites. The objectives of this project are to determine the mobile form of Pu-EDTA, and to determine the anaerobic biotransformation of Pu(IV) in the presence of EDTA.

Pu Thermodynamics: It is generally assumed that this enhanced transport of Pu in geologic environments is a result of complexation of Pu(IV) with EDTA. However, the fundamental basis for this assumption has never been fully explored. We reviewed all of the relevant published data including our own to develop reliable equilibrium constant values for formation of $Pu(OH)EDTA^-$, $Pu(OH)_2EDTA^{2-}$, $Pu(OH)_3EDTA^{3-}$, and Fe(III)-EDTA. We developed a Pu(IV)-EDTA model to predict Pu behavior in geologic environments. The mobilization of Pu(IV) by EDTA in geologic environments depends on complexation constants of Pu with EDTA, the dominant oxidation state and the nature of Pu solids, the complexation constants of environmentally important metal ions (e.g., Fe, Al, Ca, Mg) that compete with Pu for EDTA, and EDTA interactions with geomedia (e.g., adsorption, biodegradation); any of these effectively reduce EDTA concentrations for complexation. Extensive studies over a large range of pH values (1 to 14) and EDTA concentrations (0.0001 to 0.01 M) as a function of time were conducted on the solubility of 2-line ferrihydrite ($Fe(OH)_3(s)$), $PuO_2(am)$ in the presence of different concentrations of Ca ions, and mixtures of $PuO_2(am)$ and $Fe(OH)_3(s)$. Predictions based on these extensive fundamental data show that environmental mobility of Pu as a result of Pu(IV)-EDTA complexation, as reported/implied in the literature, is not possible. The data also show that in geologic environments where Pu(III) and Pu(V) are stable, the EDTA complexes of these Pu species may play an important role in Pu mobility (Rai et al. 2008).

Biological Reduction of Pu: The anaerobic reduction of Pu(IV) by two dissimilatory metal-reducing bacteria (*Shewanella oneidensis* MR-1 and *Geobacter sulfurreducens* PCA) was examined with respect to the effects of EDTA and the humic analogue (electron shuttle) AQDS. We observed a similar rate of reductive solubilization of PuO_2 in the presence of EDTA and absence of AQDS by both PCA and MR-1. In the presence of both EDTA and AQDS, our reductive solubilization of Pu occurred over a period of hours, rather than days (as in our treatments without AQDS). We found that the effect of AQDS upon reductive solubilization of $Pu(IV)O_2(am)$ in the presence of EDTA was greater for PCA than on MR-1; we speculate that this is because MR-1 produces endogenous electron shuttles, unlike PCA. In the absence of EDTA, the solubility of Pu at pH 0 decreased over time, due to an unknown mechanism, and the fraction of solid phase Pu that was Pu(III) also decreased over time, either due to oxidation or to incorporation into the acid inextractable fraction of Pu. Even these low levels of Pu reduction in the absence of EDTA are significant, because the amount of Pu solubilized still exceeds drinking water standards (15 pCi L^{-1} and 1×10^{-12} M). This research is being prepared for publication.

We conclude that Pu(IV)-EDTA is not the mobile species in the anaerobic environment, suggesting that Pu(III)-EDTA is the mobile species. The reductive solubilization of Pu(IV) by the two bacteria studied was greatly enhanced in the presence EDTA, though the absence of EDTA does not exclude the biological reductive solubilzation of toxic quantities of Pu.

*Rai, D., D.A. Moore, K.M. Rosso, A.R. Felmy, H. Bolton Jr. 2008. J. Sol. Chem, *in press.*

Stabilization of Plutonium in Subsurface Environments via Microbial Reduction and Biofilm Formation

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Microbial processes, such as bio-oxidation, *in situ* sequestration, bio-mineralization, or reaction with bacterial metabolites, may play a significant role in defining the redox speciation of plutonium and therefore determine its mobility. Our focus in this project is on Pu biogeochemistry, because Pu species comprise a significant portion of the DOE's surface and subsurface contaminant inventory, and because Pu contaminant biogeochemistry and mobility are poorly understood. The overall objective of the research presented is to study key reactions, which are known to affect major redox-active transition metals such as Fe and Mn, and determine how they affect Pu speciation and environmental mobility. Specifically we investigated the ability of dissimilatory metal-reducing bacteria (DMRB) *Geobacter metallireducens* GS15 and *Shewanella oneidensis* MR1 to reduce Pu species, and examined the effect of natural and synthetic ligands on these processes. We have also examined the equilibrium distribution of Pu and U between *Pseudomonas Putida* biofilm and iron oxide minerals.

Dissimilatory reduction of Pu species: We have demonstrated previously the ability of dissimilatory metal-reducing bacteria (DMRB) *Geobacter metallireducens* GS15 and *Shewanella oneidensis* MR1 to reduce Pu(VI)/Pu(V) to Pu(IV). Our results indicate that the product of oxidized Pu species (Pu(VI)/Pu(V)) reduction is Pu(IV) and that Pu(III) is not produced. Thermodynamic calculation show that Pu(III) production from Pu(VI)/Pu(IV) species is less favorable than Pu(IV) production. The reduction potentials of Pu(IV/V) species to Pu(IV) at neutral pH range from $E_0 = 0.59$ to 0.96 V—significantly higher than the reduction potentials characterizing the reduction of Pu(IV/V) species to Pu(III), which range from $E_0 = -0.01$ to 0.36 V. Our data indicate that although Pu(III) is thermodynamically accessible to bacterial reduction, bacteria only utilize Pu(VI)/Pu(V) to Pu(IV) reduction for their anaerobic respiration, because it is energetically more favorable. We have also demonstrated that freshly precipitated Pu(IV) hydroxide solids were accessible to bacterial reduction, but their reduction was limited to less than 8% of the available Pu. The kinetics and yield of the Pu solids reduction were affected by the presence of chelating ligands. The effect of synthetic ligands like EDTA and humic substances on Pu(IV) hydroxides and aged Pu(IV) oxides reduction was also examined.

Equilibrium distribution of Pu and U between iron oxide minerals Pseudomonas putida biofilm: Plutonium contamination is often present under oxic environments. Plutonium distribution under such environments is affected by the metabolic functions of aerobic bacteria. Key among these processes is the ability of microorganisms to dissolve minerals for nutrients acquisition. We have examined the distribution of U and Pu species between goethite and *Pseudomons putida* biofilm grown on solid growth media. We report the effect of U and Pu species on biofilm growth and the distribution of radionuclides between the biofilm components and the mineral surface.

Coupling Sorption to Soil Weathering During Reactive Transport: Impacts of Mineral Transformation and Sorbent Aging on Contaminant Speciation and Mobility

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This project aims for a predictive-mechanistic understanding of the coupling between mineral weathering and contaminant (Cs, Sr, I) transport/fate in caustic waste-impacted sediments. Specific objectives are: (1) to assess the molecular-scale mechanisms responsible for time-dependent sequestration of contaminants (Cs, Sr and I) during penetration of waste-induced weathering fronts; (2) to determine the rate and extent of contaminant release from the sorbed state; and (3) to develop a reactive transport model based on molecular mechanisms and macroscopic flow experiments that simulates observed sorption, aging, and desorption dynamics. In this study, caustic waste-weathered Hanford sediments were subjected to desorption-dissolution in a background pore water simulant.

Three glacio-fluvial sediments from the Hanford Site (WA, USA) were reacted with hyperalkaline (pH > 13) Na-Al-NO_3-OH solution over a 365-day period. Strontium uptake (q_{Sr}) was preferred over cesium at nearly all reaction times. Contaminant uptake increased with decreasing sediment particle size at early times, but that effect was erased over the long term, when all sediments showed similar mass normalized uptake after several months of reaction time. Strontium became progressively recalcitrant to desorption after 92 days, with accumulation and aging of neoformed aluminosilicates. Formation of Cs^+ and Sr^{2+} containing cancrinite and sodalite was observed after 183 days by SEM, ^{29}Si and ^{27}Al NMR, and synchrotron µ-XRF and µ-XRD. EXAFS data for $q_{Sr} \approx 40$ mmol kg^{-1} showed incorporation of Sr^{2+} into both feldspathoid and $SrCO_3$(s) coordination environments after one year. Adsorption was predominant at early times and low sorbate amount, whereas precipitation, controlled largely by sediment Si release, became increasingly important at longer times and higher sorbate amount.

Sediments weathered at several hyperalkaline conditions for 180 or 365 days were then studied in flow-through experiments that probed desorption into background pore waters to measure feldspathoid dissolution kinetics after removal of the hyperalkaline waste source. These studies indicate that the initial stage of contaminant release is governed by rapid kinetics of ion exchange, with preferential release of Sr^{2+} or Cs^+ by Ca^{2+} or K^+, respectively. However, after more than 500 pore volumes, sustained long-term release of Cs^+ and Sr^{2+} is controlled by feldspathoid dissolution. Reactive transport modeling indicates that while dissolution dominates at long times, it is also superimposed on early ion exchange processes, contributing to initial contaminant release. Hence, contaminant release includes concurrent desorption and dissolution kinetics, mirroring their measured co-occurrence during contaminant uptake from caustic solution. Quantification of nitrate precipitation into—and release from—neoformed precipitates suggests that elevated contaminant concentrations suppress feldspathoid formation, consistent with observations reported in our earlier studies of specimen clay minerals.

Development of Modeling Methods and Tools for Predicting Coupled Reactive Transport Processes in Porous Media at Multiple Scales

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The objective of our present project is (1) to develop modeling approaches and simulation tools that will predict the transport of DOE-relevant contaminants (a metalloid, oxyanion [As(III/V)] and radioactive cation [U(VI)]) in subsurface systems; (2) to study the interactions of these contaminants with synthetic subsurface media under well-controlled conditions across a range of scales, from laboratory batch to 1-D column experiments to two-dimensional 2-D soil box experiments; and (3) to investigate the scaling issues inherent in the interactions of uranium and arsenic with two major classes of subsurface materials, iron coatings and manganese oxyhydroxides, respectively.

On the modeling front, we have developed a new version of the RT3D code that is fully integrated with the updated version of the transport routines available in transport code MT3DMS. We have implemented share-memory parallel processing ideas into the RT3D code using OpenMP directives. The goal is to develop a MODFLOW-based tool that can be used to simulate the reactive transport processes occurring at the site scale. The tool will be supported within the GMS platform to facilitate the development of site-scale conceptual models. We have also derived a set of new analytical solutions for solving multispecies transport equations coupled with radioactive decay chains. These solutions were used to unravel the fundamental coupling behavior of radioactive reactive transport systems. The details of these new solution work were recently published in a two-part journal article (Srinivasan and Clement, 2008 a,b).

We have developed arsenic reactive-transport datasets in both batch and column-scale, and developed a scalable reactive-transport modeling framework to describe the datasets. This work was recently published (Radu et al. 2008). We have also completed 2-D soil box experiments to investigate how density-induced flow will affect the reactive transport characteristics of a reactive iron system. A numerical framework was used to successfully model this density-influence transport dataset (Kanel et al. 2008). Finally, we have developed batch-scale data to test the scaling characteristics of surface-complexation models used for uranium and arsenic sorption. We have hypothesized surface area and site density as fundamental scaling parameters in these metal sorption systems. We are currently exploring methods to modify the diffuse double layer models that can be used to describe the sorption of uranium and arsenic on soils with different levels iron. This work will be extended to interpret column and/or 2-D-soil-box-scale transport of uranium and arsenic.

Key Journal Articles Published in 2008:

1) Radu et al., A scalable model for predicting arsenic transport coupled with oxidation and adsorption reactions, *Journal of Contaminant Hydrology*, 95, 30–41, 2008.
2) Kanel et al., Two-dimensional transport of reactive zero nanoparticles in porous media, *Environmental Science & Technology*, 42, 896–900, 2008.
3) Srinivasan & Clement, Analytical solutions for sequentially coupled one-dimensional reactive transport problems–Part I & Part-II, *Advances in Water Resources*, 31(2), 203–232, 2008.

Anaerobic, Nitrate-Dependent Uraninite (U(IV)) Bio-Oxidation

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A proposed strategy for the remediation of uranium (U) contaminated sites is based on immobilizing U by reducing the oxidized soluble U, U(VI), to form a reduced insoluble end product, U(IV), uraninite. However, it is now recognized that microorganisms can subsequently catalyze the anaerobic oxidation of U(IV) coupled to the reduction of NO_3^-, resulting in uranium resolubilization and mobilization. Our recent studies have indicated that microorganisms capable of anaerobic nitrate-dependent bio-oxidation of U(IV) (NUox) inhabit a variety of sediments, soils, and groundwater from uranium-contaminated sites, including groundwater and subsurface sediments from the DOE ERS Field Research Center (FRC) at Oak Ridge National Laboratory (ORNL), as well as uncontaminated soils and sediments. Most probable number (MPN) enumeration revealed microbial communities exhibiting NUox ranging from 9.3×10^1 to 2.4×10^3 cells g^{-1} soil in contaminated and uncontaminated sites. Furthermore, physiological screening of a mixotrophic nitrate-dependent Fe(II) oxidizing Betaproteobacterium, *Diaphorobacter* sp. strain TPSY, isolated from Area 2 of the ERS FRC ORNL, resulted in the oxidation of 8 μM U(IV) over 24 hours, with NO_3^- serving as the electron acceptor under nongrowth conditions. Pasteurized control cultures exhibited the abiotic oxidation of 2 μM U(IV). Similarly the catalysis of U(IV) oxidation (4 μM) was observed with cultures of a previously described freshwater, autotrophic nitrate-dependent Fe(II) oxidizing Betaproteobacterium, strain 2002. Under growth conditions, strain 2002 oxidized 12 μM U(IV) in a period of 12 days.

Together with previously published results for the Deltaprotoeobacterium, *Geobacter metallireducens*, and the Betaproteobacterium, *Thiobacillus denitrificans*, and various microbial enrichments, these data indicate that microorganisms capable of anaerobic NUox are ubiquitous. Interestingly, these microorganisms are also capable of anaerobic, nitrate-dependent Fe(II) oxidation. However, nitrate-dependent Fe(II) oxidation is not necessarily synonymous with NUox, as *Azospira suillum,* a model nitrate-dependent Fe(II) oxidizing microorganism, repeatedly failed to oxidize U(IV) under similar experimental conditions. These data suggest that specific enzymes may be required for NUox metabolisms. Further physiological screening of the FRC subsurface isolate, *Diaphorobacter* sp. TPSY, revealed that *c*-type cytochromes were reduced by U(IV). Difference spectrum of U(IV) reduced minus nitrate-oxidized cytochrome content indicated absorption maxima at 422, 520, and 552. Furthermore, biochemical investigation of nitrate-dependent U(IV) oxidation in strain TPSY revealed the expression of several putative high molecular weight proteins specific to this metabolism relative to protein extracts from cultures under nitrate-reducing conditions, with and without uranium. Together, these observations suggest that oxidative microbial metabolisms in anoxic environments could have significant implications for the long-term stability of U(IV) in anoxic environments.

Viral Infection of Model Metal-Reducing Bacteria

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A proposed strategy for the remediation of uranium (U) contaminated sites is based on the immobilization of U by microbial reduction of the oxidized soluble U, U(VI), to form a reduced insoluble end product, U(IV). Previous studies have identified *Geobacter sp*. as predominant U(VI)-reducing bacteria under acetate-oxidizing and U(VI)-reducing conditions in subsurface environments contaminated with U. Examination of the annotated finished genome sequence of *Geobacter sulfurreducens* strain PCA, *G. uraniumreducens* strain Rf4, *G. metallireduceans* strain GS-15, as well as the draft genome sequence of *Geobacter sp.* strain FRC-32, has identified phage related proteins. Presence of these gene sequences indicates that model metal-reducing bacteria, *Geobacter spp.,* are susceptible to viral infection. Polymerase chain reaction (PCR) primer sets were designed to confirm the presence of viral-like sequences in the genomic DNA of laboratory cultivated *Geobacter sulfurreducens* PCA and *Geobacter* strain FRC-32. Of the six genes tested, five primers were successful amplifying a viral-like gene in *G. sulfurreducens* associated with GSU0975 (phage tail sheath), GSU2119 (integrase), GSU0989 (phage tail), and GSU2467 (phage specific recombinase). Two of the eleven predicted viral genes within the genome of *Geobacter* sp. FRC-32 have resulted in a positive PCR product 1055 and 2813. These genes are predicted to encode a Mu-like prophage tail sheath and a phage baseplate assembly protein, respectively. We are continuing to PCR-amplify all viral-like sequences within these microorganisms, as well as *G. uraniumreducens*, to identify target genes and primer sets for further analysis of active infections by following mRNA expression under metal-reducing conditions within these pure cultures. The incubation of a pure culture of *G. sulfurreducens* PCA with 1 μg mL^{-1} mytomycin C (a potent mutagen for prophage induction) during mid-log phase resulted in significant cell lysis relative to cultures that remained unamended. Samples collected following this lytic event (~44 hours) were filtered through a 0.22 μm sterile nylon filter, stained with phosphotungstic acid (PTA), and examined using transmission electron microscopy. TEM revealed the presence of viral like particles in the culture exposed to mytomycin C. Together, these results suggest the infection of *G. sulfurreducens* PCA with a lysogenic bacteriophage.

In situ Sequestration of ^{90}Sr and Uranium in the Vadose Zone through Microbial Precipitation of Phosphate Minerals

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Significant quantities of metals and radionuclides are contained in thick unsaturated zones at several contaminated sites in the western US. In many cases, this contamination has migrated to underlying groundwater, sometimes decades after being released into the subsurface. Because of the prohibitive costs associated with physically removing the contamination, an attractive remedy to this problem is to develop methods for long-term *in situ* stabilization of the contamination in the vadose zone. Our research focuses on developing a method of introducing gaseous compounds to stimulate precipitation of stable phosphate mineral phases in the vadose zone to immobilize soluble contaminants, thus minimizing further transport to groundwater. Current studies are concentrated on the use of triethyl phosphate (TEP), an organophosphate compound with a moderate vapor pressure (~400 ppm in air) that is soluble in water. Previous research has demonstrated that TEP can be used to provide a source of phosphate to subsurface microorganisms, stimulating significantly increased levels of biological activity. The ultimate goal of our project is to determine whether biological degradation of TEP can produce high enough levels of inorganic phosphate to catalyze precipitation of phosphate minerals that will scavenge strontium and uranium from unsaturated zone pore waters.

Ongoing experiments are being conducted to quantify TEP degradation rates in sediment samples from two vadose zone sites (Hanford, Washington, and the Idaho National Laboratory). After about 3 months, the TEP concentrations in the experiments are ~20% lower than in heat-sterilized samples, indicating significant biodegradation of TEP in the cultures. Increases in inorganic phosphate concentrations in the experiments do not match the decreases in TEP, suggesting that precipitation of phosphate minerals may be occurring. However, sorption of both TEP and inorganic phosphate are also possible explanations for the lack of mass balance. A series of abiotic sorption experiments are being performed to measure the potential impact of these effects. The evolution of the microbial communities during the TEP degradation experiments has been characterized using a high-density microarray (PhyloChip). The results of these microarray analyses reveal that *Proteobacteria* taxa are enriched by TEP, particularly *Methylophilaceae*, *Comamonadacea*, *Burkholderiaceae*, and *Rhizobiales*. Further work on this topic includes microarray analysis of metabolically active microbes through extraction and hybridization of rRNA. Other planned studies include evaluation of isotopic methods for monitoring microbial utilization of TEP and demonstration of radionuclide and metal sequestration induced by microbial metabolism of TEP. The result of these studies will be the basis for unsaturated column experiments designed to test different delivery methods for TEP and other nutrients, and to estimate potential rates of phosphate-mediated radionuclide immobilization in the vadose zone.

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

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Approaches are being developed in this project to upscale reactive transport models via a detailed understanding of U(VI) desorption at three scales: bench, intermediate, and field.

Uranium-contaminated aquifer sediments were collected at the Naturita UMTRA site, with grain sizes ranging from silt to gravel. A <2 mm sediment composite was prepared and dry- and wet-sieved to 6 size fractions, each of which was studied intensively at the bench-scale. In batch systems, U(VI) desorption exhibited an initial rapid desorption (about 50% towards equilibrium), followed by slow desorption that required approximately 70 days to reach equilibrium. Rates of U(VI) desorption were higher under conditions favoring formation of $UO_2(CO_3)$ complexes. Column experiments showed slow desorption of U(VI), 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 are consistent with a conceptual model in which U(VI) desorption is limited by diffusion of U(VI) (and bicarbonate) through coatings on grain surfaces and/or the intragranular space of grains and aggregates. The overall U(VI) desorption rate can be modeled by assuming there are two major reservoirs of contaminant U(VI), with diffusion resistance differing by a factor of about 20.

Two experiments have been conducted at an intermediate scale in tanks (2.4 m length) packed with the same sediments. One tank was packed homogenously with <2 mm sediments; a second tank introduced physical heterogeneity by splitting the <2 mm sediments into >250 and <250 micron dry-sieved fractions. The two-sized fractions were placed in alternating layers of different thicknesses, with a "block" of <250 micron fraction at the upgradient end. Spatial gradients of major dissolved constituents were variable in time and space (differently in each tank), and kinetic hindrances to U(VI) desorption were exhibited during stop-flow events. Although U(VI) content in the two tanks was similar, U(VI) release rates differed significantly—for example, 7.3 (homogenous) versus 4.9 μmoles/L (heterogeneous) during the first pore volume of elution.

Reactive transport modeling is currently being conducted to evaluate the effects of chemical and physical heterogeneities on the U(VI) release rates. Small-scale field experiments were conducted under both natural- and forced-gradient conditions to evaluate the importance of groundwater velocity on U(VI) desorption rates. Both experiments were conducted by injecting 0.6 m^3 of 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 to 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 five 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 and including ion exchange reactions. A strategy for upscaling the rates of U(VI) desorption from the bench scale to the intermediate and field scales is currently being developed, based in part on a detailed knowledge of the spatial distribution of intra-granular porosity.

The Hanford 300 Area Groundwater Uranium Plume: Insights from U and Sr Isotopes

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The 300 Area at the Hanford Site in Washington is situated directly along a 2 km stretch of the Columbia River. Past operations in the 300 Area included the disposal of chemical and radioactive waste resulting from fuel-rod fabrication and research processes into a series of settling ponds and disposal trenches. These waste fluids included U, Zr, Cu, as well as a mixture of acidic and basic solutions. The waste disposal resulted in a persistent groundwater U plume in the 300 Area, with U concentrations of greater than 100 ppb, that communicates with the Columbia River. 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 timescale. The persistence of the groundwater U plume is likely due to replenishment from contaminated vadose zone sediments, the complex and slow release of U from those sediments, as well as the dynamic groundwater chemistry in response to incursions of Columbia River water due to highly variable river stages (see review in Zachara et al. 2007, and references therein).

Here we report on results of isotopic studies of the groundwater within and around the vicinity of the 300 Area, and of vadose zone sediment samples from beneath the process ponds. Sr isotopic ratios ($^{87}Sr/^{86}Sr$) of dissolved Sr bear on the hydrologic stratification of the groundwater, and the relative importance of groundwater sources, including the Columbia and Yakima rivers, and infiltration from the vadose zone. The hydrogen and oxygen stable isotopic compositions of water also provide monitors of groundwater source (e.g., Columbia influence) and vadose zone processes (e.g., evapotranspiration).

Isotopic analyses of dissolved groundwater U and vadose zone sediment-leachates provide constraints on contaminant U sources and types. For example, vadose zone U sources can be compared to groundwater U plumes to assess the sources of continuing U supply to groundwater. To the extent that there are multiple sources of U, isotopic measurements allow us to de-convolve and apportion those sources and estimate the fluxes of contaminant U from sediment to groundwater, and from groundwater to river. Analyses of 300 Area groundwater reveal that U in the plume is a mixture of processed enriched U fuel and natural U fuel. Comparison of the groundwater to Columbia River water allows us to estimate the flux of U from the 300 Area to the Columbia River, and constrain where the different U components are entering the river. Na-carbonate/bicarbonate leachates of vadose zone sediments from beneath the North Process and South Process ponds have U isotopic compositions that allow us to model how the vadose zone and groundwater U may be related.

Mechanism of Uranium and Technetium Reduction by Members of the Genus Shewanella

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Uranium reduction. *Shewanella putrefaciens* strain 200 requires cytochrome *c* for anaerobic growth on a wide range of electron acceptors, including metals and radionuclides. *S. putrefaciens* employs the System I cytochrome *c* maturation pathway (Ccm) to attach the heme cofactor to apocytochrome *c*. Recent studies on uranium (U(VI)) respiration by *S. putrefaciens* demonstrated that a conserved histidine residue (H108) in the ABC-transporter permease CcmB was required for both Ccm and anaerobic growth on electron acceptors with mid-point redox potentials (E_0) below a threshold value of 0.36V. In the present study, addition of cystine to the growth medium restored anaerobic growth, but not Ccm activity, to the previously generated mutant CCMB1 (H108Y random point mutation in CcmB). H108 was subsequently replaced with alanine (H108A), leucine (H108L), lysine (H108K), tyrosine (H108Y) or methionine (H108M) by site-directed mutagenesis. The site-directed mutants were grouped into three classes based on anaerobic growth capability, cytochrome *c* content and periplasmic redox condition (measured by free thiol (-SH) content): (1) H108Y and H108K were unable to grow anaerobically on electron acceptors with $E_0 < 0.36$V, produced periplasmic cytochrome *c* at levels <10% of the wild-type strain, and contained periplasmic (–SH) content at levels two-fold greater than the wild-type strain; (2) H108A and H108L retained the ability to grow on electron acceptors with $E_0 < 0.36$V, produced periplasmic cytochrome *c* at levels <20% of the wild-type strain, and contained periplasmic -SH content at levels 50% greater than the wild-type strain; and (3) H108M retained the ability to grow on electron acceptors with $E_0 < 0.36$V, produced periplasmic cytochrome *c* at near wild-type levels, and contained wild-type levels of periplasmic SH. –SH concentrations correlated inversely with the anaerobic growth rates and periplasmic cytochrome *c* content of the site-directed mutants. The addition of cystine to the growth medium restored anaerobic growth capability to the H108Y and H108K mutants. These results demonstrate that *S. putrefaciens* CcmB requires either histidine or a hydrophobic residue capable of coordinating heme iron in a low spin state (i.e., methionine) at amino acid position 108 for maintenance of periplasmic redox homeostasis and production of mature cytochrome *c*.

Technetium reduction. A rapid mutant screening technique was also developed to identify *S. oneidensis* respiratory mutants unable to reduce Tc(VII) as anaerobic electron acceptor. The Tcr mutant screening technique was based on the observation that wild-type *S. oneidensis* produced a black Tc(IV) precipitate on its colony surface during growth on Tc(VII)-amended agar, while colonies arising from mutagenized cells did not. Tcr mutants unable to produce the black precipitate were subsequently tested for anaerobic growth on an array of three electron donors and 13 alternate electron acceptors. The Tcr mutants displayed a broad spectrum of anaerobic growth deficiencies, including several that were unable to reduce Tc(VII) with hydrogen or lactate as electron donor, yet retained the ability to reduce Tc(VII) with formate. Genetic complementation analysis is currently under way to identify the genes required for Tc(VII) reduction activity.

Heterogeneity in Bioreduction and Resulting Impacts on Contaminant Dynamics

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Biogeochemical processes governing the fate and transport of contaminants within the subsurface exhibit a high degree of spatial heterogeneity, owing, in large part, to variability in physical structure. In response to the probability of coupled processes governing reactive transport of contaminants, the objective of our project is to unravel biological and chemical heterogeneity in metal reduction within structured media.

Iron(III) (hydr)oxides have a profound effect on contaminant dynamics, being a dominant substrate for metal sorption. They additionally serve as electron acceptors in anaerobic bacterial metabolism, and as a result undergo dissolution and mineralogical transformation with the onset of reducing conditions. We (and others) have observed the rapid and near complete conversion of 2-line ferrihydrite (supported on quartz-sand) to goethite and magnetite under advective flow. The predominant factor controlling (bio)mineralization of ferrihydrite-sand is the surface loading of Fe(II)—a mechanism supported by abiotic experiments. Reaction of ferrihydrite with Fe(II) results in the rapid transformation to goethite at low and magnetite and high surface concentrations. However, within diffusively controlled hydrologic environments, dissimilatory iron reduction can result in a buildup not only of Fe(II) but also bicarbonate alkalinity, resulting in additional reduction products, such as siderite and green rust within batch systems.

In structured soils and sediments, individual aggregates form a network of interconnected microenvironments. Solutes move preferentially (by advection) through macropores and slowly (by diffusion) into intra-aggregate micropores, leading to mass-transfer limitations in solute transport. As a consequence of the physical heterogeneity, biogeochemical environments comparable to both column (advective) and batch (diffusive) systems are present. We used a combined experimental and modeling approach to qualitatively and quantitatively understand redox transformations at the microscale. Microbial distribution and resulting heterogeneity in biogeochemical processes were examined within flow-through reactors containing a single artificial aggregate made of ferrihydrite coated-sand and inoculated with *S. putrefaciens*; a continuous pulse of 3 mM lactate was injected along a simulated external macropore, and both oxic and anoxic advecting waters were investigated. Temporal and spatial heterogeneity in biotransformation of iron results within the aggregate. Within anoxic advecting waters, a slight and uniform transformation results in approximately 20% (mol Fe) of ferrihydrite evenly distributed as goethite and magnetite after 9 days of reaction. This distribution of mineral phases remains within the outer portion of the aggregate; however, progressing toward the aggregate interior, increasing proportions of goethite result—after 36 days of reaction, goethite becomes the dominant product. Additionally, and surprisingly, siderite also results within aggregate interiors and increases in concentration with increasing reaction time. Uranium, when distributed through the aggregate initially, accumulates near the perimeter of the aggregate and is deposited as a uraninite phase. Our results demonstrate the large variation in biotransformation of metals within structured media and the controlling influence on mass transfer limitations on reaction pathways.

Transcriptomic and Proteomic Analysis of *Desulfovibrio vulgaris* Sulfate-Reducing Biofilms

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Desulfovibrio vulgaris ATCC 29579 is a sulfate-reducing bacterium (SRB) commonly studied in the context of metal corrosion and heavy-metal bioremediation, and SRB populations are commonly observed in pipe and subsurface environments as surface-associated populations. To compare the physiological states of *D. vulgaris* biofilm and planktonic cells, transcriptomic and proteomic analyses were done on biofilms formed upon glass slides within a CDC reactor under sulfate-reducing conditions.

Transcript and protein expression profiles were unique, but sets of genes had similar trends in both transcript and protein levels, namely genes associated with energy conservation. An annotated *ech* hydrogenase, formate dehydrogenase, and pyruvate ferredoxin oxidoreductase had increased levels of transcript and protein in the biofilm samples. Several other hydrogenases and formate dehydrogenases also showed an increased protein level for biofilm cells and proteins for each step in the reduction of sulfate to sulfide. Decreased transcript and protein levels were observed within the biofilm for putative *coo* hydrogenases, as well as a lactate permease and *hyp* hydrogenases. Genes annotated for ATP synthase were down expressed at both the protein and transcript level. Genes annotated for amino acid synthesis and nitrogen utilization were also among the most dominant changers within the biofilm state, namely an increase in putative proteins for the synthesis of arginine, cysteine, methionine, and histidine. Alternatively, enzymes annotated for the synthesis of aromatic amino acids showed decreased protein levels in the biofilm. Both transcript and protein levels for ribosomal proteins were notably decreased within the biofilm cells. Stress-response proteins, including heat shock proteins, universal stress proteins, and superoxide dismutase and catalase, all had increased transcript and proteins levels. Various methyl-accepting chemotaxis proteins were increased on both the transcript and protein level, as well as genes annotated for iron sequestration such as ferritin, bacterioferrtin, and *feo*A.

Various proteins were also increased within biofilm cells for flagella synthesis such as FlaB3, FlaD, and FlgE. The results indicated that biofilm cells may have an alternate flux of carbon and energy that may influence metal-reducing and metal-interacting capacity. In addition, genes associated with amino acid production suggested a survival response of surface-adhered cells. Also, "stress" response genes, such as, *hsp*, *usp*, and *feo*A, could play important roles in slower growing cells. The results will provide insight for better understanding and control of the growth of surface-adhered, sulfate-reducing populations.

Microbial Community Structure and Dynamics During Accelerated Uranium Bioremediation

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Enhanced reductive precipitation of U(VI) through stimulation of indigenous microorganisms is an attractive, low-cost strategy for *in situ* remediation of contaminated groundwaters and sediments. The amount of organic carbon (OC) supplied determines not only the amount of electron donor available for bioreduction of U(VI), but also affects the resulting concentration of aqueous (bi)carbonate generated by microbial respiration. Increased (bi)carbonate concentration promotes U(VI) desorption and alters thermodynamics permitting U(IV) oxidation under highly reducing conditions.

With this knowledge we designed a long-term column study to investigate the impact of electron donor concentration and form on the accumulation of bi(carbonate) and the resulting stability of bioreduced U. We investigated the interaction among OC form, OC concentration, and time on the development of microbial populations, in an effort to identify factors leading to sustained radionuclide immobilization.

OC was supplied as acetate or lactate at four different concentrations (100 mM, 30 mM, 10 mM, 3 mM OC equivalents), and columns were sampled at three time points. In the columns receiving high OC supply, the first time point (5.9 pore volumes, 120 days) corresponds to a phase of net U-reduction, and the latter two time points (12.1 pore volumes, 350 days and 17.1 pore volumes, 580 days respectively) correspond to stable periods of U(IV) reoxidation and U(VI) remobilization. DNA was extracted from column sediments, 16S rRNA genes were amplified, and the microbial communities were analyzed using a high-density phylogenetic microarray (PhyloChip).

OC concentration, not OC form, had the largest impact on microbial community structure, despite the fact that communities were supplied with fermentable and nonfermentable substrates. Time was the second most important variable for community structuring, with community rank abundance curves converging to a similar pattern in terms of richness and evenness regardless of OC concentrations, OC supply form, or differences in the identities of community members. While known U-reducing bacteria were present in all columns at all time points, the dynamics of these organisms varied with OC concentration and form, suggesting niche differentiation within this functional group. However, U-reducing bacteria continued to increase in relative abundance from 350 to 580 days, despite continued and stable U reoxidation demonstrating the difficulty of simply relating phylogeny, function, and system phenotype.

These data show that the initial rate of electron donor supply during accelerated bioremediation in subsurface sediments strongly impacts microbial community development. Uranium remobilization, however, appears to occur irrespective of electron donor form, and despite the presence of multiple species of U-reducing bacteria. Our new project will use column studies, with sediments from three IFC sites, to assess the relative importance of sediment geochemistry and community seed on the functional capacity and microbial community composition resulting from conditions intended to augment uranium immobilization.

Biogeochemical Mechanisms Controlling Reduced Radionuclide Particle Properties and Stability

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Uranium and technetium are the major risk-driving contaminants at Hanford and other DOE sites. Laboratory studies have demonstrated that microbial reduction results in the formation of poorly soluble nanoparticles of hydrous oxides, $UO_{2(s)}$ and $TcO_{2(s)}$, which are believed to limit mobility of these radionuclides in the environment. The mechanisms of microbial reduction of U and Tc have been the focus of considerable research in the ERSP. Recent investigations at PNNL have focused on resolving biogeochemical processes controlling reactivity and persistence of ^{99}Tc in subsurface environments. The goal of this research project is to elucidate the principal biological and geochemical mechanisms that govern the chemical form and physical distribution of these redox reactive contaminants.

In contrast to U(VI), the microbial reduction of $Tc(VII)O_4^-$ is generally considered to be catalyzed by hydrogenase. Due to our recent identification of metal-reducing *Shewanella* in Hanford Reach Columbia River sediments where ^{99}Tc is predicted to migrate into in the future, we investigated the role of putative *Shewanella oneidensis* MR-1 hydrogenases in $Tc(VII)O_4^-$ reduction. Experiments confirmed that the NiFe hydrogenase was required for the H_2-driven reduction of Tc(VII), presumably through a direct coupling of H_2 oxidation and Tc(VII) reduction. A combination of genetics and *in vitro* assays with purified cytochromes was used to deduce that the cytochromes localized to the outer membrane also catalyzed the reduction of $Tc(VII)O_4^-$ to nanoparticulate $TcO_2 \cdot nH_2O$. Non-H_2-driven Tc(VII) reduction was slower than H_2-driven reduction, suggesting that electron donor type may influence rates of biogenic Tc(VII) reduction and the location of TcO_2 nanoparticles within the cell envelope.

Previous research demonstrated that, in addition to the direct enzymatic mechanisms described above, TcO_4^- is also subject to reduction by biogenic Fe(II), the rate and extent of which are controlled by the form and distribution of Fe(II) within sediments. To investigate competitive direct and indirect biological Tc(VII) reduction processes and subsequent impacts on physical distribution and chemical speciation of Tc(IV), experiments were conducted with ferrihydrite (FH), TcO_4^- and several species of metal-reducing bacteria. Bioreduction resulted in a mix of crystalline Fe phases including goethite (α-FeOOH) and magnetite (Fe_3O_4), depending upon organism type. Tc(VII) was effectively reduced to Tc(IV), but the physical distribution and chemical form were variable and dependent on organism type. Tc(IV) was present mainly as a Tc-Fe phase associated with a fine-grained Fe phase, believed to be magnetite or untransformed FH. For some organisms, there was a significant accumulation of nanoparticulate $TcO_2 \bullet nH_2O$ within the cell envelope that was likely the result of direct enzymatic reduction. The Fe biomineral-associated Tc phase consisted of monomers and dimers of Tc(IV) coordinated to Fe-O as inferred from Tc EXAFS analysis. The final concentration of aqueous Tc was related to the distribution of end-products with the Tc-Fe phase supporting lower solubility ($\sim 2 \times 10^{-9}$ M) than the biogenic TcO_2 (1.0×10^{-7} M). These results indicate that in subsurface environments with active microbial metal reduction and microbial reducible Fe(III), heterogeneous electron-transfer reactions can lead to variable Tc(IV) products that differ in their physical distribution and solubility.

Developing Highly Sensitive Micro-Biosensors for *in-situ* Monitoring Mercury and Chromium (VI) Contaminants by Genetically-evolving and Computer-designing Metal-binding Proteins

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The Department of Energy's environmental cleanup operations are facing enormous challenges in remediating metal and radionuclide contaminants. Adding to this challenge is the lack of overall knowledge with respect to distribution, movement, and transformation of those subsurface contaminants, which makes it difficult to implement good *in situ* remediation strategies. One of the keys tasks for gaining significant understanding of the mobility and fate of contaminants in the subsurface requires the development of novel techniques for detecting and long-term monitoring of the contaminants, both before and after remediation efforts. The development of biosenors in recent years has offered new, promising techniques for heavy metal detection.

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 biosensors that can be used for accurate detection and monitoring of heavy metal contaminants at DOE sites. The principal objectives of our research are: (1) using directed protein evolution technologies to engineer novel metal-binding protein with high specificity and binding-affinity to mercury(II) or chromium(VI) ions; (2) building low-cost microscale biosensors that can be deployed to the subsurface for *in situ* detection and monitoring of mercury(II) and chromium(VI) contaminants, based on the engineered proteins; (3) screening for better hosting microbial strains that could lead to longer biosensor lifetime; and (4) further expanding protein genetic-engineering technology to design biosensors for detecting other specific heavy metal and radionuclides contaminants. The resulting novel metal-binding proteins engineered in our plan will provide the basis for developing a new generation of low-cost, simple-to-use, long-term, *in situ* metal/radionuclide detectors.

Under the goal described above, various whole-cell biosensors composed of metal binding proteins (metalloregulator MerR and metal transporter MerT) and different fluorescent proteins (emGFP, eYFP, eBFP and mCherry) were recently constructed with *Escherichia coli* as host strain. These basic whole-cell biosensors have a wide range of mercury (II) detection (0.5 nM–10,000 nM). By introducing the bio-amplification genetic circuit based on the gene cascade expression system of P_{RM}-*cI* from bacteriophage λ, and *Pm-XylS2* from *Pseudomonas putida*, we have increased the detection sensitivity of the whole-cell biosenors by ~2–10 fold in our tested conditions. In addition, the fabrication of cheap, portable fluorescence-based detectors is under way.

Radionuclide Sensors for Subsurface Water Monitoring

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The objective of this project is to continue development and investigation of novel sensor concepts, materials, and analytical approaches for sensitive and selective determination of α- and β-emitting radionuclide contaminants in water. Contamination of the subsurface by radionuclides is a persistent and vexing problem for the Department of Energy. Detection of α- and β-emitting radionuclides in water is difficult, due to their short ranges in condensed media. Prior to this team's research under the EMSP program, no radionuclide sensors for non-gamma-ray emitting radionuclides existed for groundwater monitoring. We have shown that the combination of separation chemistry and radiometric detection in a compact flow-through unit enables sensitive detection of α- and β-emitting radionuclides in water. Our team is particularly interested in sensing approaches that minimize the use of reagents for sensor regeneration or renewal; and in investigating the effects of matrix components and co-contaminants on sensor performance. Our ongoing work has identified a number of scientific challenges in the areas of sample matrix, selective preconcentration chemistries, sensor materials, and radiometric detection principles that must be addressed to develop effective *in situ* sensor probes. Our research plans include:

- Develop preconcentrating minicolumn radionuclide sensors as equilibration-based sensors for *in situ* monitoring for a range of key contaminants, including ^{99}Tc , ^{90}Sr, and ^{129}I , and to develop new sensor concepts for tritium. Investigate separation chemistries for these radionuclides that will enable reproducible and reversible analyte binding in chemically untreated groundwater.

- Develop a fundamental understanding of equilibration-based sensing theory and perform detailed experimental characterization of matrix and co-contaminant effects on sensor performance. These studies will provide information for evaluating tradeoffs in sensor design that improve selectivity and lifetime in the presence of matrix interferences and co-contaminants.

- Develop radiometric flow-cell designs, digital signal processing and pulse shape discrimination methods for more selective radiometric detection of radionuclides of interest. These developments will enhance the selectivity of the radiometric detector for the analyte of interest.

- Perform rigorous analytical characterization and calibration of sensor prototypes to validate design rules and tradeoffs, and characterize quantitative performance.

- Educate students in modern radiochemistry and DOE waste cleanup challenges.

Spectroelectrochemical Sensor for Pertechnetate Applicable to Hanford and Other DOE Sites

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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}Tc that is applicable to characterizing and monitoring the vadose zone and associated groundwater. The single focus is pertechnetate, TcO_4^-, which is considered to be the dominant species in the vadose zone and ground water. 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 will build 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:

- Fluorescence offers a means of dramatically increasing the sensitivity of the spectroelectrochemical sensor, and we have demonstrated a limit of detection ($<10^{-12}$ M) that is 100 times lower than that needed for pertechnetate.

- TcO_4^- preconcentrates in sensor films containing anion exchange polymers and can be electrochemically reduced. This is the first step in operation of a spectroelectrochemical sensor for TcO_4^-.

- Prepared lower oxidation-state Tc-complexes fluoresce at ambient temperatures in sensor films, and these complexes exhibit reversible redox processes, leading to fluorescence modulation, which is the second step in operation of a spectroelectrochemical sensor.

- The spectroelectrochemical sensor and associated instrumentation for either absorbance or fluorescence modes are portable and easily transported to and used at DOE sites.

Our continued work incorporates three specific tasks: *Task 1, Refinement of the chemically-selective reagent-containing sensor film for TcO_4^- sensing; Task 2, Development of the TcO_4^- reductive trapping mechanism; Task 3, Development and evaluation of prototype sensors*. Tasks 1 and 2 will pave the way to development of prototype sensors and associated instrumentation. These will be used to evaluate and improve critical performance characteristics, such as limit of detection, range, response time, and reversibility. Optimized prototypes will be used to demonstrate sensor performance on TcO_4^- standards and samples from the vadose zone, and subsurface water at the Hanford Site.

Exploring Feedbacks Between Flow Characteristics and Biogeochemical Transformations During Biostimulation

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Ongoing work within the U(VI)-contaminated alluvial aquifer at the Rifle, CO, Integrated Field Challenge Site focuses on investigating the efficacy of biostimulation for facilitating microbial reduction of U(VI) to U(IV). At the site, a series of biostimulation experiments have been conducted within different flow cells by controlled co-injection of acetate and bromide. We hypothesized that the delivery of amendments could be strongly influenced by the heterogeneity of the subsurface, and that the resulting biochemical transformations could in turn produce precipitates and biomass that could alter subsequent flow and bioreduction. Here, we explore the feedbacks between flow characteristics and biogeochemical transformations at the Rifle site using characterization and reactive transport modeling, based on datasets acquired during two biostimulation experiments performed in 2002 and 2003.

Characterization of hydrological processes was performed using two different approaches. We first analyzed the bromide breakthrough data, using an approach we developed to capture the complex injection functions associated with the two biostimulation experiments. The results indicate that the velocity fields are spatially variable, and that the fields transition over time towards lower velocities close to the injection wells and higher velocities further away from the injection wells. We then performed inverse modeling to estimate the 2-D permeability distribution for both the 2002 and 2003 experiments. We developed a depth-averaged transport model using a geostatistical approach and hydraulic data available from boreholes. Using the TOUGH2 family of codes, we simulated the injection of bromide and water according to the measured time-varying rates. Permeability values were then estimated using an optimization algorithm that seeks to minimize the difference between the simulated and measured bromide breakthrough data at the monitoring well locations. In agreement with the bromide breakthrough analysis, the inversion study indicated that permeability reduction occurred near the injection wells in response to the acetate injection.

To understand how and to what extent biogeochemical reactions could potentially change the hydrological properties of the aquifer, 2-D reactive transport modeling was performed using CRUNCHFLOW to simulate the coupled biogeochemical, microbiological, and hydrological processes. The reaction network was chosen based on column experiments performed using the Rifle sediments, and the model was parameterized using heterogeneity estimates obtained from the hydrological inversion. Modeling results indicated that the biogeochemical reactions indeed led to the accumulation of biomass and precipitates, primarily calcite and iron sulfide. The amounts of biomass and precipitation reached a maximum (~1% of pore space) close to the injection wells, where the concentrations of injected acetate are largest. The simulations also suggested that negative correlation between sediment geochemistry and physical heterogeneity could lead to localized increases in transformational end products.

This characterization and modeling study suggests that bioremediation treatments can lead to the development of transformational end products, which can vary spatially and impact the local-scale flow. This hypothesis is supported by time-lapse surface complex resistivity data collected during another Rifle bioremediation experiment, which indicate a temporal variation in the preferential delivery of amendments. Although laboratory studies have documented pore-clogging processes, to our knowledge this is the first systematic documentation at the field scale of such processes.

A Thermodynamically Based Model for Predicting Microbial Growth and Community Composition Coupled to System Geochemistry

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In this project, we are developing an approach for modeling subsurface environments that couples thermodynamic descriptions for microbial growth with associated geochemical reactions. Although originally developed for use in better understanding bioimmobilization of metals and radionuclides via reductive precipitation, the modeling approach is potentially useful for exploring alternative bioimmobilization strategies and for exploring interactions between microbial communities and system geochemistry in in a variety of basic and applied biotechnology research settings.

We define a synthetic microbial community as a collection of microbial groups; each with a growth equation derived from bioenergetic principles. The number of groups is arbitary and can include any energy-yielding growth process. The growth equations and standard-state free energy yields for each proposed microbial group are appended to a thermodynamic database for geochemical reactions, and the combined equations are solved simultaneously to predict the effect of added substrates on microbial biomass, community composition, and system geochemistry. The approach was used to predict the results of laboratory and field bioimmobilization experiments for radionuclides and metal contaminants at the S-3 ponds at Oak Ridge National Laboratory (U, Tc), the Old Rifle site (U,V), and at the Hanford 100 H site (Cr). Predicted effects of ethanol, acetate, and lactate addition on contaminant solubility and oxidation state, major ion geochemistry, mineralogy, microbial biomass, and community composition were in general agreement with experimental observations, although the available experimental data precluded rigorous model testing. The proposed modeling approach represents a tractable way to model complex microbial communities (simulations were performed using 35 microbial groups that included the main microbial metabolisms identified in these enviornments). In particular, all simulations were completed with a single set of only four microbial growth parameters and suggest that thermodynamic descriptions of microbial growth provide realistic descriptions of observations made during ERSP field experiments. Model simulations also provide insight into the long-standing difficulty in transferring experimental results from the laboratory to the field and from one field site.

Chromium Isotopes as Indicators of Hexavalent Chromium Reduction

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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, because it is both the dominant mechanism of natural attenuation and the major means of *in situ* remediation for Cr(VI). Our laboratory experiments suggest that Cr isotope measurements are highly effective as a reduction indicator, but we are just beginning to apply these measurements extensively in field studies. Reduction of Cr(VI) causes isotopic fractionation. The Cr(III) reaction product is enriched in lighter Cr isotopes, while the remaining Cr(VI) becomes progressively enriched in heavier isotopes as reduction proceeds. It appears that Cr(VI) reduction can be quantified by measuring changes in the $^{53}Cr/^{52}Cr$ ratio, but this approach is new and still under development.

The proposed work will begin to apply this new technology at two DOE sites. The project objectives focus on three major questions: (1) at Idaho National Laboratory (INL), is Cr(VI) in groundwater being reduced and immobilized naturally, and can this reduction be quantified through the use of Cr stable isotope measurements? (2) How well can Cr stable isotopes be used as a key diagnostic indicator of the dynamics of in situ redox manipulation (ISRM) barriers like those deployed at Hanford? (3) Can calibration factors for the Cr isotope approach be determined for the conditions at each specific site, and do these factors fall within a reasonably narrow range, such as that defined by our previous laboratory work?

This project has been active since June 2007. Much of the effort to date has focused on training a new graduate student and demonstrating our ability to obtain high-precision $^{53}Cr/^{52}Cr$ measurements on groundwater samples with concentrations as low as 1 microgram Cr(VI) per liter. Measured $^{53}Cr/^{52}Cr$ ratios are expressed as parts per thousand, or per mil, deviations from the standard ratio of 0.11339 for NIST SRM-979 (e.g., $\delta^{53}Cr = 1.00$‰ means the measured ratio is 1.00 per mil greater than the standard). Precision, demonstrated by results of duplicated sample analyses from INL and other sites, is about ±0.15 per mil.

We have determined $\delta^{53}Cr$ of Cr(VI) in four groundwater samples from INL. One well from a contaminated zone in a perched water body yielded $\delta^{53}Cr = 0.76$‰. Previous work suggests that contaminant Cr has $\delta^{53}Cr$ close to +0.3, so the sample shows little elevation in $\delta^{53}Cr$ above its starting value and thus provides no evidence for substantial Cr(VI) reduction. Two other samples yielded $\delta^{53}Cr$ values of 1.00‰ and 1.40‰. These values are slightly elevated relative to those assumed for contaminant Cr, and suggest small amounts of reduction have occurred. A fourth sample, assumed to be uncontaminated groundwater, yielded $\delta^{53}Cr = 1.24$‰. This presumably represents natural Cr released from aquifer rock by weathering.

Future work will include analysis of arrays of groundwater samples from INL and Hanford. A laboratory component of the project will examine the various chemical and microbial reaction pathways that lead to Cr(VI) reduction, and determine the amount of $\delta^{53}Cr$ occurring for a given amount of Cr(VI) reduction (i.e., isotopic fractionation factors). These will then be used as calibration factors for interpretation of the field data.

Biogeochemical Processes Responsible for the Enhanced Transport of Plutonium under Transient Unsaturated Conditions

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Well defined solid sources of $Pu^{III}Cl_3$, $Pu^{IV}(NO_3)_4$, $Pu^{IV}(C_2O_4)_2$, and $Pu^{VI}O_2(NO_3)_2$ were placed in lysimeters containing vadose zone sediments and left exposed to natural environmental conditions (including the growth of annual weed grasses) for 2 or 11 years. We recently showed that Pu mobility was limited, <1-cm/yr, in these lysimeters, because Pu existed almost exclusively in the oxidation state of +4, the strongest binding form of Pu. However, Pu mobility was much greater than expected: reactive transport modeling clearly demonstrated that some resolubilization of Pu(IV) occurred under oxidizing conditions. The mechanism for this resolubilization is not known and is the subject of this project.

Studies were initiated to measure the release rate of Pu and the changes in the Pu oxidation states from the Pu sources in the lysimeters with the intent to develop a reactive transport model source-term. The Pu(III) and Pu(IV) sources had identical Pu-concentration depth profiles and similar Pu release rates. Source release data indicate that $Pu^{IV}(C_2O_4)_2$ was the least mobile, whereas $Pu^{VI}O_2(NO_3)_2$ was the most mobile. SXRF revealed that Pu was very unevenly distributed on the sediment, and that Mn concentrations were too low (630 mg kg^{-1}) and perhaps of the wrong mineralogy to influence Pu distribution. Pu-XANES analysis conducted on 27 sediment samples from the $Pu^{IV}(NO_3)_4$ and $Pu^{III}Cl_3$ amended lysimeters contained essentially identical Pu distributions: approximately 37% Pu(III), 67% Pu(IV), 0% Pu(V), and 0% Pu(VI). Although Pu has been known for decades to exist in the +3 oxidation state under microbially induced reducing conditions, to our knowledge, this is the first observation of steady-state Pu(III) in natural sediments. Based on thermodynamic considerations, Pu(III) has a wide potential distribution, especially in acidic environments, and as such may warrant further investigation.

A previously developed reactive transport model with surface-mediated redox reactions successfully simulated the lysimeter results below the source, assuming a steady, net downward flow. To simulate more realistic field conditions, a transient variably saturated flow model (using 11 years of daily rainfall data) with root water uptake was developed and coupled to the reactive transport model. Overall, the hydraulic and geochemical transient analysis showed results nearly identical to the much simpler steady flow analysis. Modeling also supported the importance of surface-mediated redox processes, with the most important variable affecting transport being Pu oxidation state. However, no model studied was able to produce the *upward* Pu transport observed in the data (Pu moved upward 20.6 cm to the surface sediments). The hydraulic and chemical mechanisms tested as potential causes for this upward migration (evaporation, hysteresis, and air-content-dependent oxidation) yielded little or no enhancement of the upward Pu movement. Plant uptake was the only mechanism to yield appreciable Pu upward movement. Our analysis indicated that roots would absorb water in their immediate vicinity, with the bulk of the upward water movement occurring in the root and stem xylem. It was predicted that Pu would accumulate in the plant leaves and ultimately be deposited on the ground surface when seasonal plant death occurred. This process was verified using TIMS analyses, which showed that the Pu isotopic ratios on the surface soils were those of the buried weapons-grade source material and not from atmospheric deposition.

Coupled Biogeochemical Processes in the Soil and Soil-Plant Systems Responsible for Enhanced Transport of Plutonium in the Vadose Zone

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Physical/chemical phenomena that occur in nature are almost always coupled to each other. It is part of what makes natural flow and transport processes complex and nonlinear. For the past several years, and now in the present research project (DE-FG02-07ER64401), Clemson University and the Savannah River National Laboratory (SRNL) have been trying to understand the coupled chemical and physical processes that produced the results of long-term plutonium (Pu) lysimeter experiments conducted in the vadose zone at the SRNL. Each lysimeter of interest contained a filter paper spiked with a different reduced Pu solution, and they were left exposed to natural environmental conditions (including the growth of annual weed grasses) for 11 years. The resulting Pu activity measurements on lysimeter cores showed anomalous activity distributions below the source, with migration of Pu above the source, all the way to the soil surface.

Our present understanding of the results is based on the following scenario. A very small amount of what started as a reduced Pu source (highly adsorbed on mineral surfaces) was oxidized (relatively small adsorption). Both of these species interacted chemically while moving downward with the net infiltration. The small amount of Pu that was in the oxidized state moved out ahead of the reduced Pu, producing the enhanced downward transport observed in the experiments. It was proposed initially that the unexpected upward Pu transport was caused by upward water flow in the soil during periods of high transpiration. Presumably, flow at times would be upward because most of the grass roots were above the source. However, a quantitative analysis of this hypothesis indicated that very little water actually moved upward through the soil resulting from a rapid lowering of soil hydraulic conductivity with even small decreases in water content. Our analysis indicated that roots would absorb water in their immediate vicinity, with the bulk of the upward water movement occurring in the root and stem xylem. Even though one would expect the Pu to be strongly adsorbed to xylem cellulose, the relatively rapid xylem flow velocities during hot wet periods (estimates of up to 600 cm/hr) would be more than adequate to transport Pu ions or complexes over long distances. In fact, it was predicted that Pu would accumulate in the plant leaves and ultimately be deposited on the ground surface when seasonal plant death occurred. Recently, it has been verified by SRNL isotope ratio analysis that a Pu activity residue on the lysimeter soil surfaces contains the weapons-grade Pu used to formulate the source solutions.

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

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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 Field Research Center (FRC) in Oak Ridge, TN, 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.

The utility of transcripts for dissimilatory (bi)sulfite reductase (*dsrA*) genes and for *Geobacteraceae* citrate synthase (*gltA*) genes as molecular proxies for sulfate- and Fe(III)-reducing prokaryotes, respectively, was demonstrated in FRC subsurface sediments. Transcript abundance was correlated with the rate of the corresponding electron transport pathway in sediment microcosms. Phylogenetic analysis of *gltA* mRNA clone sequences demonstrated that groups closely related to *Geobacter uraniumreducens* and *Geobacter* FRC-32 are active and abundant in microcosm sediments. In addition, phylogenetic analysis of sulfate-reducer-specific 16S rRNA clone sequences, retrieved from RNA isolated from *in situ* core samples, indicated that populations closely related to the *Desulfobacteraceae* family are active and abundant in the FRC subsurface.

A systematic approach was applied to cultivate novel nitrate-respiring prokaryotes from FRC sediments using a modified synthetic groundwater medium. Nitrate-reducers were enriched in culture treatments in which the nitrate concentration, electron donor type, and electron donor concentration were varied. At least 16 pure cultures have been obtained. The microbial community composition of the enrichments and the identity of the isolates are currently being examined through screening of the 16S rRNA gene sequences. In parallel, next-generation high-throughput cultivation methods have been developed and tested on contaminated FRC groundwaters. Two bacterial strains (tentatively identified as *Ralstonia* and *Paenibacillus*) were isolated from a low pH high nitrate well that had resisted previous attempts at cultivation of bacteria.

The form and oxidation state of Fe(III) minerals in FRC sediments is being characterized using a suite of physical and wet chemical methods. Deficiencies were observed with the extraction and speciation of Fe minerals using the conventional ferrozine method. Improved wet chemical extraction and speciation techniques were developed to more accurately monitor Fe mineral transformation in the subsurface.

Upscaling Pore-Scale Lattice-Boltzmann Modeling of Multicomponent Reactive Transport to the Continuum Scale

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The objective of this work is to investigate the role of pore-scale heterogeneity on macro-scale continuum representations of reactive flows in porous media. To this end, multicomponent reactive transport processes are simulated at the pore scale based on the lattice Boltzmann (LB) model we recently pioneered. The LB model for fluid flow and solute transport are equivalent to solving the incompressible Navier-Stokes and advection-diffusion equations at the pore scale, respectively. Reactions included in the simulations involve both homogeneous aqueous complexing reactions and heterogeneous reactions with minerals. Heterogeneous reactions include mineral precipitation and dissolution, ion exchange, and surface complexation. These latter reactions are incorporated into the LB method through boundary conditions imposed at the mineral surface. The model can provide detailed information on local fields, such as fluid velocities, solute concentrations, and mineral compositions and amounts, as well as the evolution of pore geometry resulting from chemical reactions. The pore-scale results are then volume-averaged over a representative elemental volume (REV) and compared to single and multiscale continuum model simulations. Simulations are carried out for various 2-D and 3-D synthetic, structured, and realistic media describing transport of a tracer and reaction kinetics, to analyze multiscale effects.

Generally, it is found that sufficiently fast reaction rates lead to concentration gradients at the pore scale and thus are sensitive to the local velocity field. We also find that, in general, a multiscale continuum formulation is required to fit volume-averaged pore-scale results. 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, provides parameters needed for constitutive relations appearing in the multiscale continuum formulation.

In certain cases, multiscale processes may be fit using a single continuum model employing effective parameters that are not directly measurable. To illustrate this, we develop a multiscale continuum formulation for reactive transport in a hierarchical porous medium, consisting of a primary continuum coupled to multiple secondary continua, either in series or parallel. Multiscale phenomena are captured by taking into account isolated pores described by the secondary continua, which are coupled to the primary continuum fluid by diffusion. An analytical solution is derived for the stationary state of a single component system with linear reaction kinetics. This solution is shown mathematically to be equivalent to a single-continuum formulation, with an effective surface area that is a function of the various parameters defining the reactive system. It is suggested that a multiscale continuum approach may help explain the observed discrepancy between laboratory and field-derived reaction rates, by explicitly representing distinct transport domains through separate interacting continua, which could be responsible for the formation of preferential pathways.

High-Resolution Simulation of the Hanford 300 Area

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Numerical modeling is a critical tool to the U.S. Department of Energy for evaluating the environmental impact of remediation strategies for subsurface legacy waste sites. Unfortunately, the physical and chemical complexity of many sites overwhelms the capabilities of even most state of the art groundwater models. Of particular concern are the representation of highly heterogeneous stratified rock/soil layers in the subsurface, and the biological and geochemical interactions of chemical species within multiple fluid phases. There is clearly a need for higher-resolution modeling (i.e., increased spatial and temporal resolution) and increasingly mechanistic descriptions of subsurface physicochemical processes (i.e., increased chemical degrees of freedom). Effectively harnessed, high-performance computation can answer this need, enabling higher fidelity simulations, increased chemical complexity, and incorporation of multiscale effects through multiple interacting continua.

As part of DOE's SciDAC-2 program, high-performance computation is being applied to better characterize and predict the movement of radionuclide contaminants at the Hanford 300 Area, using the massively parallel code PFLOTRAN, a multiscale, multiphase, and multicomponent reactive flow and transport code founded upon PETSc data structures and solvers. Challenges in modeling the Hanford 300 Area include accounting for fluctuations in the Columbia River stage, which occur on an hourly basis with several-meter variations, implementing realistic inland boundary conditions on the computational domain, and implementing a realistic subgrid scale model for the U(VI) source term. We present preliminary results for transport of a hypothetical tracer plume within the 300 Area, illustrating the impact of river forcing on the geohydrology of the site. Future research will include U(VI) geochemistry simulated within multiple interacting continua at the site.

Proteomic Characterization of the Changes in Community Structure and Dynamics before and after Amendment at DOE Relevant Sites

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Microbial remediation of metals is based on the ability of microbes to transform metals from one oxidation state to another. While this function is conferred to the organism by genes encoded in its genome, each gene must be expressed into a functioning product (i.e., a protein) for the microbe to perform its function in nature. Such is also the case on a broader scale in microbial communities, where microbes interact, compete, and act upon the changing environment. Thus, integral to the improvement of microbially mediated contaminant transformation at field sites is the in-depth characterization of the components of the communities—the activity and function of each of the components and how they interact with each other. However, the complexity of the community and in many cases the absence of a sequenced metagenome present challenges to this type of characterization.

In the presence of a metagenomic sequence, the correct identification of peptides from such a complex collection of proteins can hinder the complete characterization of the microbial community. We have developed a model to estimate the probability that a peptide was correctly identified by our process. Higher-level inference related to the presence of different proteins and communities will be achieved through the use of the above-calculated probabilities, and the theoretical relationship between communities, proteins, and peptides, by modeling the ambiguity introduced by each of these levels in the identification process of our pipeline.

In the absence of metagenomic sequence, closely related organisms can be used as a basis for characterization. We have demonstrated the utility of this approach using the groundwater communities from the uranium contaminated flood plain in collaboration with the ERSP Rifle IFC project. The 16S rDNA methodology revealed several *Geobacter* species from which a database of observed peptides was created, using genome sequences for specifically identified *Geobacter* species and genome sequences from near neighbor species. This database was used to study the proteome response of the *Geobacter* species to an acetate injection into the groundwater at the uranium-contaminated Rifle site. Preliminary findings suggest that the manner in which the genome sequences were used (a concatenated *Geobacter* sequence, a condensed *Geobacter* sequence, etc.) to generate the database of observed peptides played an important role in evaluating this response. In spite of this finding, the specific proteome of one *Geobacter* species, possibly *G. bemedjiensis* or a closely related species, appeared to dominate this response. These initial studies demonstrated that proteomic characterization of microbial communities is feasible, but requires a significant integrated effort, involving experts in the areas of microbiology, proteomics technology development, and bioinformatics.

Microscopic Mass Transfer Controls Local Geochemistry and Uranium(VI) Reactivity

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Uranium in the subsurface sediments can concentrate in microenvironments where mass fluxes are dominated by diffusive mass transfer. Characterization has revealed that sorbed U(VI) typically exists in complex, microscopic, intragrain domains in Hanford sediments. Grain-scale, microscopic mass transfer is expected to control the rate and extent of U(VI) release from the intragrain to advective domains. The objective of this research is to investigate the mechanisms and physical properties of the microscopic mass transfer process and its role in controlling local geochemistry and U(VI) reactivity in the contaminated sediments.

Molecular dynamics (MD) simulations have been performed to calculate ion diffusion coefficients in porous media consisting of pores at nano- to microscales. In contrast to the traditional assumption that diffusion coefficient was a constant at the pore scale in porous media, the MD calculations showed that diffusion coefficients parabolically increased with increasing distance from mineral surface, because of electrostatic attractions between diffusing ions and atoms in the mineral structure. Such surface effects disappeared when the pore size increased to 20–100 nm, depending on solution composition. A percolation-based model has been developed to evaluate how the spatial heterogeneity of intragrain pore diffusion coefficients affects the apparent diffusive mass-transfer rates that can be measured macroscopically. The calculation has revealed that for a nonreactive tracer, the apparent diffusion coefficient is the arithmetic average of the intragrain diffusion coefficient distribution.

Column leaching experiments and numerical simulations using multicomponent, dual-domain reactive transport models have been performed to evaluate the influence of microscopic mass transfer on the evolution of intragrain geochemistry and U(VI) release kinetics. Comparison of the experimental and modeling results indicated that diffusive mass transfer could significantly constrain the supply of reactants and removal of products, leading to the significant difference of pH, carbonate, calcium, and other major ion concentrations between the intragrain and advective domains. The geochemistry of major ions in the intragrain domains, in turns, affected U(VI) aqueous speciation reactions, which subsequently affected the U(VI) solubility, dissolution kinetics, and apparent rates of U(VI) release from the sediment to aqueous phase. Accurate modeling of U(VI) release from the sediment required understanding not only U(VI) geochemistry and mass transfer, but also the effect of mass transfer on the geochemistry of major ions in microenvironments.

Phosphate treatment of the U(VI)-contaminated sediments significantly suppressed the rate of intragrain U(VI) release, apparently resulting from the "sink" effect of the secondary U(VI)-phosphate precipitates and/or calcium-phosphate precipitates that decreased the U(VI) mass-transfer rates from the sediment to the advective domain. The rate of U(VI) release, however, gradually returned to pre-phosphate treatment conditions as fresh groundwater dissolved and removed the secondary phosphate minerals. Increasing the phosphate concentration in the sediment treatment retarded the U(VI) rebounding.

U(VI) Reduction by Versaphilic *Anaeromyxobacter Dehalogenans* Strains

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Anaeromyxobacter spp. are versaphilic delta-Proteobacteria commonly found in soils and sediments. *Anaeromyxobacter* isolates share the ability to grow via fumarate, nitrate, nitrous oxide, and ferric iron reduction, and many isolates respire chlorinated phenols. *Anaeromyxobacter* spp. consume oxygen and all isolates tested to date reduce soluble U(VI) to immobile U(IV). Detailed experiments with *Anaeromyxobacter dehalogenans* strain 2CP-C demonstrated growth with U(VI) as electron acceptor, but the growth yields were lower than predicted based on the energetics of the U(VI)/U(IV) redox couple. The reasons for the lower-than-expected growth yields are unclear, but may be explained by the biomolecular mechanisms involved U(VI) reduction, which are under investigation.

Several *Anaeromyxobacter* strains were isolated from the ERSD Integrated Field-Scale Subsurface Research Challenge (IFC) site (i.e., the FRC site) in Oak Ridge, TN. The isolates shared highly similar 16S rRNA genes and were all capable of U(VI) reduction; however, distinct U(VI) and nitrate reduction rates distinguished strains from each other. Strain-specific, quantitative, 16S rRNA gene-targeted real-time polymerase chain reaction (PCR) demonstrated that multiple *Anaeromyxobacter* strains coexist at the Oak Ridge IFC, and, importantly, that the *Anaeromyxobacter* population responded to biostimulation with ethanol. Contour plots established that enhanced U(VI) bioreduction was associated with an increased abundance of *Anaeromyxobacter* spp., suggesting that native *Anaeromyxobacter* spp. contribute to U(VI) reduction. Further, the *Anaeromyxobacter* population increased in size following the intrusion of oxygenated groundwater, suggesting that these bacteria adapt quickly to changing redox conditions, respire oxygen *in situ*, and thus protect UO_2 from reoxidation.

The genome of strain 2CP-C has been sequenced, and whole genome microarray experiments explore the *Anaeromyxobacter* transcriptome of cells grown in chemostats with different electron acceptors. The goal of these gene expression arrays is to identify process-specific biomarkers (e.g., for U(VI) reduction) for enhanced monitoring of the active *Anaeromyxobacter* population.

Molecular Analysis of the *In Situ* Metabolic State of Subsurface *Geobacter* Species

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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. To date, studies have focused on the study site in Rifle, CO, in which *Geobacter* species appear to be the primary agents for reductive precipitation of uranium during *in situ* bioremediation of the uranium-contaminated groundwater. Previous studies have demonstrated that quantifying transcripts for several key genes could aid in estimating the rates of *Geobacter* metabolism during uranium bioremediation and, surprisingly, identified Fe(II) as a key limiting nutrient.

New studies in the last year focused on quantifying transcripts associated with: electron donor limitation; phosphate and nitrogen availability; and microbial growth rates in the subsurface. For example, in the 2007 Rifle field experiment, acetate concentrations in the groundwater were artificially manipulated and transcript levels for two classes of acetate-transporter genes, *aplI* and *aplII*, were quantified over time. The expression of these two genes clearly changed in response to changes in acetate concentrations in the groundwater, suggesting that they may be useful indicators of when acetate availability is limiting rates of uranium bioremediation. Studies conducted with *Geobacter uraniireducens*, an isolate from the Rifle site, indicated that, of a variety of genes, transcript abundance of *rpsC* correlated best ($r^2 = 0.94$) with growth rates. Therefore, expression patterns of this gene were used to estimate growth-rate values of *Geobacter* species during the 2005 and 2007 *in situ* uranium bioremediation studies at the Rifle site. The number of *rpsC* transcripts were directly affected by acetate concentrations in the groundwater, and based on transcript abundance, it was estimated that *in situ Geobacter* growth rates ranged from a generation time of 95 hours prior to acetate injections, to 20 hours, when acetate concentrations were at their peak. There was substantial heterogeneity within the 2007 field plot in ammonium availability (0-400 µM). To diagnose how this impacted on the metabolic state of *Geobacter*, we quantified transcript abundance for an ammonium transporter gene, *amtB*, and the nitrogen fixation gene, *nifD*. Transcripts for *amtB* increased linearly with decreasing ammonium concentrations, a trend that was not shared by *nifD* expression, which remained relatively constant until ammonium concentrations decreased below detection limits, at which point *nifD* transcription increased, documenting the onset of nitrogen fixation. Quantifying expression of *pstB* and *phoU*, the two *Geobacter* genes expected to be most highly upregulated under phosphate limitation, suggested that although these genes were being expressed in response to high phosphate demand during accelerated growth of *Geobacter* species following acetate additions, phosphate was probably not a limiting nutrient.

These results demonstrate that monitoring transcript levels for key metabolic genes is providing an unprecedented insight into the metabolic status of the subsurface microbial community catalyzing important microbe-metal interactions.

Outer-Surface Components Involved in Electron Transfer to Fe(III) Oxides in Geobacter sulfurreducens

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The interactions of dissimilatory metal-reducing microorganisms with Fe(III) oxide minerals are expected to play an important role in controlling the fate and transport of metal and organic contaminants in the subsurface, under both natural attenuation and engineered bioremediation strategies. However, little is known about the mechanisms by which *Geobacter* species, important agents of subsurface Fe(III) reduction, transfer electrons to Fe(III) oxides. For example, it was previously proposed that electrically conductive pili, known as microbial nanowires, could be the final conduit for electron transfer between the outer-surface of *G. sulfurreducens* and Fe(III) oxides, but it is difficult to directly track electron transfer throughout the cell and onto Fe(III) oxides, necessitating the development of alternative biophysical techniques to evaluate extracelular electron transfer.

In one approach to further investigate the nanowire hypothesis, a gold electrode was masked with a non-conductive polymer that contained an array of 100 nm diameter pores extending from the outer surface to the surface of the electrode. This arrangement prevented direct contact between the cell surface and the electrode, but pili should be able to extend from the cells, through the pores, and contact the electrode surface. When inoculated with *G. sulfurreducens* at 25°C, this masked system produced current well above background levels and current production was temperature-dependent, consistent with a biological process. These studies are the first direct demonstration of long-range electron transfer in a microorganism that does not produce soluble electron shuttling compounds.

In another approach, a biofilm of *G. sulfurreducens* was grown over a 50 µm nonconducting gap separating two gold electrodes. This permitted electrochemical interrogation of the biofilm. Although biofilms have previously been reported to be nonconductive, the *G. sulfurreducens* biofilm was conductive, which is also consistent with a long-range conductivity mechanism in this organism. The two-electrode technique is expected to have broad application in the study of the electron transfer properties of proteins displayed on the outer cell surface. Analysis of a number of *G. sulfurreducens* strains revealed that thin filaments, similar in size and shape to the previously described conductive pili, could be produced under different environmental conditions, but that these filaments were not comprised of the pilA subunit protein. Preparations of these filaments were enriched in the outer surface *c*-type cytochrome OmcS, suggesting a close cytochrome-filament association. A novel purification scheme yielded OmcS in a redox-active state. The purified OmcS was able to donate electrons not only to soluble electron acceptors, such as Fe(III) citrate and anthraquinone-2,6-disulfonate, but also to insoluble Fe(III) and Mn(IV) oxides. These results suggest an alternative mechanism for extracellular electron transfer that does not require pili. Additional evidence for an alternative route for extracellular electron transfer was the finding that a strain of *G. sulfurreducens* that was adaptively evolved for enhanced Fe(III) oxide reduction continued to reduce Fe(III) oxide if the gene for PilA was deleted. This was associated with continued display of the outer-surface *c*-type cytochromes OmcS and OmcE. These results further demonstrate the importance of multiple redox-active components in contributing to Fe(III) oxide reduction in the subsurface.

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

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This research addresses one of the ultimate goals of the DOE Environmental Remediation Sciences Program, which is to develop tools that will make it possible to predict the fate of subsurface contaminants under either natural attenuation or engineered bioremediation conditions. Our goal is to, for the first time, 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 which it is known that *Geobacter* species can account for over 90% of the microbial community.

In this first year of studies, a framework integrating the genome-scale metabolic model and transport process was developed and initial numerical simulations of microbial U(VI) reduction in subsurface sediments were carried out. The model incorporated the flow of groundwater under anaerobic conditions and the dynamics of growth and respiration of *Geobacter sulfurreducens* with acetate as the electron donor and Fe(III) and U(VI) as the electron acceptors. In contrast to existing models of bioremediation, this approach accounted for changes in microbial metabolism that arise in response to local geochemical conditions. The effect of potential physiological variables, such as differences in growth yields and geochemical conditions, were evaluated with the model.

Hybrid *in silico* models that integrate uptake kinetics with genome-scale models of metabolism were developed and used to analyze the influence of heterogeneities in the availability of ammonium and acetate on the community dynamics of *Geobacter* and *Rhodoferax* species at the ERSP Rifle, CO study site. The models help explain why *Rhodoferax* species can be important components of the microbial community at portions of the Rifle site, prior to acetate injection, when ammonium is available. However, *Geobacter* species predominate at all locations once the subsurface is amended with high concentrations of acetate. To obtain realistic uptake parameters for acetate by *Geobacter* species living in the subsurface, uptake was examined in pure cultures over a range of acetate concentrations. These studies revealed that there are multiple acetate uptake systems, one with a half-saturation constant of only 5 micromolar, but others with much higher half-saturation constants. This array of high affinity-low velocity uptake systems, as well as low affinity-high velocity uptake systems, helps explain how *Geobacter* species can survive in the subsurface prior to the addition of acetate, and then can rapidly respond to acetate additions during *in situ* uranium bioremediation. Analysis of uptake parameters for other nutrients likely to influence growth of *Geobacter* species in the subsurface is ongoing.

This research represents the critical first steps in producing coupled microbial-reactive transport models for predicting fate and transport of contaminants that will eventually aid in the stewardship of subsurface environments contaminated not only with uranium and other radionuclides, but also other classes of contaminants, such as chlorinated solvents.

Experimental and Modeling Strategies for Laboratory-Scale, Field-Relevant Sedimentary Samples

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The objective of this research is to improve the conceptual and quantitative understanding of processes influencing the transport of radionuclides at the DOE's Hanford Reservation. The major issues at Hanford include scale-dependence of transport parameters due to alternating layers of contrasting hydraulic conductivity, and rate-limited mass transfer. Because these processes are a function of sedimentary layering and pore structure, we have used "intact" or undisturbed sediment samples to quantify the fate and transport of various contaminants. Samples were collected in vertical and horizontal orientations in two sizes (0.025 m × 0.05 m and 0.2 m × 0.25 m) to determine, respectively, hydraulic parameters and contaminant transport parameters. Hydraulic conductivity as a function of water content is being determined at the University of Tennessee using a centrifuge technique. In the transport experiments on the larger samples, a pattern of increased dispersivity was observed when flow was parallel versus across beds, and under unsaturated conditions versus saturated conditions. At very low water contents, the transport of U(VI) and CoEDTA exhibited decreased reactivity when flow was parallel to beds due to extensive flow bypassing. This was confirmed by dye trace experiments on the same samples. At intermediate water contents, however, contaminant reactivity did not exhibit a distinct pattern, most likely because variations in mineralogy encountered by the flowpaths may result in either increased or decreased reactivity.

In these previous experiments, flow was either parallel to or across beds in separate samples: in essence, they were a 1-D system. New experiments will utilize intermediate-scale intact samples (at least 0.5 m × 1.0 m × 0.3 m) in which both horizontal and vertical (i.e., 2-D) flow can be observed within the same sample. Techniques for intermediate-scale sample collection previously used in Hanford's 200 Area will be presented to prepare for the next generation of samples to be collected from the 300 Area. Examples of techniques for quantifying advection, diffusion, and mass-transfer processes in the new intermediate-scale samples will be presented in order to facilitate discussions to find the most appropriate methods for use in the new 300 Area samples.

The Phytoremediation of Ionic and Methylmercury

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We have made significant progress toward our goal of engineering highly productive plant species with magazines of genes that will enable them to extract, tolerate, and hyperaccumulate mercury aboveground for subsequent incineration and storage of mercury-laden ash. Mercury pollution (e.g., ionic (Hg(II)), methylmercury, MeHg, CH_3Hg^+) is a worldwide problem, seriously affecting the health of human and wildlife populations. MeHg is inherently more toxic than metallic Hg(0) or ionic Hg(II) mercury. Because MeHg is efficiently biomagnified up the food chain, it poses the most immediate threat. Our current research focuses on identifying and testing transgenes controlling the tolerance, chemical speciation, electrochemical state, transport, aboveground binding, and vacuolar storage of mercury. As part of earlier DOE ERSP funded research, we successfully engineered several plant species to use the bacterial *merB* gene, *methylmercury lyase,* to convert MeHg to less toxic Hg(II), and to use a highly modified bacterial *mercuric ion reductase* gene, *merA,* to further detoxify Hg(II) to the least toxic metallic form Hg(0). All these plants germinate, grow, and set seed at normal growth rates on levels of MeHg or Hg(II) that are lethal to wild-type plants. Hence, these first efforts to identify genes that increase tolerance were quite successful.

The focus of our ongoing research is to increase the rates of mercury uptake, transport aboveground, and hyperaccumulation in leaf vacuoles. We have cloned and tested more than 20 genes from bacterial, fungal, and plant sources in transgenic plants and several produced useful and exciting phenotypes. Two of the several *Arabidopisis high affinity zinc transporter (ZIP)* genes tested increased mercury uptake in yeast, but only one, *ZIP7,* appears to enhance root mercury uptake in transgenic plants. Serendipitously, we have discovered an important epigenetic mechanism by which the transcription of the *ZIP* genes is controlled at the level of chromatin remodeling. Plants co-expressing *gamma-glutamylcysteine synthetase (ECS)* and *glutathione synthetase (GS)* have increased levels of mercury accumulation aboveground as a result of higher concentrations of glutathione and other thiol-peptides. *Arabidopsis* has a family of 14 glutathione conjugate pumps (GCPs) with the potential to enhance vacuolar storage of $(GS)_2$-Hg complexes. We have found that four of these are elevated in expression upon exposure to mercury, and we have cloned them to use in our phytoremediation strategy. In parallel, we have shown that the yeast GCP, *YCF1,* enhanced plant tolerance to mercury, but like the endogenous Arabidopsis genes, *YCF1* was poorly expressed in transgenic plants. Highly mutagenized forms of *YCF1* are now being examined to increase the specific activity of mercury vacuolar transport. During the last several months that remain in this project, we will test magazines of five to six well-characterized transgenes in *Arabidopsis*. Ultimately, these magazines of genes will be combined on a minichromosome transferred to field-adapted plants like cotton wood and sterile switch grass for the phytoremediation of mercury.

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

Exploratory Science Element

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From 1957 to 1992, the US DOE conducted 828 underground nuclear tests at the Nevada Test Site (NTS) (DOE, 2000). The Underground Test Area (UGTA) Environmental Management Project utilizes wells associated with the resulting blast cavities ("hot wells") to monitor radionuclide migration. UGTA also affords a unique opportunity to investigate the microbial colonization of radioactive subsurface habitats. As the temperature and pressure history of underground nuclear tests differs from that of radionuclide processing and disposal sites, these habitats may contain novel micoorganisms of utility for bioremediation. The initial objectives of this project are logistical (technical and regulatory infrastructure). Our first scientific objective is to verify the *presence or absence* of microorganisms in water-containing blast cavities.

The 2008 NTS hotwell sampling campaign has begun and will extend through summer 2008. Our team collected its first samples on 02/06/2008. These were pumped from a 9 7/8-in post shot re-entry hole, U4u PS#2a, 1,620 ft below ground surface (ft bgs) in the vicinity of the Dalhart blast cavity. Dalhart (<150 kilotons; DOE, 2000) was detonated in 1988 at 2,100 ft bgs. The re-entry hole was drilled in 1990 and completed with a 2 7/8-in tube with a slotted interval from 1548–1644 ft vertical depth. The hole has been sampled six times, most recently on 02/06/2008. The radiological impact of Dalhart in well samples is evidenced by tritium activities as high as 60×10^6 pCi/L. The well was purged in 2003 (7,058 gallons) and again in February, 2008 (5681 gallons) prior to sampling. Samples for DNA analysis were obtained by filtration, and unfiltered samples for cultivation were stored at 4^oC. The next steps, including DNA extraction, polymerase chain reaction (PCR) amplification of the 16S rRNA gene, and clone library construction will be performed in the Radiochemistry Laboratory at University at Nevada at Las Vegas. Concurrently, relevant microbial physiotypes will be enumerated by dilution cultivation. Radiological and water chemistry analysis by LLNL will be used to inform media design. In addition to the hotwell samples, LLNL has stored samples from 2006/2007 of what appears to be microbially reduced tunnel water. Representative microorganisms from both habitats will be screened for the ability to interact with radionuclides and examined for radiation resistance.

Reference: DOE (2000), United States Nuclear Tests: July 1945 through September 1992, DOE/NV-209 (Rev. 15), Department of Energy, Las Vegas, NV

Nucleation and Precipitation Processes in the Vadose Zone during Contaminant Transport: Formation of Uranium(VI)-Silicates

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We are investigating the nucleation of U(VI)-silicate phases in aqueous solutions under conditions characteristic of contaminated subsurface sites at Hanford and Oak Ridge. Although U(VI)-silicate minerals are known geologically, virtually no data exist on how these and similar phases form, especially as nanoparticles or colloids, near the earth's surface. The goal is to delineate their formation kinetics and thermodynamic stabilities for use in modeling U(VI) mobility in the environment using reactive-transport approaches.

We have explored the effect of pH (from 2 to 9) in solutions with a fixed initial ratio of U:Si = 1:2 for 1 day and 6 weeks of aging at room temperature and 4 days of aging at 150°C on the formation of U(VI)-silicate coordination complexes, using High-Energy X-ray Scattering (HEXS), Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR), powder X-ray diffraction (XRD), and solution thermodynamic modeling[1,2]. Most precipitates showed no evidence of Bragg diffraction in the XRD data. Room-temperature samples above pH 3.1 all had similar Fourier transforms of their HEXS data, but hydrothermally treated precipitates varied in HEXS correlations at longer distances. Only the hydrothermal sample at pH 5.1 had Bragg reflections, indexable as soddyite ($(UO_2)_2SiO_4 \cdot 2H_2O$). The hydrothermal sample at pH 6 showed similar HEXS correlations, which evolved in samples at increasing pH into correlations more consistent with Na-boltwoodite ($Na(UO_2)(SiO_3OH) \cdot 1.5H_2O$). Based on the results, we proposed a model for solid formation that includes a uranyl silicate building block, or synthon, that preorganizes in solution and is the backbone structural unit needed to nucleate larger particles. The synthon includes three uranyl ions in a silicate-bridged oligomer with correlation lengths extending out to about 0.5 nm. Solids formed from room-temperature solutions of pH 4 to 9, aged for 6 weeks, had ATR-FTIR spectra that revealed changes in the uranyl and silicate vibrations as a function of pH, that were consistent with a shift in mid-range structural linkages from those similar to soddyite to those more like Na-boltwoodite. Six weeks of aging at ambient temperature is enough time for the synthons to rearrange and adopt mid-range structural linkages characteristic of crystalline phases predicted by thermodynamic modeling.

Work in progress includes new experiments extending to pH 11, U:Si ratios of 2:1, 1:1, 1:2, and 1:5, and other aging times, as well as comparison of EXAFS spectroscopic analyses with HEXS and ATR-FTIR results. Column flow experiments under simulated rainfall conditions are also under way to evaluate uptake of U(VI) in synthon form on quartz and feldspar surfaces.

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[2]Gorman-Lewis D., Skanthakumar S., Jensen M. P., Nagy K. L., and Soderholm L., FTIR characterization of amorphous uranyl silicates, in review.

The *Desulfovibrio* "Pangenome" as a System for Studying the Mechanisms of Mercury Biotransformation

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Anaerobic sulfate-reducing bacteria (SRB) are a diverse group of microorganisms that play an important role in the global sulfur and carbon cycles. They are also involved in a wide range of metal ion biotransformations, with implications in the cycling of industrial heavy metal pollutants. Among those, some SRB can transform inorganic mercury into the more toxic methylmercury, which accumulates in the food chain and has become a global environmental problem. The molecular mechanism of methylmercury biosynthesis is unknown. To study this process, we are combining comparative genomics with molecular physiology and are using the *Desulfovibrio* genus as a model system. Some members of this genus are able to methylate mercury, whereas others are not. We have sequenced the genomes of two methylating species, *D. africanus* and *D. desulfuricans* ND132, to near completion (~95% based on occurrence of bacterial core genes) using the 454 shotgun approach. The metabolic potentials inferred from the sequences of these two organisms were compared to those of several related *Desulfovibrio* species that were shown to lack the capacity of methylmercury synthesis and for which completed genomes have been publicly released (*D. desulfuricans* G20, *D. vulgaris* DP4 and *D. vulgaris* Hildenborough). The two strains that can methylate mercury have slightly larger genomes (~3.9–4.2 Mbp versus ~3.7–3.8 Mbp). Among the ~1,900 types of conserved protein domains (based on Pfam classification) present in the *Desulfovibrio* "pangenome," we have identified several dozen that are specific to the two strains that methylate mercury.

We are complementing the computational analysis of these specific genes with gene expression microarrays, mutagenesis procedures, and functional complementation to elucidate the mechanism of mercury methylation in *Desulfovibrio*. Preliminary growth studies toward a genetic system have thus far shown antibiotic resistance to only kanamycin and ampicillin of seven antibiotics tested. A complex medium with yeast extract, 0.5% NaCl, lactate as the carbon and electron donor, and sulfate as the electron acceptor yields 1×10^9 *D. africanus* cells/mL in 48 hours. We are currently determining transformation parameters. Proof-of-principle testing has targeted three candidate loci in *D. africanus* ATCC19997 for potential roles in Hg methylation of which orthologs were either not found in nonmethylating *Desulfovibrio* species or did not share synteny with nonmethlyators. These studies will provide insight into the molecular mechanisms for methylmercury production by *Desulfovibrio* species, and a comprehensive comparative genomic analysis of *Desulfovibrio* will also provide insight into the evolution of metabolic strategies and environmental adaptation in this genus of SRB.

Functional Community Characteristics and Mercury Contamination in Streams

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Several streams located on the Department of Energy's Oak Ridge Reservation in Oak Ridge, TN, have been exposed to large discharges of mercury. The effects of this contamination have been evident in studies of mercury resistance genes. We used a functional gene array (FGA) to examine the response of streambed microbial communities to various levels of mercury exposure. The FGA included 23,864 probes for more than 14,000 microbial genes involved in the cycling processes for nitrogen (e.g., denitrification and nitrogen fixation), carbon (e.g., cellulose degradation), and sulfur (e.g., dissimilatory sulfur reduction), as well as methane oxidation and reduction, organic contaminant degradation, and metal reduction and resistance. We hypothesized that there is a greater diversity of genes related to pollutants at the contaminated sites.

Results from one site in East Fork Poplar Creek that was contaminated with mercury (33.3 µg/g) and numerous other contaminants, and one control site in Hinds Creek with much lower mercury concentrations (65 ng/g) indicated elevated gene frequencies in all general categories at the contaminated site. Genes involved in metabolism (sulfate reduction, denitrification, carbon utilization) were about 2-fold higher. Genes involved in metal resistance and contaminant degradation were approximately 3-fold higher. The greatest difference between the two sites was in the frequency of RuBisCO genes, which was much higher at the contaminated site.

A subsequent study examined six sites (including the two previous sites) where total mercury concentrations in the sediment ranged from 71.4 to 39,100 ng/g. There was a poor correlation between mercury concentrations in the stream sediments and in the water ($R^2 = 0.51$). This poor correlation is consistent with the complex relationship between mercury concentrations in the stream sediment and water that is likely influenced by geochemical factors and mercury speciation. Soluble reactive phosphorous (SRP) in the stream water ranged from about 3 to 460 µg/L. SRP concentrations were highest in East Fork Poplar Creek, downstream from the Oak Ridge sewage treatment plant, and were lowest near the west end boundary of the Y-12 site in Bear Creek. For the samples analyzed to date, the FGA appears to be able to detect differences in the diversity of genes for specific functions. Also, genes related to contamination are elevated to a greater degree at the contaminated site than are general metabolic indicators.

Centrifugal Determination of Unsaturated Hydraulic Properties for Individual Sedimentary Layers from the Hanford Subsurface

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The overall goal of this research is to provide new insights into the measurement and prediction of hydraulic properties for partially saturated layered sediments at the Department of Energy's (DOE) Hanford site. The primary objectives are to:

- Introduce the transient flow centrifuge method developed by the oil and gas industry to the environmental/DOE community.
- Quantify the spatial variation and anisotropy of the capillary pressure-saturation, $\theta(\psi)$, and unsaturated hydraulic conductivity, $K(\theta)$), functions measured on samples from individual sedimentary layers.
- Test the unique, but often assumed, relationship between $\theta(\psi)$ and $K(\theta)$.
- Provide forward predictions of upscaled effective hydraulic properties for the multi-layered sedimentary sequence based on the composite medium approximation (COMA).

Transient flow centrifuge experiments are being carried out on 88 undisturbed sediment samples that were collected inside the Environmental Restoration Disposal Facility (ERDF) at ~21 m below surface level in August, 2007. The sediment face (1.2 m high by 9 m wide) exposed four lithological units. Core samples (4.8×10^{-5} m^3) were taken from 13 horizontal levels cut into this face. On each level, three vertical and three horizontal core samples were taken in order to investigate the degree of anisotropy. The samples were obtained by carving out horizontal and vertical columns with dimensions slightly larger than the core diameter, and then pushing the cores over the exposed columns with a minimum of force.

Basic laboratory characterization includes grain-size distribution, particle density, bulk density, and saturated hydraulic conductivity. The centrifuge experiments are being performed with a Coretest URC 628 centrifuge (0.09 m arm length). By allowing the fluid saturation and capillary pressure profile to equilibrate at a number of different angular velocities (*multirate transient flow centrifuge experiment*) the $\theta(\psi)$ function is determined. The Brooks and Corey capillary pressure-saturation model is then fitted to these data. In a separate *single-rate transient flow centrifuge experiment*, the production is used to independently determine $K(\theta)$; the $K(\theta)$ model parameters are known and fixed, while the parameters specific to the $K(\theta)$ function are estimated inversely, based on a numerical simulation performed with the UC-analyze code.

The individual $\theta(\psi)$ and $K(\theta)$ functions, combined with a detailed mapping of the thickness of individual layers, will be used to predict large-scale anisotropic effective hydraulic properties for the multi-layer sedimentary sequence using COMA. A new DOE-ERSP proposal is being developed to test these predictions against effective hydraulic properties for large multilayered samples measured in the Idaho National laboratory geocentrifuge (2-m arm length).

Our quantitative results are directly applicable to the Hanford subsurface. Additionally, our conceptual approach should advance the scientific agenda of the Environmental Remediation Sciences Division (ERSD) by providing reasonable strategies for measuring and predicting water fluxes within heterogeneous vadose zones of contaminated sites throughout the western U.S.

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

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Uranium (U) contamination is the primary radioactive metal contaminating DOE subsurface environments at many sites and is of particular concern because of its carcinogenicity. One potential method of treating U contamination is by using sulfate reducing bacteria (SRB) to reduce soluble U(VI) to insoluble U(IV) (as uraninite, UO_2).

Our published HR-TEM, SAED, and EDS analysis data showed that SRB transformed U(VI) into colloidal uraninite with particle diameters of 3–5 nm. Further, we showed that under lactate-limited sulfate-reducing conditions, this biogenic uraninite can be oxidized by common Fe(III)-(hydr)oxides such as hematite, goethite, and ferrihydrite. In addition to the oxidation of biogenic uranium under biotic conditions, we also studied the oxidation of aqueous-phase biogenic uraninite by Fe(III)-(hydr)oxides under abiotic conditions. These aqueous uraninite particles were separated from SRB using 0.2 μm membrane filter, and treated with hematite and ferrihydrite under strict anaerobic conditions. Results show that Fe(III)-(hydr)oxides oxidized uraninite; however, the reactivity of biogenic uraninite was greater with ferrihydrite as compared to the more crystalline structure of hematite. Thermodynamic calculations also indicate congruent favorability of these reoxidation reactions. We are currently investigating mechanisms of reoxidation of reduced uranium.

We hypothesized that *the oxidation of uraninite nanocrystals by Fe(III)-(hydr)oxides under sulfate reducing conditions would occur through the direct contact of uraninite nanocrystals with (1) Fe(III) minerals; (2) Fe(III)-chelating compounds produced by SRB which have solubilized Fe(III) from Fe(III)-(hydr)oxide solids; or (3) electron shuttle compounds that accept electrons from uraninite nanocrystals and transfer them to Fe(III)-(hydr)oxides.* We determined that our lab strain *Desulfovibrio desulfuricans* G20 had a significant siderophore production capability; 80mg/L G20 cells produced siderophores with a minimum Fe(III) binding capacity equivalent to a 5 µM desferroxamine B solution. In collaboration with Dr. Abbie Richards and Dr. Matthew Fields (both at MSU), we are exploring the role of siderophore production by SRB in the uraninite-Fe(III) reactions. Dr. Fields has shown through gene arrays that *Desulfovibrio vulgaris* Hildenborough is also a siderophore producer. Dr. Abbie Richards is an expert in siderophore separation and characterization. She will be assisting us in separating and testing the effects of various SRB-generated siderophores, from both species, on uraninite-Fe(III)-(hydr)oxide redox reactions. These results may have strong implications for field application of *in situ* biological reduction of U(VI).

An Integrated Assessment of Geochemical and Community Structure Determinants of Metal-Reduction Rates in Subsurface Sediments

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The objective of our research is to examine the importance of microbial community structure in influencing uranium reduction rates in subsurface sediments. If the redox state alone is the key to metal reduction, then any organisms that can utilize the oxygen and nitrate in the subsurface can change the geochemical conditions so metal reduction becomes an energetically favored reaction. Thus, community structure would not be critical in determining rates or the extent of metal reduction unless community structure influenced the rate of change in redox. Alternatively, some microbes may directly catalyze metal reduction (e.g., specifically reduce U). In this case, the composition of the community may be more important and specific types of electron donors may promote the production of communities that are more adept at U reduction. Our results will help determine if the type of electron donor or the preexisting community is important in the bioremediation of metal-contaminated environments subjected to biostimulation.

In a series of experiments at the DOE Field Research Center (FRC) site at Oak Ridge, we have consistently shown that all substrates promoted nitrate reduction, while glucose, ethanol, and acetate always promoted U reduction. Methanol only occasionally promoted extensive U reduction, which is possibly due to community heterogeneity. There appear to be limitations imposed on the community related to some substrates (e.g., methanol and pyruvate). PLFA indicated different communities with methanol. TRFLP and clone libraries indicated distinct differences among communities, even in treatments that promoted U reduction. Thus, there is enough metabolic diversity to accommodate many different electron donors resulting in the U bioimmobilization.

Coupling Biogeochemical Process Evaluation for Conceptualizing Trichloroethylene Co-metabolism

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The focus of this project is to determine the diverse biogeochemical processes involved in the microbial transformation of trichlorethene (TCE) in the Test Area North (TAN) contaminated aquifer at the Idaho National Lab. Our primary goals are to define the microbial processes involved in TCE co-metabolism and to quantify the contribution of these processes to TCE remediation within the medial zone of the TAN plume. Ultimately, the studies are designed to provide multiple lines of evidence to determine whether co-metabolism via the methane monooxygenase pathway is contributing to natural attenuation of TCE within this zone of the plume. Flow-through *in situ* reactors (FTISRs) containing crushed basalt were incubated *in situ* for 238 days at TAN-35. After removal of the FTISRs, basalt and residual water collected from the reactors were immediately sampled and distributed for specific analyses. Aliquots of basalt were subjected to a comprehensive suite of analyses, including community proteomics, gene expression, metabolic activity, microbial community structure, and kinetics of TCE degradation. In addition to FTISR experiments, stable carbon isotope measurements and PhyloChip analyses were done on a set of groundwater samples collected from the source area of the TCE plume to the leading edge of the defined medial zone. The results to date provide a comprehensive and multi-tiered analysis of co-metabolic TCE remediation, and support the contribution of this process to the attenuation of TCE at this site. Incubation experiments performed with ^{13}C-TCE added basalt and groundwater from the FTISRs indicate that the microorganisms in the FTSIRs are capable of co-metabolically degrading approximately 7.5 µg of TCE per liter of groundwater. A novel time-course cell lysis method was developed for proteomics, allowing increased peptide coverage that resulted in a greater number of total proteins being detected. This demonstrated the presence and activity of aerobic methanotrophs in the medial zone. Confirmation of methanotrophic activity was achieved through combined use of targeted enzyme biochemical probes and fluorescent *in situ* hybridization (FISH), which consistently identified methanotrophs and active sMMO enzyme both in the planktonic phase (groundwater) and biofilm (basalt) communities derived from the reactors. Real-time polymerase chain reaction (PCR) technique identified ~3000 copies of mmoX per g of basalt; while RT-PCR confirmed that the mmoX subunit was being actively transcribed. Stable carbon isotope ratios of dissolved inorganic carbon (DIC) and dissolved methane indicated increased levels of methane oxidation, with distance from the source area (-55% to -28% for methane; >8% to -13% for DIC) corresponding to increased dissolved oxygen concentrations.

These data corresponded well with site geochemistry and microbial assessments for community composition (PhyloChip) and activity (FISH) that identified a gradient of methanogenic to methanotrophic populations within the contaminant plume. Currently, the FTISRs are been redesigned to allow *in situ* stable isotope tracer studies, intended to derive TCE degradation rates (and methane incorporation rates) as close to *in situ* conditions as possible. Re-installation of the FTISRs in the aquifer is scheduled for March 2008.

Hyphenated Techniques for Determining pH-Dependent Pore-Scale Uranium (VI) Speciation

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Accurate prediction of uranium (U) bioavailability and mobility, both of which are key components to understanding risk at a contaminated site, depends on the speciation of uranium. Unfortunately, U(VI) speciation can be highly variable in geochemically and hydrologically heterogeneous systems. We are investigating U(VI) sorption using flow-field flow fractionation (Fl-FFF), hyphenated with inductively coupled plasma mass spectrometry (ICP-MS) and ICP-optical emission spectrometry (ICP-OES), to increase our understanding of pore-scale U(VI) solution complexation reactions within the context of intermediate-scale aquifer heterogeneity.

FFF is a technique that separates colloids in aqueous suspension by their relative diffusivities, as represented by their hydrodynamic diameter. With online ICP-MS detection, uranium associated with different molecular-size fractions can then be determined in real time as the various size fractions of the sample elute. Uranium in a natural aquifer sample might be dissolved, bound to nanoparticulate material, and/or bound to macromolecules such as natural organic matter (NOM). These fractions elute at different rates, so a relationship between size and uranium content can be determined. The concept is tested by investigating uranium speciation in relatively well-characterized systems. An additional advantage of this technique is that the analysis requires only 10 to a few hundred microliters of sample.

Thus far, we have optimized the method and investigated pH-dependent interactions between U and synthesized hematite nanoparticles (~60 nm in diameter). These characterizations will be compared with results from equilibrium speciation models and distributions obtained from batch sorption experiments, using more traditional separation techniques (e.g., centrifugation, filtration). Future experiments will involve further method development and measurement of U complexation with fulvic and humic acid. Upon complete validation of the method, we will determine uranium binding with less well-characterized ligands such as natural organic matter and cell surfaces from 2-D flow experiments and field samples.

Fluid Flow, Solute Mixing, and Precipitation in Porous Media

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One approach to reducing the risks associated with toxic or radioactive metal contaminants in the subsurface is to immobilize the metals *in situ*. The mechanisms by which metals can be immobilized include both changes in speciation, where the metals are incorporated into, or sorbed onto, mineral phases; and isolation, where the metals are shielded from the mobile phase by encapsulation or flow diversion. In our project, we have been investigating how mineral-forming reactants can be transported and mixed in porous media to stimulate the nucleation, growth, and deposition of minerals. The research issues concern: (1) the nature of solute transport and mixing in porous media when two or more reactants are involved in the formation of mineral phases; (2) the mixing geometry, flow, matrix heterogeneity, and coupling between precipitation and permeability and how they affect the spatial distribution of mineral phases, and (3) the impact of precipitation on a mobile, sorbing contaminant metal. In this project, we are using calcium carbonates as the model mineral phases, and strontium as the model target contaminant.

Two mixing geometries have been constructed in a quasi two-dimensional flow cell. In one system, a solution containing dissolved calcium ($CaCl_2$) is injected parallel to a solution containing a carbonate salt ($NaHCO_3$). Mixing occurs at the solution/solution interface where calcium carbonate precipitates are formed, the permeability of the precipitation zone is reduced, and dispersive mixing between the solutions is reduced. The formation of a narrow, self-focusing precipitation zone, and its impact on solute concentration gradients, have been simulated with smoothed particle hydrodynamic modeling of the transport, mixing and precipitation processes at the pore scale, and also continuum (grid-based) simulations. The second mixing geometry involves sequential addition of the two solutions, such that the mixing occurs via longitudinal dispersion and migrates in the direction of flow.

Chemical gradients are formed as a consequence of mixing and precipitation. Therefore, saturation states and ratios between the ions that form precipitates also vary over space. The effect of saturation states and Ca^{2+}/CO_3^{2-} ion ratios on the rates of precipitation and the co-precipitation of Sr^{2+} were studied, using constant-composition batch experiments. Experimental results suggest that both Sr uptake and precipitation rates increase with increasing carbonate/ calcium ratios. X-ray absorption spectroscopy has been used to determine whether the mechanism of Sr uptake is by co-precipitation (vs. absorption, encapsulation, or formation of $SrCO_3$) and laser ablation-inductively coupled plasma mass spectrometry has been used to show the degree of Sr concentration at the surface of the seed material (Iceland spar calcite). An important issue for the immobilization of trace metals in newly precipitated minerals is the stability of the mineral surfaces in mixing zones where volume-averaged conditions may not be representative of local conditions as a consequence of mixing/precipitation dynamics. A system that appears to be supersaturated, based on volume-averaged (bulk) measurements of chemical activities, may in fact have localized regions of undersaturation. The potential for surfaces of precipitated phases to be dynamic has been suggested by model simulations, and such potential has implications for the long-term stability of immobilized contaminants.

Subsurface Bio-Immobilization of Plutonium:
Experiment and Model Validation Study

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A concurrent experimental and modeling study centers on the interactions of *Shewanella alga BrY* with plutonium, the key contaminant of concern at several DOE sites. The project goal is to understand the long-term stability of bio-precipitated "immobilized" plutonium phases under changing redox conditions in biologically active systems. Our hypothesis is that stable plutonium phases will prevail where bioreduction occurs.

Experimentally, we established key abiotic and biotic interactions in the *S. alga*-plutonium system. Of the higher-valent plutonium species, only $Pu(V)O_2^+$ persists due to reduction of $Pu(VI)O_2^{2+}$ by organic species typically present in biologically active systems. PuO_2^+ forms weak aqueous complexes that are bioavailable, shows high toxicity towards *S. alga*, and has relatively low adsorption onto biomass. Direct enzymatic reduction of PuO_2^+ by *S. alga* occurred under all anaerobic growth conditions investigated, and aqueous Fe^{2+} and Fe(II/0) phases caused its abiotic reduction in near-neutral systems. Reduction of PuO_2^+ by Fe^{2+} is a key reduction pathway for *S. alga* systems in which Fe^{3+} is an electron acceptor. The relative contributions of enzymatic and biogenic Fe^{2+} processes in the overall reduction of Pu(V) plutonium are the focus of ongoing experimental efforts. Under the conditions of our batch experiments, the result of bioreduction was the formation of insoluble Pu(III) precipitate. Future experimental plans are to establish the fate of Pu(V) when iron oxide solid (rather than aqueous Fe^{3+}) is the electron acceptor, better establish the mechanisms leading to Pu(III) versus Pu(IV) and the final bioreduction product, and establish the potential for biomobilization of the plutonium precipitates.

Our modeling activities have addressed several upgrades of the CCBATCH biogeochemical model: (1) including Pu and U speciation data, (2) allowing anaerobic growth utilizing Fe^{3+} as electron acceptor, (3) determining the bio-available form of Fe^{3+}, and (4) incorporating biosorption of Pu(V) onto *S. alga*. For (3), we upgraded CCBATCH to allow selected complexes of Fe^{3+} to be primary electron acceptors and carried out modeling experiments with different ratios of Fe^{3+} and NTA. Results indicate that $Fe(OH)_2NTA^{-2}$ is the bioavailable form. For (4), we observed that most of the $Pu(V)O_2^+$ is complexed to the phosphoryl group on *S. alga*, owing to its large affinity constant compared to other ligands present in the growth media. An important feature is that the functional groups grow at the same rate as *S. alga*. Integrating (1)–(4), we used CCBATCH to model the reduction of Pu(V) by *S. alga*, while accurately capturing the production of total Pu^{3+} and its speciation in the presence and absence of chelating agent. Future work will involve predicting which Pu precipitates will prevail and their stability within long-term, continuous transport systems.

The Structure of Environmental Water on Surfaces and Interfaces: The Role of Hydroxyls in the Wettability of Surfaces

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The goal of this project is to determine the molecular structure, growth, and reactive properties of water films on mineral surfaces, usually oxides, carbonates, and pyrites. Using a unique photoelectron spectrometer developed in 1999–2002 in my group at the Lawrence Berkeley National Laboratory[1], we study the initial stages of water adsorption on surfaces of metals and oxides. The novelty of this spectrometer is its ability to operate under ambient gas pressures and temperatures (5 Torr, 300 K). X-ray Photoelectron and Absorption Spectroscopy (XPS and XAS) of films in equilibrium with the vapor allow us to construct isotherms and isobars as a function of relative humidity. The structure and growth of water on TiO_2 [2], SiO_2[3], Cu_2O, Al_2O_3[4] and Cu[5] were studied and linked to the initial formation of hydroxyl groups that served as nucleation sites for the subsequent molecular adsorption. An important result from our experiments is the observation that molecular adsorption takes place only after the surface becomes saturated by OH species, which act as growth seeds. In the case of TiO_2, this is connected with the energy of the H-bond between OH and H_2O being higher than that between two water molecules[2]. Surface hydroxyls form by reaction of H_2O with O-vacancies, always present on the surface after preparation. On SiO_2 we found that the molecules in the first two layers have a dipolar orientation that is random or parallel to the surface. The film structure, however, becomes similar to that of macroscopic liquid water after its thickness reaches 4 to 5 molecular layers. On clean Cu, metal adsorption of water was observed only on the (110) oriented surface, where H_2O could adsorb dissociatively, producing OH groups. On the compact (111) orientated surface, no adsorption could be observed, and the surface remained dry in the presence of 1 Torr of water.

In the future, we plan to study the effect of pre-adsorbed films (organic and inorganic) on wetting properties of the substrate. Exchange reactions between ions in the sample substrate (minerals) and ions in a solution deposited on the surface will be studied, because of their relevance to the vadose layer exchange reactions involving noxious ionic species. We have also started a series of experiments to determine how surficial organic films containing alcohols, acid, and amine groups affect the adsorption of water.

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Mechanistically Based Field-Scale Models of Uranium Biogeochemistry from Upscaling Pore-Scale Experiments and Models

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Effective environmental management of DOE sites requires reliable prediction of reactive transport phenomena. A central issue in prediction of subsurface reactive transport is the impact of multiscale physical, chemical, and biological heterogeneity. Heterogeneity manifests itself through incomplete mixing of reactants at scales below those at which concentrations are explicitly defined (i.e., the numerical grid scale). This results in a mismatch between simulated reaction processes (formulated in terms of average concentrations) and actual processes (controlled by local concentrations). At the field scale, this results in apparent scale-dependence of model parameters and inability to utilize laboratory parameters in field models. Accordingly, field modeling efforts are often restricted to empirical estimation of model parameters by fitting to field observations, which renders extrapolation of model predictions beyond fitted conditions unreliable.

The objective of this project is to develop a theoretical and computational framework for (1) connecting models of coupled reactive transport from pore-scale processes to field-scale bioremediation, through a hierarchy of models that maintain crucial information from the smaller scales at the larger scales; and (2) quantifying the uncertainty that is introduced by both the upscaling process and the incompleteness of field-scale characterization.

We are performing detailed experimental characterization of water flow and biofilm development at the pore scale using magnetic resonance imaging (MRI). We then simulate selected reactive transport processes at the pore scale in the experimentally measured pore geometries. We are using two general approaches to simulate flow and transport at this scale: (1) mesh-free particle methods (smoothed particle hydrodynamics, or SPH) and (2) traditional grid-based methods (computational fluid dynamics or CFD). Simulations of flow, solute transport, and mixing, as well as reactions including mineral precipitation and biofilm growth, have been performed. Upscaling to derive Darcy-scale process descriptions and effective parameters is being executed using volume averaging techniques, with the closure problem being solved numerically in complex realistic pore geometries. Future work will incorporate the upscaled models (and estimates of associated uncertainties) into a geophysically conditioned stochastic model of facies distributions at the field scale, and applied to the Hanford Integrated Field Challenge site.

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

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Past activities conducted by the United States Department of Energy (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 because of 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. A promising complementary or alternative approach for the *in situ* remediation of DOE contaminants is to develop biomimetic redox catalysts that can be delivered to the specific subsurface locations where contaminants reside.

The objectives of this new exploratory research project are to evaluate (1) the effectiveness of metal reduction and immobilization (e.g., precipitation) using metalloporphyrin catalysts; (2) the roles of solubilizing, encapsulating, and complexing agents in enhancing, limiting, or controlling the reaction rates in the catalyzed reaction systems; and (3) the use of nanotechnology for environmental applications through implementation of nano- and molecular-scale materials incorporated within a support containing nanoscale porosity. Of particular interest to us in this project is elucidating the role that solution chemistry (e.g., pH, hardness, alkalinity, presence of organic and inorganic ligands, presence of other metal contaminants) will play in the metalloporphyrin-catalyzed reduction reactions of U(VI) and Cr(VI).

Activities initiated upon the beginning of this project have included:

- Development of analytical methodologies to quantify aqueous solution concentrations of Cr(VI) and U(VI), plus the concentrations of their respective reduced forms as needed.
- Chemical speciation modeling to elucidate important Cr and U species, specifically for:
    - Identifying important dissolved Cr(VI) and U(VI) species expected to be present at DOE sites.
    - Subsequent elucidation of relative reduction rates for different Cr(VI) and U(VI) species.
    - Predicting the likely species of Cr(III) and U(IV) formed upon reduction.
- Identifying appropriate bulk reductants for use in reducing Cr(VI) and U(VI), taking into account expected pH conditions and expected reaction rates.
- Identifying appropriate soluble and insoluble metalloporphyrins for use as catalysts.

Characterization of Random *Shewanella oneidensis* Mutants Deficient in Adhesion to Iron Oxide Surfaces

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Shewanella oneidensis MR-1 utilizes a variety of terminal electron acceptors under anaerobic conditions, including insoluble Fe(III)-oxides. Although relatively little is known about the mechanisms of bacterial iron reduction, it appears that the bacterium must be in close proximity to, or in contact with (adhered to), the iron oxide surface for efficient electron transfer to occur. Thus, the mechanisms of bacterial adhesion are of primary interest when describing bacterial iron oxide reduction processes. Previous studies have shown that the presence or absence of cell surface features such as outer membrane proteins (presented at the ERSP PI Meeting 2007), lipo-, and exopolysaccharides greatly influence bacterial adhesion. In *Shewanella*, several outer membrane proteins such as flagellin and GspD (part of the Type 2 secretion system) have been shown to be required for wild-type adhesion, while exopolysaccharide capsules have been shown to reduce adhesion to iron oxide surfaces, and probably also limit electron transfer because of physical interruption of the cell-iron oxide surface contact. The goal of this work is to identify novel components involved with *Shewanella* adhesion to iron oxide surfaces to elucidate the mechanism of adhesion.

Changes in the cell surface, whether natural or effected (i.e., mutagenesis), can be expected to alter bacterial adhesion. In this study, a rapid adhesion assay was designed with the aim of identifying randomly generated *S. oneidensis* MR-1 mutants with adhesion deficiencies towards iron oxides. Using the assay, twenty mutants were identified that were significantly adhesion deficient (≤ 85 %) compared to wild type *S. oneidensis* MR-1. Of these 20 mutants, 11 were growth-limited in the utilization of at least one soluble anaerobic electron acceptor, even when growth was at wild-type levels under the growth conditions of the adhesion assay (fumarate reduction). This suggests that adhesion is greatly dependent on the overall metabolic fitness of the cell. The most severely adhesion-deficient mutant was found to be nonmotile, where the loss of adhesion was likely due to the lack of a fully operational flagellum. Two severely adhesion-deficient strains that retained motility and did not express growth deficiencies on alternative electron acceptors were further characterized by epifluorescence microscopy, employing a fluorescently labeled lectin, concanavalin A, ATR-FTIR, and low-temperature scanning electron microscopy to identify changes in the cell surface relative to wild type cells. These adhesion-deficient strains were found to produce greater quantities of exopolysaccharide on their cell surface relative to wild type cells. Genotyping of the identified adhesion mutants is currently under way.

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

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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 of field-scale transport processes. During the second year of our three-year project, we have performed extensive characterization at the study site, developed a multiscale characterization framework, and performed numerical experiments.

To complement the borehole and crosshole data collected at the study site during the first year of our study, we have performed hydrofacies and geophysical characterization. Analysis of core and CPT data suggests that the study site can be described by two lithofacies (L); one having more mobile flow characteristics (L=0) and one having less mobile characteristics (L=1). A 3-D surface P-wave seismic reflection survey was completed at the SRS P-Area study site. The resulting seismic data have been processed as a 3-D data cube. Interpretation of the processed 3-D seismic cube is revealing significant details about the lithostratigraphy of the study site.

A multiscale characterization framework has been developed and tested to estimate the relative proportion of the lithofacies and the lithofacies type given the following data sets: (1) large-scale surface-based geophysical data; (2) small-scale crosshole geophysical data; and (3) small-scale wellbore data (such as from geophysical logs, flowmeter logs, and core samples). Using a Bayesian framework, we derive a conditional probability distribution of the unknown variables at each point in space conditioned to the available data. Synthetic case studies have been performed using domains that represent the P-Area. These studies suggest that the approach can be used to estimate the spatial distribution of hydrofacies using disparate and multiscale datasets.

Numerical dual-domain modeling experiments have been conducted using synthetic permeability fields to reveal nondimensional relationships between multidomain transport parameters (porosities, mass transfer coefficient, dispersivity) and practical measures of field-scale heterogeneity. An initial round of experiments involved 30 high-resolution realizations of 30 different normalized configurations. Optimal model parameters were determined by empirical fitting of breakthrough curves. Four transport formulations were considered: (1) the traditional advection-dispersion equation, (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 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. These studies suggest that optimal dual-domain values can be defined from field characterization data.

During our third year, we will expand characterization while applying the above methodologies to the composite set of field data derived from the SRS P-Area study site.

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

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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 *in situ* stabilization mechanism for divalent trace ions, such as the short-lived radionuclide strontium-90, is co-precipitation in calcite. Calcite, a common mineral in the arid western U.S., can incorporate trace metals by forming solid solutions. The rate of trace metal incorporation is susceptible to manipulation using either abiotic or biotic means. We previously demonstrated that increasing the calcite precipitation rate by stimulating the activity of urea hydrolyzing microorganisms can result in significantly enhanced Sr uptake. In limited field experiments, we showed that with nutrient amendments, we could stimulate ureolytic activity and calcite precipitation in groundwater. A particularly attractive characteristic of this approach is that it accelerates naturally occurring processes, rather than drastically altering *in situ* environmental conditions. This enhances the sustainability of the remediation results. The specific objectives of our research are to:

- Assess the field-scale relationships between introduced reagents, changes in urease activity, calcite precipitation rates, mineral distribution, and hydrogeological-geochemical heterogeneity, under conditions representative of DOE sites where ^{90}Sr contamination occurs.
- Assess the sustainability of the treatment.
- Evaluate the potential for geophysical methods to detect and monitor the spatiotemporal distribution of urea hydrolysis and calcite precipitation in the subsurface at field-scale.
- Develop modeling tools to enable optimal design of *in situ* remediation strategies based on engineered calcite precipitation, with particular attention to the effects of precipitation on flow re-routing.

We are conducting integrated field, laboratory, and computational research to evaluate (a) the relationships among urea hydrolysis rate, calcite precipitation rate, and trace metal partitioning under environmentally relevant conditions; and (b) the coupling between flow/flux manipulations and calcite precipitate distribution and metal uptake. The primary emphasis is on field-scale processes, with the laboratory and modeling activities designed specifically to support the field studies. Field experiments are planned in perched water (vadose zone) at the Vadose Zone Research Park (VZRP) at the Idaho National Laboratory. The VZRP is an uncontaminated field research site that mimics conditions at the nearby Idaho Nuclear Technology and Engineering Center, where significant ^{90}Sr contamination has been identified in the perched water and aquifer below the facility. We will use a flow cell consisting of five 18 m deep wells spaced 1 m apart and arranged in a five-spot pattern. Preliminary hydrological and geophysical investigations are being integrated with our biogeochemical methodology as we design our initial field experiments, which are planned for the spring and summer of 2008.

Promoting Uranium Immobilization by the Activities of Microbial Phosphates

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The overall objective of this project is to examine the activity of nonspecific phosphohydrolases present in naturally occurring subsurface microorganisms, for the purpose of promoting the immobilization of radionuclides through the production of uranium U(VI) phosphate precipitates. Specifically, we hypothesize that the precipitation of U(VI) phosphate minerals may be promoted through the microbial release and/or accumulation of PO_4^{3-} as a means to detoxify radionuclides and heavy metals. An experimental approach was designed to determine the extent of phosphatase activity in bacteria previously isolated from contaminated subsurface soils collected at the DOE Field Research Center in Oak Ridge, TN (FRC). Our recent publications (Martinez et al., 2007 *Environ. Microbiol.*, and Beazley et al., 2007 *Environ. Sci. Technol.)* have demonstrated that FRC *Bacillus* and *Rahnella* cultures, grown aerobically in minimal media containing 10 mM glycerol 3-phosphate (G3P) as the sole carbon and phosphorus source, and 15 mM NO_3^- as the sole N source, are capable of biomineralizing 73% and 95% of soluble U(VI), respectively, when challenged with 200 μM uranyl acetate in a range of pH between 4.5 and 7.0.

During this phase of the project, we initiated anaerobic biomineralization studies with FRC *Rahnella* sp. Y9602. Following 5 days of anaerobic growth at pH 5.5, more than 1 mM soluble PO_4^{3-} was liberated into the culture media, resulting in the biomineralization of 96% soluble U(VI). High concentrations of nitrite (2.5 mM) measured in these incubations suggested that *Rahnella* sp. respired on nitrate as the terminal electron acceptor. XANES measurements identified the oxidation state of the uranium in the mineral phase to be entirely U(VI), indicating the absence of uranium reduction. Further measurements by EXAFS and synchrotron source XRD identified the mineral formed both aerobically and anaerobically to be chernikovite [$H_2(UO_2)_2(PO_4)_2$].

In addition to the anaerobic studies, slurry incubations with contaminated FRC Area 3 soil were conducted in simulated groundwater containing 10 mM G3P at pH 5.5. After 10 days, the slurries accumulated up to 4 mM soluble PO_4^{3-}, indicating the presence of phosphatase-active bacteria. Total DNA was extracted from the slurries and 16S diversity determined by high-density oligonucleotide microarray (PhyloChip).

Our research suggests that the remediation of U(VI) via precipitation of U(VI) phosphate minerals may be enhanced in both aerobic and anaerobic conditions by the phosphatase activity of facultative subsurface microorganisms, such as *Rahnella* sp., particularly in areas where high levels of nitrate and low pH may inhibit uranium reduction.

Mercury in Aquatic Ecosystems on the Oak Ridge Reservation— Paradoxes Provide a Key to a More Complete Understanding of the Global Mercury Cycle

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The accumulation of methylmercury (MeHg) in aquatic organisms is a serious problem, arising from both the global transport of gaseous mercury and localized industrial contamination, such as on the Oak Ridge Reservation (ORR), where ~300,000 kg of mercury (Hg) were lost to water, soil, and sediments from 1950 to 1963. This poster provides an overview of the extent of mercury contamination at the ORR and apparent paradoxes related to Hg concentrations in water, sediments, and aquatic biota. Conversion of elemental and oxidized Hg to the highly toxic methylmercury (MeHg) in aquatic ecosystems is a significant global problem. Paradigms for MeHg production, developed from the extensive study of Hg transport, transformation, and bioaccumulation in systems affected by atmospheric mercury deposition, often fail to describe the behavior of mercury at sites contaminated by industrial or other local sources, where inorganic Hg concentrations may be orders of magnitude higher. Multiple Hg-contaminated aquatic ecosystems at the ORR provide excellent examples of the paradoxical differences between sites contaminated by local versus global sources. Some unresolved paradoxes include:

1. In the East Fork of Poplar Creek, which drains a heavily Hg contaminated area at the Y-12 National Security Complex, concentrations of dissolved Hg in water decrease with increasing downstream distance, yet waterborne MeHg increases. Although waterborne Hg concentrations have declined from ~1.8 to 0.2 ppb since 1985, Hg in fish has remained virtually unchanged at the site.

2. Watts Bar Reservoir (downstream from East Fork Poplar Creek) has experienced Hg loading of 3 to 6 times background levels as a result of inputs from the ORR, yet Hg concentrations in fish are among the lowest in the TVA system.

3. In contaminated ponds and impoundments around the ORR, Hg concentrations in fish are poorly correlated with the sediment Hg concentrations. Some of the highest Hg levels in fish are found in an abandoned local quarry that has little Hg contamination. Similarly, although Bear Creek receives Hg inputs in its headwaters from sources within Y-12, waterborne Hg levels in lower Bear Creek are virtually indistinguishable from regional background. However, Hg levels in fish are 3 times greater than background values.

Therefore, improved understanding is urgently needed to elucidate the chemical and biological mechanisms controlling Hg speciation and transformation of mercury in these environments. Investigating the causes of the apparent paradoxes associated with mercury on the ORR will advance the science and enable the development of a predictive model that may be universally applicable to both locally and globally contaminated sites.

Environmental Hydrogen and Other Fixed Gas Production and Consumption in Groundwater

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The objective of this research is to develop a methodology to monitor the major dissolved gases in groundwater that are indicative of key metabolic processes, including respiration (CO_2), methanogenesis (CH_4 and CO), hydrogen production (H_2), denitrification (N_2O and N_2), and oxygen consumption (O_2). Such metabolic gases occur coincident with contaminant availability changes, such as uranium reduction.

An *in situ* passive sampling and gas chromatographic protocol was developed for analysis of the major and several minor fixed gases in groundwater. A gas-tight syringe, mated to a short length of silicone tubing, was equilibrated with dissolved gases by immersing in groundwater and then used to transport and to inject a 0.5 mL gas sample into a gas chromatograph. Using argon carrier gas, a HayeSep DB porous polymer phase, and sequential thermal conductivity and reductive gas detectors enabled good sensitivity for He, Ne, H_2, N_2, O_2, CO, CH_4, CO_2, and N_2O. Within 4 days of immersion in groundwater, passive samplers, when initially filled with either He or air, attained the same and constant gas composition at the Oak Ridge Field Research Center (FRC) site in an area heavily impacted by uranium, acidity, and nitrate. Between June 2006 and July 2007, 12 permanent groundwater wells were used to test the passive samplers in groundwater contaminated by a group of four closed radioactive wastewater seepage ponds. Over a thousand passive gas samples from these wells averaged 56% CO_2, 32.4% N_2, 2.6% O_2, 2.6% N_2O, 0.21% CH_4, 0.093% H_2, and 0.025% CO—with an average recovery of 95 ± 14% of the injected volume.

In addition to the direct evidence for denitrification (N_2O) and methanogenesis (CH_4) offered by the abundances of these gases, their isotopic compositions are being measured to determine their mechanism(s) of generation. For example, the isotopic composition of H_2 dissolved in two groundwater samples, impacted by the closed S3 storage ponds, was measured at δ^2H = -586 and -536 ‰. Such isotopic composition is consistent with a radiolytic, rather than a microbial, origin for H_2. In contrast, the $\delta^{13}C$ and δ^2H of the methane gas is consistent with its microbial origin. Further measurements of the isotopic composition of various dissolved gases are ongoing.

Polymer-Encapsulated Soils for Nondestructive Monitoring of Uranium and Thorium *In Situ* Behavior in Groundwater

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The objective of this research is to develop a nondestructive and inexpensive technique to monitor the long-term performance (immobilization) of soil contaminants, particularly uranium and thorium, which are prevalent at the Oak Ridge Field Research Center (FRC), in their natural groundwater setting. Whether uranium immobilization is achieved through active soil bioremediation, chemical amendment, or through natural attenuation, measurements of the degree and permanence of contaminant immobilization are critical to the assessment of long-term site performance. A range of contaminated soils have been encapsulated in polyacrylamide hydrogels and observed nondestructively during uptake and release of uranium and thorium following *in situ* placement within various FRC groundwater monitoring wells. These permeable environmental leaching capsules (PELCAPs) are assayed nondestructively and repeatedly for uranium, thorium, and many constitutive elemental concentrations, using x-ray fluorescence (xrf) spectroscopy. These polymer-encapsulated soils behave quite similarly to nonencapsulated soils for uranium and other elemental chemical interactions; the hydrogel matrix has been found to be quite inert to contaminant interactions, posing only a short (<1 day) diffusion delay in equilibrium kinetics. PELCAPs, containing various uranium- and thorium-contaminated soils from the FRC, have been placed in uncontaminated or end-state groundwater to determine their degree and kinetics of mobilization. Other PELCAPs containing initially uncontaminated soils have been placed in contaminated groundwater wells, to follow uptake of uranium and thorium and their subsequent release when the PELCAPs were moved to uncontaminated groundwater. Such uptake and release of contaminants allows the complete process of natural attenuation to be followed, enabling a realistic assessment of long-term site performance when no remedial actions are undertaken. Soils, which have received various treatments to enhance uranium immobilization, are undergoing similar long-term performance assessment to compare directly with naturally attenuated soils. PELCAPs, containing soils from other DOE sites, are undergoing similar uptake and release observations within FRC groundwater as are PELCAPs containing an assortment of uranium-adsorbing materials (specific resins, activated charcoal, a polyphosphate, limestone, and hydrated Portland cement) for comparative purposes. The nondestructive xrf analysis of hydrogel-encapsulated soils is directly applicable to many pertinent environmental contaminants, including Hg, Ba, As, Zn, Se, Cr, Cd, as well as many radionuclides when using gamma spectroscopy.

Anaerobic Reduction and Transformation of Fe-Hydroxides

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The microbially mediated reductive dissolution of Fe(III)-oxyhydroxides plays an important role in regulating the redox state of subsurface soils and aquifers, and controls the mobility of redox-sensitive contaminants found at many of the DOE sites. The objective of this project is to improve our understanding of what controls these transformations and how quickly they take place. This, in turn, provides the basis for understanding how Fe speciation affects contaminant mobility.

The reductive dissolution of Fe-hydroxides by dissimilatory iron-reducing bacteria (DIRB) causes structural changes as a result of the dissolution process. The reductive dissolution, however, also has an indirect effect as a result of the production and re-adsorption of ferrous iron onto the surfaces of remaining Fe-hydroxide surfaces, where further structural transformations take place via electron transfer processes. However, the detailed mechanisms and pathways of such microbially induced and Fe(II)-catalyzed transformations of Fe-hydroxides are still largely unknown. As a first step to study such complex reaction processes kinetically in a microporous flow environment, the microbially produced electron shuttle, AH_2DS, was directly injected into capillary tubes packed with ferrihydrite-coated glass spheres. Synchrotron X-ray Absorption Spectroscopy (XAS) was carried out on the reacted Fe-hydroxides particles along the packed capillary tube on SSRL beamline 11-2, so as to quantitatively identify the secondary mineral phases formed and their spatial distributions. Preliminary results showed goethite as the major mineral phase formed during the reductive dissolution by AH_2DS, with a distinct front of 1–2 mm width developing in the case of low flow velocities. The reactive transport code CrunchFlow was used to simulate the spatial distribution of the goethite formed along the packed capillary tube and to further interpret the kinetics of the transformation process. Parallel experiments using inorganic ferrous ions injection solution were also performed to study the kinetics of abiotic Fe(II)-catalyzed transformation of Fe-hydroxides. The phase transformations were monitored by synchrotron XRD method on the ALS beamline 11.3.1 and 10.3.2. The reaction products were further examined by Transmission Electron Microscope at LBNL's National Center for Electron Microscopy (NCEM). The study results showed a complete conversion from ferrihydrite to magnetite at 20 mM and 2 mM ferrous iron injection concentrations, with no intermediate phases detected. The transformation rates are rapid compared to those obtained in traditional bench reactors. Rather than following a constant rate, the transformation reaction was slow at the beginning, but increased rapidly thereafter, following a power rate law. The overall fast transformation process indicates that a topotactic structural rearrangement resulted from the inorganic Fe(II) injection without significant dissolution and recrystallization. The transformation process conforms to a solid-state transformation mechanism, and the rate profiles showed a dispersive kinetic behavior.

Nitrate Enhanced Microbial Cr(VI) Reduction

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A major challenge for the bioremediation of radionuclides (i.e., uranium, technetium) and metals (i.e., Cr(VI), Hg) is the co-occurrence of nitrate as it can inhibit metal transformation. 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 organisms, *Geobacter metallireducens* (Mechanism 1), *Sulfurospirillum barnesii,* (Mechanism 2), and *Desulfovibrio desulfuricans* (Mechanism 3) to investigate each of these mechanisms.

Our objectives are to (1) to investigate the role of Cr(VI) concentration in the kinetics of both growth and reduction of nitrate and Cr(VI) in these three organisms; (2) to develop a profile of bacterial enzymes involved in nitrate transformation (e.g., oxidoreductases) of each organism, using a proteomic approach; (3) to investigate the function of periplasmic nitrite reductase (Nrf) as a chromate reductase by determining the kinetics (K_m, V_{max}, K_{cat}) of Cr(VI) reduction and other biochemical characteristics of Nrf from the three different organisms; and (4) to develop a strategy to maximize microbial chromium reduction in the presence of nitrate.

The growth kinetics of the cells grown on nitrate with and without Cr(VI) were determined. For the initial set of experiments, 100 μM Cr(VI) was used. As previously demonstrated, cultures of *S. barnesii* simultaneously respired nitrate and reduced Cr(VI). All Cr(VI) had been reduced within five days. Cells harvested during log growth phase (48 hours) and stationary phase (96 hours) and prepared for proteomics analysis. *D. desulfuricans* exhibited the characteristic lag phase when the cells were grown on nitrate in the presence of Cr(VI). Interestingly, however, this lag phase became attenuated as the cultures were maintained and transferred onto media containing Cr(VI). Again, all the Cr(VI) was reduced within five days. Cells were harvested during log growth phase (48 hours) and stationary phase (96 hours) and prepared for proteomics analysis. For *G. metallireducens*, growth on nitrate was greatly inhibited by the presence of Cr(VI); however, this inhibition was reduced with addition of ferric citrate (1.5 mM). Cells were harvested at 72 hours and prepared for proteomics analysis. For all three organisms, 2-D gel electrophoresis revealed differences in protein expression between cells grown on nitrate with Cr(VI) and with nitrate alone. The difference in protein profile was most striking for *D. desulfuricans*, indicating significant changes are induced by chromium. Candidate proteins were excised from the gels and processed for mass spec analysis. MALDI-TOF mass spectra of selected trypsin digested proteins were performed, and we have tentative identification of several peptides. The NrfA homolog of *S. barnesii* was identified in PAGE gels and confirmed by MS-MS analysis.

Integrating the Molecular Machines of Mercury Detoxification into Host Cell Biology

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NABIR RESEARCH OBJECTIVES: The bacterial mercury resistance (*mer*) operon unctions 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 how these components interact with each other and with the host cell to restore function in cells exposed to Hg compounds.

Regulation of *mer* Operon Expression: Our NMR with ^{19}F-Tyr-substituted MerR (*J. Mol. Biol. 371:79*) had shown that MerR's allosteric changes are distinctly metal-specific when it is bound to the operator, MerO, rather then when it is free. Now we find by calorimetry in physiological conditions (5 mM glutathione, pH 7) that MerO-bound MerR has 10-fold lower affinity for Zn(II) than for Hg and almost undetectable Cd(II) binding. Analytical ultracentrifugation shows that Cd(II) or Zn(II) cause free MerR to multimerize (also seen by calorimetry). XAFS shows that Cd(II) and Zn(II) establish distinct coordination arrangments with free MerR. The emerging picture is a unique regulator with two levels of coordination-driven, metal-ligand control (affinity and allostery) each modulated by interaction with the DNA operator. MerR also contacts RNA polymerase (RNAP); cross-linking now shows the MerR domains that abut various (RNAP) subunits. We are modelling the MerR-MerO-RNAP complex with D. Vassylyev (UAB) and will analyze complex assembly kinetics with D. Bornhop (Vanderbilt). In the year ahead, we'll examine the anti-activator, MerD, alone and complexed with MerR, MerOP, RNAP. We'll also pilot transcriptional microarrays of *E.coli* chromosomal expression in response to Hg(II).

MerA Core Interactions with NmerA: To stabilize their interaction, an Hg(II)-crosslinked complex of NmerA with a non-reducing mutant Core was made and is in crystallography trials to define key contacts in the inter-domain Hg-ligand-transfer step. The cleavage of NmerA from full length MerA has proved remarkably robust to point and deletion mutageneses and even substitution of homolog MerP for NmerA; all manipulations yield no change in the extent of cleavage nor the length of resulting Core. It's also becoming clear that, despite recombinant-NmerA's stability, as a domain of MerA when NmerA is cleaved it is completely degraded as if by ATP-dependent proteases, although prior genetics indicated no single one is responsible. In cells over-expressing MerA, antibody to full MerA detects stable 30-50 kDa proteins, apparently products of incomplete translation. Because all of these shorter proteins also react with NmerA-specific antibody, its cleavage appears not to be co-translational. A fraction of full length MerA is membrane-bound even in cells lacking the full *mer* operon, and we're now examining whether NmerA cleavage is associated with the membrane, which would implicate a distinct set of proteases.

Hg(II) Membrane Transporters: *In vitro* MerT binds 2 Hg(II) as expected for its 2 pairs of cysteines and in vivo, strains co-expressing MerT and Core or full MerA are being evaluated for their resistance phenotypes as an indication of their ability to take Hg(II) from MerT. In the coming year, this work will include MerC and MerF transporters.

MerB and its Interactions with Core MerA The crystal structure of MerB (*in preparation*) implicated conserved Asp99 as an acid/base catalyst in protonolysis and, indeed, an Ala99 mutant of MerB is inactive. In addition, the fluorescence of active-site proximal residue, Trp95, proves quite sensitive to binding/release of Hg(II) and is valuable as a probe for kinetic investigation of the ability of NmerA to extract MerB's Hg(II) product from wild type and the three Cys to Ser mutants of MerB. We also have identified several conditions for production of crystals of the MerB mutant C96S stably complexed with its substrate phenylmercuric acetate (PMA). Solving this structure will locate PMA's organic side-chain; only the post-protonolysis Hg(II) product was detected in the wildtype crystal structure. In the coming year, we will complete these studies and use our kinetic data for interactions of all *mer* proteins to model their working in the cell with W. Burgos (Penn State).

Identifying Biomarkers and Mechanisms of Toxic Metal Stress with Global Proteomics

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Growing 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'll use AMT-FTICR-MS global proteomics both to identify Hg- and RHg-damaged cellular proteins and follow their recovery with and without the *mer* operon.

Efforts in the *Miller Lab* focus on the bioinformatic and *in vitro* aspects of the work: (1) developing software tools to predict and, more importantly, analyze mass spec data for Hg-peptide adducts arising in Hg-treated cells; and (2) preparing mercurated adducts of pure defined proteins and peptides as models to establish the characteristic behavior in mass spec fragmentation needed to identify the adducts. On the bioinformatic side, we first developed tools to predict the most likely Hg(II)-peptide adducts in proteins of the *E. coli* genome, based on available structures and structural models. However, our major effort has turned to analyzing fragmentation of model mercurated proteins and peptides to determine how best to establish the signatures of Hg-adducts in global proteomics samples. We'll present analyses of two models: Hg-peptide adducts prepared from pure synthetic peptides and peptides obtained by trypsinolysis of specific Hg-adducts generated from proteins of the *mer* operon.

For the *in vivo* work, the *Summers Lab* constructed strains based on the first sequenced *E.coli* K12 strain, MG1655, and a derivative of it carrying the widely found, sequenced HgR plasmid NR1. Neidhardt's MOPS medium used in all other *E.coli* -omics work has been benchmarked for these strains, and three assays of cellular redox- or Hg(II)-damage have been implemented to monitor the effects of cell treatments before sending them for mass spec proteomic analysis. These assays are (1) quantification of total cellular thiols using the sensitive fluorescent Measure-IT reagent (Molecular Probes); (2) qualitative estimation of protein-bound thiols by reaction with an iodoacetamide-derivative of the fluorescent dye BODIPY, followed by SDS-PAGE; and (3) qualitative estimation of oxidation-induced protein carbonylation by derivatization with DNP (OxyBlot procedure) which is then detected by an anti-DNP antibody in proteins separated electrophoretically. A set of untreated cells and a set of cells treated with varying concentrations of phenylmercuric acetate (PMA, a safer surrogate for methylmercury) and also a Hg(II) treated set have been prepared, analyzed by Summers' lab and the untreated and PMA-treated sets have been sent as cell pellets for mass spec analysis at PNNL.

The *Lipton Lab* has completed an AMT-FTICR-MS analysis of the untreated set, MG1655 and MG1655(NR1), that identified 3-fold more chromosomal proteins (64% of ORFs) than the existing 2-D analysis of *E.coli* and 53% of plasmid-encoded ORfs. This is the first global proteome ever done on this workhorse organism of molecular biology and also the first on a large conjugative plasmid. It is currently being repeated for the untreated condition and extended to the PMA-treated preparations; PMA should give less complex peptide patterns since it is monovalent and will not crosslink protein thiols as Hg(II) can. For the year ahead, we will analyze cells treated with the ethylmercury compound merthiolate, and also cells treated with hydrogen peroxide, to distinguish the redox shock proteome, which we expect will partially overlap the Hg(II) exposure proteome, as noted above. We will also begin longitudinal experiments, using stable isotopes (^{15}N, ^{13}C) to follow proteins' recovery from Hg/PMA exposure.

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

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One potential remediation strategy for metal-contaminated subsurface environments is the deployment of microbes for biotransformation of the oxidized mobile metals to largely insoluble, reduced forms. Success of such a strategy depends on knowledge of the structure, dynamics, and activity of the autochthonous microbial community. Mass spectrometry (MS) based proteomics has tremendous potential for unraveling the molecular protein details of active 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. We have constructed laboratory microcosms derived from Cr(VI)-free and Cr(VI)-contaminated soils from the DOE Hanford site to test the effects of nutritional amendments on microbial composition, gene expression (as evidenced by proteome analysis), and concomitant chromate reduction. We have constructed ssu-rRNA clone libraries from the native Hanford soil as the first step in: (1) determining the phylogenetic composition of microbes populating the soils of interest, (2) establishing a baseline phylogenetic profile of Cr(VI)-free and Cr(VI)-contaminated Hanford soils for comparison with nutritionally amended soil microcosms, and (3) providing a benchmark to guide our efforts to optimize conditions for the most efficient methods of soil proteome analysis.

Towards these goals, we have employed a new method for isolating community genomic DNA (cgDNA) from low-biomass samples that enables recovery of at least 50 to 100 times as much cgDNA as we have recovered with any of the commercial soil extraction kits routinely used. Another integral part of this project is the characterization of a variety of different microbial and protein extraction procedures to identify the most effective and quantitative method for isolating proteins for metaproteome profiling. In one such set of experiments involving a sterilized Cr(VI)-contaminated microcosm spiked with the chromate reducer Pseudomonas putida F1, we were able to detect 28% of the 6544 candidate proteins predicted by genome annotation. Our analysis indicated that there were a greater number of up-regulated proteins compared to down-regulated proteins in the contaminated sample. Proteins that were up-regulated included those involved in iron metabolism, stress response regulation, general metabolism, and hypotheticals. In another experiment using a native, unsterilized, uncontaminated Hanford soil sample, we were able to detect proteins from P. putida F1 and KT224, Corynebacterium efficiens, Rhodopseudomonas palustris and several species of Geobacter. We are currently analyzing the data from our initial clone libraries to determine how well our protein extraction protocol represents the diversity of the community, so that we can continue to close the gap between expected and observed protein content in these soils. This, in turn, will enable a better of understanding and application of the microbial community response to chromate challenge.

Microcantilever Sensors for *In Situ* Subsurface Characterization

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Analysis of subsurface contaminants by traditional sample collection followed by laboratory analysis has a number of limitations that can be overcome by deploying inexpensive, miniature, real-time sensors capable of multianalyte detection with speciation. Microfabricated cantilever sensors offer several advantages, including low-power consumption, *in situ* and real-time operation, extreme high sensitivity, and integration into arrays for multianalyte detection. The adsorption of analyte molecules on a cantilever surface results in cantilever bending when adsorption is confined to a single side, due to a differential in the surface stress between sides. Chemical selectivity can be achieved by immobilizing a self-assembled monolayer on the cantilever surface that has an affinity for a particular analyte. Recently, it was observed that a differential surface stress can also be created, as a result of redox reactions initiated by applying an electrochemical potential to a microcantilever with a thin film of metal on one side. There is particular interest in analyzing and speciating two broad categories of metallic contaminants: heavy metals (lead, chromium(VI), mercury, zinc, beryllium, arsenic, cadmium, and copper) and transuranic elements (uranium, neptunium, and plutonium). Although these two general groups and the members of each group are chemically different, they generally do not have sufficiently orthogonal molecular-recognition characteristics to be monitored using molecular recognition alone. However, many of these metallic elements are electrochemically active, providing a second mode of selectivity and means of preconcentration using stripping techniques. These methods can be used to replace or complement the methods we previously developed for species such as Cs, Hg(II), methyl-Hg(II), Cu(II), Cr(VI), and Cd(II) using analyte-specific SAMs immobilized on gold-coated silicon cantilevers using thiol chemistry

We are particularly interested in the analysis and speciation of metallic and inorganic mercury, as well as organic mercury containing compounds such as methyl- and dimethylmercury. By adding the capabilities of electrochemical analysis to microcantilever-based sensors, we will be able to make rapid and sensitive measurements using a sensing platform ideally suited for multiplexing and remote deployment. There are broad applications for this sensing technology, and it will be used to characterize the microbial generation of methylmercury. In ORNL programs, the rapid, inexpensive, sensitive analysis and speciation of mercury content is an essential component. While sensitive electrochemical detection of Hg(II) and Hg(0) are well understood, and we have previously demonstrated a microcantilever-based method for detecting methylmercury, we have yet to combine the methods on a single microcantilever sensor. This sensor, once developed for a single microcantilever, will then be able to be deployed in an array format.

Metagenomics-Enabled Understanding of the Functions and Activities of Microbial Communities at ERSP Field Research Center at Oak Ridge, TN

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Metagenomics, pyrosequencing, quantitative polymerase chain reaction (PCR) techniques, and terminal restriction fragment polymorphism (TRFLP) were used to study patterns of community composition and diversity in Area 3 sediments of the Field Research Center (FRC) at Oak Ridge, TN. The 16S rRNA gene was amplified using universal primers targeting the bacterial domain for TRFLP and the V4 region for pyrosequencing. Six sediment samples were analyzed by pyrosequencing at an average of ten thousand reads per sample. Pyrosequencing revealed that the microbial communities were dominated by members of the *Proteobacteria* (mostly from Delta and Beta classes). Known uranium reducers were detected in the following order of frequency: *Desulfovibrio, Geobacter, Anaeromyxobacter Desulfosporosinus*, *Shewanella* and *Deinococcus*. Diversity patterns were also studied before and after reintroduction of oxygen in the system. Minor differences were detected, and the impact of oxygen differed across samples, suggesting differences in oxygen distribution. Community profiles from TRFLP and pyrosequencing were correlated to multiple environmental variables using canonical correspondence analysis (CCA). Five environmental variables (out of twelve used) explained the microbial diversity on the CCA: they were pH, nitrate, sulfate, uranium, and sodium. This result suggests a system heavily influenced by pH and electron acceptor availability.

Metagenomic analyses targeted the groundwater community from contaminated Area 3 to determine the effects of chronic exposure to contaminants on microbial community structure. The sample was obtained from a site (FW106) experiencing long-term exposure to high levels of uranium and other heavy metals, nitric acid, and organic solvents. Community analysis indicated very low species diversity (~13 OTU) dominated by denitrifying γ- and β-proteobacteria. Metabolic reconstruction of the dominant γ-proteobacterial species revealed adaptations for specific geochemical parameters, including the following: denitrification pathways; pathways for degradation of organic compounds (such as1,2-dichloroethene, acetone, butanol, methanol and formaldehyde); and accumulation of multiple heavy-metal efflux systems (czcABC, czcD, cadA-family, mer operon genes, etc.). Genomic analysis suggested that lateral gene transfer is the predominant mechanism of introducing genetic variation in the community, resulting in the lateral acquisition of, for example, an acetone carboxylation pathway and heavy metal efflux systems. The sample was compared to a second groundwater metagenome from a pristine FRC site (FW301) to determine differences between the two communities. In contrast to the low species diversity of FW106, the FW301 is represented by multiple phyla, including all five classes of proteobacteria—Planctomycetes, Chloriflexi, Actinobacteria, Acidobacteria, Bacteroidetes and Firmicutes. Abundance profiling of geochemical and cytochrome genes between FW106 and FW301, and between FW106 and the acid mine drainage (AMD) metagenome, show distinct environmental signatures between the samples. This analysis verified the previous observation of accumulation of heavy metal other toxin efflux mechanisms in FW106, as well as an accumulation of specific c-type cytochromes in FW106 that may be important in heavy-metal resistance.

Biochemical Mechanisms and Energy Strategies of *Geobacter sulfurreducens*

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To provide the scientific understanding required to allow DOE sites to incorporate relevant biological, chemical, and physical processes into decisions concerning environmental remediation, a fundamental understanding of the controls on micro-organism growth in the subsurface is necessary. The research aims to determine the survival properties of *Geobacter* under prolonged starvation. These properties will be addressed by (1) monitoring cell number and morphology during prolonged starvation, (2) determining their energy requirements to maintain a growing versus a nongrowing population and (3) examining global gene expression patterns during growth and starvation phases to determine the metabolic strategy of *Geobacter*.

The long-term survival studies have been started and are ongoing. We have been monitoring total cell counts and are characterizing the ability of *Geobacter* to survive long periods of nutrient deprivation. *Geobacter* was grown in either fumarate or ferric citrate media [1] and counted by acridine orange staining. Similar to results with *E. coli* [2], *Geobacter* maintains a population of approximately 10^6/mL even under long-term starvation conditions for over a year. While some microbes are known to decrease in size upon prolonged starvation, this has yet to be observed with our starved *Geobacter* cultures. The determination of the time course of *Geobacter* growth, entry into stationary phase and into prolonged starvation phase, provides information on when to sample total RNA for DNA microarray studies to determine global gene expression patterns. These studies will be initiated the next year.

To determine maintenance energy and growth yield values in chemostats using solid-phase electron acceptors, we are collaborating with Wayne Curtis of Chemical Engineering here at Penn State. Curtis is constructing the chemostats such that solid phases can be added to the cultures. Meanwhile, we have initiated batch culture studies to determine parameters to be used for chemostat cultures. Because of the slower growth rate on solid substrate and concerns with slow dilution rates in the chemostat, we have examined conditions to maximize growth rate. The effect of chelators upon growth rate with iron oxides and Mn oxides have been characterized in preparation for the chemostat studies.

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Uranium Spatial Distribution and Partitioning within Alkaline Waste Plumes in the Hanford Vadose Zone

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The accidental overfilling of waste liquid from tank BX-102 at the Hanford Site in 1951 put about 10 metric tons of U(VI) into the vadose zone. Our research goal is to understand the status of this spilled U in the subsurface. In contrast to other studies characterizing the sediment from borehole samples collected underneath tank BX-102, we conducted laboratory simulations of the spilling event in sediment columns using synthesized waste solution, and followed up with analyses of samples aged for different lengths of time. Steady-state conditions were basically achieved after aging for one year at 70˚C.

Main findings are as follows: As the plume propagated through the sediment, the pH decreased greatly (as much as 4 units) at the moving plume front. Massive amounts of U-colloids formed at the plume front during initial waste liquid filtration. Total U concentrations (aqueous and colloid) within the plume front exceeded the source concentration by up to 5-fold. Uranium colloid formation and accumulation at the neutralized plume front could be one mechanism responsible for the highly heterogeneous U distribution observed in Hanford's contaminated vadose zone.

The tests of infiltration flow rates indicate that kinetic limitations on sorption and precipitation permit practically unretarded U transport at flow rates $\geq$5 cm/day. Given the very high hydraulic conductivity of Hanford formation sands (10^2 to 10^4 m day^{-1}), low-permeability zones within the sediment might have been critical in slowing transport of high concentrations of U during initial release into the Hanford vadose zone. Predictions based on equilibrium K_d partitioning of U would greatly underestimate the extent of U migration.

A surprisingly high U concentration (0.022 M) was found in the aged plume body liquid phase. This value can serve as the upper limit of the liquid-phase U concentration zone in the field. The U effective K_d (sorption and precipitation) is low within the plume body, ≤ 1 (l/kg), because of the alkaline pH and high concentrations of bicarbonate, but as high as 10^4 (l/kg) in the plume front zone. The aforementioned findings were obtainable only through laboratory experiments, coupling flow and geochemical reactions under field-relevant conditions.

Novel, Intelligent Nanoparticle-Hydrogel Sensors for *In-situ* U(VI) Detection

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Rapid, *in-situ* quantification and characterization of radionuclides (such as uranium) in the subsurface environment is essential for achieving rapid turn-around time of analytical results and making timely decisions in environmental monitoring and remediation. However, current analytical techniques often require lengthy analytical time, chemical handling, and expensive instrumentation. The goal of this exploratory research was to develop simple, inexpensive photonic hydrogel-based sensors to sensitively and selectively detect uranium in contaminated groundwater. Intelligent nanoparticle-hydrogel-based sensing materials, in the form of visual color strips (similar to pH test papers), were synthesized and used to detect uranium at low concentrations. The technique was developed on the basis of colloidal photonic crystals made of highly charged and monodispersed SiO_2 nanoparticles, which diffract light according to Bragg's law. The SiO_2 photonic crystals were immobilized into a polymer network to form stable, robust polymer hydrogel films, using a UV-light induced photo-polymerization with acrylamine as monomer and N', N-methylenebisacrylamide as a crosslinker. To selectively adsorb U(VI), the hydrogels were further functionalized by grafting specific functional groups such as phosphonate in its network. The functionalized hydrogel thin film contained ~90–95% water, so that U(VI) ions were capable of diffusing freely into and adsorbing onto the hydrogel. This causes the crystal lattice spacing to change or the strip color to change, allowing quantitative detection of contaminant concentrations in water. Our preliminary results showed that the sensor strips respond to U(VI) concentrations at ppm levels in water. Future studies are needed to further optimize the synthetic conditions and surface functional groups, so that we can achieve the detection limit at ppb concentration levels. Ultimately, simple, inexpensive, intelligent nanoparticle-hydrogel-based testing strips are to be developed for sensitive detection of a range of contaminant metals and radionuclides in groundwater, with reduced analytical costs and turnover time.

Molecular-Level Investigations of Nucleation Mechanisms and Kinetics of Formation of Environmental Nanoparticles on Mineral Surfaces

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Environmental nanoparticles are frequently poorly crystalline or metastable structures, whose kinetics of formation and growth are poorly understood. Further, the sorption or growth of nanoparticles on mineral surfaces may control the mineral surface's reactivity and modify its ability to influence contaminant transport. Either a passivating or activating effect in reactivity can be created. In this work, our intent is to determine the molecular nature of the nucleation of nanoparticles on surfaces, the kinetics of surface nucleation and growth, and the effect of crystal surface topology using new synchrotron-based techniques.

We have combined: (1) state-of-the-art crystal-truncation rod diffraction (CTR) and grazing incidence x-ray absorption fine structure spectroscopy (GIXAS) techniques to investigate the three-dimensional molecular-scale geometry of silicate monomer sorption on the r-plane of hematite; with (2) a new grazing-incidence small angle x-ray scattering (GISAXS) methodology to explore the initial development of environmental nanoparticles on various mineral surfaces. With this method, we can study interface reactions like precipitation on the nanoscale, in the presence of bulk solution. This study also includes complementary techniques such as atomic force microscopy (AFM), bulk SAXS, dynamic light scattering (DLS), XRD, and TEM.

From both nonspecular and specular CTR data, the silicate adsorption geometry on hematite surfaces can be obtained in three-dimensions. The best-fit model is a monodentate mononuclear complex, perhaps analogous to silicate-containing natural mineral structures. Current MD simulations and DFT calculations are examining the energetic stability of this complex, as well as possible surface silicate polymerization reactions. Complementing this work, the first Si K-edge GIXAS data have been collected and analyzed.

In the other part of our study, using *in situ* GISAXS, we have been able to observe the nucleation and growth of iron oxide nanoparticles on quartz surfaces. The growth mode changes after a short period, and two types of kinetics are observed. Large changes in the topological distribution of the nucleated nanoparticles occur as a function of solution ionic strength, and this is being modeled as a diffusion-controlled heterogeneous nucleation reaction. The GISAXS technique provides statistically improved morphological information of environmental nanoparticles compared with AFM and SEM, and allows real-time geochemical kinetics analysis of nanoparticle growth and reactions.

By using this arsenal of newly developed state-of-the-art techniques, we intend to investigate the mechanisms and kinetics related to the nucleation and growth of nanoparticles on surfaces having varying step density (i.e., varied surface topologies), as well as the presence of heavy-metal ions or organic compounds, at different temperatures.

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

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The chemical stability of phosphate minerals (autunite and apatite) is a seminal issue governing the success of phosphate-based remediation for *in situ* immobilization of uranium. There is limited understanding about the effect of microbial activity and the ability of microorganisms to utilize phosphate minerals a nutrient sources under oligotrophic conditions. We hypothesize that the biotic durability of autunite and apatite minerals will decrease relative to baseline abiotic conditions, thereby reducing the longevity of phosphate remediation technology for *in situ* remediation of uranium. The objectives of this investigation are to: (1) evaluate changes to the microbial community between pre- and post-polyphosphate remediation, (2) determine the affect of dominant microbial metabolites on the long-term durability of autunite and apatite, and (3) incorporate a kinetic-rate equation into current reactive transport codes to account for the biotic contribution to autunite and apatite weathering, enabling more accurate predictions of the long-term fate of phosphate amendments for the *in situ* immobilization of uranium. This abstract summarizes work completed after one year of this project.

Sampling, Preparation, and Characterization of Bacterial Consortia. Leveraging ongoing Hanford activities, including the EM-22 directed polyphosphate remediation demonstration, we have collected a number of sedimentary cores prior to the polyphosphate injection. Solutions of microbial metabolites from various borehole locations and depths have been cultured and are being characterized, and solutions of bacterial degradation products and metabolic exudates have been produced, collected for characterization, and are being used in testing to evaluate the effect on autunite and apatite durability.

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 autunite and apatite serve as baselines against which we can compare the effects of microorganisms.

Effect of Hanford Micro-organisms on the Durability of Autunite. A series of static tests were conducted utilizing a natural microbial extract from the Hanford site in the presence of calcium meta-autunite, $Ca_2[(UO_2)(PO_4)]_2 \cdot 3H_2O$, to quantify the reaction progress (mol kg^{-1}), as indexed by release of uranium into solution, to evaluate the longevity of autunite in the presence and absence of two soluble phosphate amendments, sodium trimetaphosphate and sodium tripolyphosphate.

Effect of Microbial Metabolites on the Dissolution Kinetics of Autunite and Apatite. Lactic acid and pyruvic acid have been identified as significant bacterial metabolites. Single-pass flow-through (SPFT) experiments have been conducted to quantify the effect of lactic and pyruvic acid on the dissolution kinetics of apatite and autunite, under the pH range of 7 to 10 and temperature range of 5 to 90°C.

Future Activities. We will: (1) identify changes in the microbial community from pre- and post-amendment microorganisms extracted from 300 Area sediments; (2) quantify the dissolution kinetics of autunite and apatite in the presence of microbial metabolites and exudates from pre- and post-amendment microorganisms extracted from 300 Area sediments; (3) calculate the contribution of microbial metabolites to the dissolution of autunite and apatite; (4) utilize LI-PAS and NMR, combined with the MALDI-TOF measurements, to provide the required information needed to determine the aqueous speciation of uranium and phosphate in the presence of microbial metabolites; and (5) incorporate the results obtained from the experimental strategy into a chemical affinity-rate law to provide credible estimates of radionuclide release.

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

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Previous studies have found that organic acids, organic bases, and detergent-like chemicals change surface wettability. The wastewater and NAPL mixtures discharged at the Hanford site contain such chemicals, and their proportions likely change over time due to reaction-facilitated aging. The specific objectives of this work are to (1) determine the effect of organic chemical mixtures on surface wettability, (2) determine the effect of organic chemical mixtures on CCl_4 volatilization rates from NAPL, and (3) accurately determine the migration, entrapment, and volatilization of organic chemical mixtures.

Four representative organic mixtures (MIX1-MIX4) and two binary organic mixtures were created from chemicals discharged at the Hanford site. The organic mixtures (MIX1-MIX4) include different compositions of carbon tetrachloride (CCl_4), DBBP, TBP, DBP, and lard oil. A representative wastewater was also prepared with nitric acid, various nitrate salts, and sodium hydroxide. Surface tension results for the wastewater equilibrated with the organic mixtures are markedly lower than the corresponding value for wastewater and pure water equilibrated with CCl_4. Interfacial tension results for all four organic mixtures and the wastewater were lower compared to pure CCl_4 and water or wastewater. Similarly, the contact angles of organic mixtures on quartz surfaces were reduced relative to pure CCl_4. Binary mixture results indicate that the DBBP and TBP compounds in the mixtures, and not the lard oil, control changes in interfacial tension. Hence, chemical components discharged with CCl_4 at the Hanford site do affect chemical wettability relative to pure CCl_4.

We conducted four flow-cell experiments in a 100 cm long, 80 cm high, and 5.5 cm wide 2-D flow cell in order to account for the impact of the organic mixtures and wastewater on DNAPL distribution and migration in a layered vadose zone, which contained a fine lens surrounded by coarser sand. Sands were presoaked in wastewater and packed into the flow cell. After packing, either the organic mixture with the lowest interfacial tension or pure CCl_4 was released into the flow cell. After the NAPL distribution reached equilibrium conditions (no apparent movement), soil vapor extraction (SVE) was applied. In one experiment with pure CCl_4 and wastewater, the CCl_4 penetrated the bottom of the fine sand lens and reached the water-saturated zone. In another experiment with the organic mixture and wastewater, the mixture did not migrate beyond the fine sand lens.

During the coming year, we will complete measurements of contact angle for all mixtures, and measurements of interfacial tension and contact angle for the biologically aged mixtures. Hanford groundwater sediments were previously obtained, and a microbial culture for biologically aged mixtures was developed. During the coming year, we will also measure capillary-pressure saturation profiles of all fresh NAPL-wastewater mixtures at PNNL. Micromodels have been fabricated, and NAPL entrapment in these systems has been started. We will perform mass-transfer experiments using pure CCl_4 and water, as well as organic and wastewater mixtures. Modifications to incorporate multicomponent mass transfer into the STOMP simulator will be completed. We will compare flow-cell experiments and micromodel experiments with STOMP simulations. STOMP will also be used to simulate NAPL migration and NAPL entrapment at the 3-D field scale.

Technetium and Iron Biogeochemistry in Suboxic Subsurface Environments with Emphasis on the Hanford Site

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This project is seeking to understand abiotic and biotic processes in Hanford's unconfined aquifer that control groundwater redox state, and the fate and transport of redox-sensitive, polyvalent technetium (^{99}Tc; $t^{1/2} = 2.13 \times 10^5$ y). As a project hypothesis, we speculate that the cessation of operational water discharges to Hanford's vadose zone in 1997 will decrease oxygen saturation in groundwater in the future, allowing reductive attenuation of mobile Tc(VII) to less soluble Tc(IV) forms by ferrous-containing aquifer solids and metal-reducing bacteria. Various lines of research are being pursued in support of this hypothesis. Here, we report on a model system study in which we sought to evaluate the relative importance of biotic versus abiotic reductive processes on Tc aqueous concentrations, post-reduction molecular speciation and mineral association, and reoxidation rate. The study contacted Tc(VII) with biogenic magnetite, and ferrihydrite plus MR-1 (*Shewanella putrefaciens*) and H_2 or lactate. Ferrihydrite with co-precipitated Tc(IV) was synthesized and contacted with Fe(II)$_{aq}$, bioreduced AQDS, and ferrihydrite plus MR-1 and H_2 or lactate under anoxic conditions. Final Tc concentrations were measured after 24 days and extensive chemical, mineralogic, microscopic, and spectroscopic measurements were performed on the resulting solids. Kinetic oxidation experiments were performed on all solids containing Tc(IV). Important findings were as follows: (1) heterogeneous abiotic reduction dominated Tc valence transformations; (2) the molecular speciation of Tc(IV) was similar to Tc(IV)-ferrihydrite in all systems; (3) MR-1 produced small amounts of Fe(II) that caused ferrihydrite recrystallization to goethite—the Fe(II)-sorbed goethite was a strong heterogeneous reductant of Tc(VII); and (4) the Tc(IV)-ferrihydrite coprecipitate and the Tc(IV)-magnetite association were much slower to oxidize than was $TcO_2 \cdot nH_2O$. New experiments using ferrous-containing mineral solids from Hanford sediments (e.g., clinochlore, pyroxenes, and Fe(II) basaltic glass) and Hanford microbial isolates (including various *Shewanella* strains) are under way to study Tc valence transformations under more relevant conditions.

Further Development and Applications of GeoChip
for Microbial Community Analysis

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Microarray technology provides the opportunity to identify thousands of microbial genes or populations simultaneously. The objective of this study is to further develop and apply a comprehensive functional gene array (GeoChip) to detect and monitor microbial communities at DOE-ERSP sites during bioremediation processes. Based on GeoChip 2.0, a new-generation 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 HUMMER, 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 phylogenic markers, such as *gyrB*. Finally, a software package has been developed to facilitate the management of such a complicated array, especially for data analysis and future update.

Here, we have used GeoChips to successfully analyze microbial functional structure in a variety of environments: (1) Microbial communities within a pilot-scale test system established for biostimulation of U(VI) reduction in the subsurface by ethanol injection were examined during the long-term U(VI) reduction phase. The microarray data indicated that both FeRB and SRB populations reached their highest levels during U(VI) reduction, and that U concentrations in the groundwater were significantly correlated with the total abundance of c-cytochrome and *dsrAB* genes. Mantel test analysis of microarray and chemical data indicated a significant correlation between the U concentration and total c-cytochrome or *dsrAB* gene abundance.

(2) In a different study of the same system, the effects of dissolved oxygen (DO) and ethanol on the stability of the bioreduced area were examined. Detrended correspondence analysis (DCA) of detected genes showed a shift towards a different community structure after ethanol injections resumed compared to the periods of starvation and exposure to DO. The overall results indicated that ethanol was the main factor affecting community structure.

(3) In a third study, groundwater monitoring wells along a contamination gradient were examined. Diversity of nitrate-fixation genes decreased in NO_3^--contaminated wells while signal intensities of metal resistance and reduction genes increased in heavily contaminated wells. Sulfate-reduction genes had greater diversity and greater signal intensity in more contaminated wells. CCA analysis showed pH was an important variable, while NO_3^- and U correlated with the highest contaminated well. (4) We have also used GeoChip to examine a UMTRA site (Rifle, CO). Cluster analysis showed samples in the same locations grouped together, regardless of geochemistry. CCA analysis of environmental parameters and functional genes indicated Fe^{2+} was the most significant geochemical variable for community structure.

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.

**Integrated Field-Scale Subsurface
Research Challenges (IFC)**

The Hanford 300 Area IFC Well-Field Configuration and Construction To Accommodate Site Characterization, Field Injections over Varying Flow Paths, and Groundwater Monitoring during Field Experiments

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The Hanford 300 Area IFC is located at the former site of a waste pond that contained residue from reactor fuels fabrication. The pond fill was excavated and backfilled, but uranium persisted in the vadose zone beneath the pond footprint and migrated in groundwater to the Columbia River. The site is influenced by the short and long-term rise and fall of the Columbia River, which causes river water infiltration into the near shore environment, and causes significant changes to the hydraulic gradient and flow direction beneath the IFC. At low river stage, groundwater flow is predominantly toward the southwest, but at high river stage, flow is predominantly toward the southeast.

The well field layout and construction plan was designed to accommodate the following objectives: (1) provide sediment samples at spacing sufficient to characterize the distribution of reactive phases within the test plot; (2) allow injection and monitoring over the predominant flow directions; (3) permit detection and monitoring of an injection plume during its reaction with the aquifer phases, including its spatial resolution and its chemical evolution; and (4) allow rapid sample collection and automated electrode measurements of injected plumes, with the ability to adapt data collection to changing experimental needs.

The well field was laid out to consist of an equilateral triangular array of wells, oriented with one side approximately north-south, with an overall length along a side of approximately 60 m. There will be 35 wells, at a nominal spacing of 10 m, completed through the saturated zone in the Hanford Fm., occurring over a depth of approximately 10–17 m. In addition, three three-well clusters will be installed within the well field footprint to allow depth-discrete water sampling in the Hanford Fm. Several monitoring and sampling systems will be installed during and after well construction.

The completed wells will be 4 inches in diameter. During construction, sediments will be removed, either by coring or by fixed-interval grab sampling, for characterization of solid-phase properties such as grain-size distribution, mineralogy, and cation exchange capacity. Core samples will be removed from seven wells for detailed description, analysis, and experimentation with continuous materials, and grab samples will be described and catalogued for further analysis and distribution to interested researchers.

At the vadose zone infiltration site, the wells will be completed with porous cups and an array of electrical resistivity sensors, outside the casing, to allow monitoring and sampling during infiltration experiments. All of the wells completed through the saturated zone will be instrumented with electrical resistivity and thermocouple electrodes. These sensors will be installed on the outside of the well screen during well completion for continuous monitoring during experimentation. Each well will also include a centrally controlled water sampling pump, and an array of conductivity, pH, bromide, pressure, and temperature electrodes, installed within the screen.

Reactive Transport Processes of Uranium and Planned Field Experiments for the Hanford 300 Area IFC

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The Pacific Northwest National Laboratory and a group of collaborators are using the Hanford 300 Area uranium (U) plume in waste management area 300-FF-5 as a site for an Integrated Field-Scale Subsurface Research Challenge (IFC). This location contains the second highest U inventory of any Hanford site, and the seasonal and long-term dynamics of the U-plume have defied prediction. Multiscale mass-transfer processes appear to cause complex behaviors at the site and are our scientific theme. A series of forefront science questions on mass transfer are posed that relate to 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. Scientific hypotheses focus on multiscale mass-transfer processes in the vadose zone, capillary fringe, and saturated zone; their influence on field-scale U(VI) biogeochemistry and transport; and their implications to natural attenuation and remediation. The site experiences seasonal changes in groundwater flow direction and chemical composition, allowing for the study of chemical and mixing gradients, seasonal adsorption/desorption events, and flow paths of different trajectory. Laboratory adsorption and desorption measurements of U(VI) on/from contaminated site sediments show strong kinetic behavior that is expected to be amplified in the field as a result of physical and chemical heterogeneities. A series of injection and passive monitoring experiments, that exploit the marked variations in water composition and U concentration that occur across the site, will be performed in an extensively monitored well-field over the next several years to investigate field-scale adsorption/desorption kinetics and causal factors. A three-dimensional geostatistical model will be established for the experimental site from extensive characterization measurements on borehole samples and surface and down-hole geophysical measurements. This geostatistical model along with laboratory-parameterized geochemical models will drive reactive transport simulations of the field experiments using multiple codes that differ in their treatment of mass transfer process and their scaling relationships.

Geohydrology of the Hanford 300 Area and Pre-Modeling of IFC Experiments

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Pacific Northwest National Laboratory

The focus of the Hanford 300 Area IFC is on mechanisms responsible for the long-term persistence of a groundwater uranium plume, in spite of the excavation and removal of contaminated sediments from all known source areas. This presentation gives an overview of the geologic and hydrologic characteristics of the 300 Area and preliminary modeling activities associated with the design of the IFC well field and experiments. Data from an automated well network for water-level monitoring were used in conjunction with particle tracking calculations, and with considerations for monitoring by geophysical methods (crosswell ERT and GPR), to design the configuration of the IFC well field. Small-scale, high-resolution 3D models of the IFC experimental domain are being developed using the STOMP simulator. A 3D uranium plume-scale STOMP model of the 300 Area has also been developed and calibrated. Comparisons of observed and simulated water levels and selected pre-experiment modeling results are presented.

Characterization and Data Management at Hanford's 300-Area IFC Site

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A multidisciplinary group of researchers is using Hanford's 300-Area IFC to address knowledge gaps in multiscale mass-transfer processes in relation to uranium transport. An essential component in the interpretation of planned experiments and the development of mechanistic models for predicting future plume behavior is a detailed model of subsurface properties that reflects site heterogeneity. The main objective of this project is to develop a quantitative 3-D model of the hydrostratigraphy of the IFC site to guide experimental design and aid in refining the site conceptual model. A secondary objective is to combine historic site data into a database that can be used with mechanistic models to progressively improve the quantitative understanding of mass transfer processes as new IFC data become available. Over the past year, historic information from approximately 100 wells has been collected from diverse sources scattered among multiple scientists and organizations. Historic and more recent data include borehole geophysical logs, grain size distributions, aqueous geochemistry from water samples, water levels, weather data, topographic information, facility information and interpretations of contacts between the main hydrostratigraphic units. These data are being organized into a web-accessible, relational database that will be the primary repository for historic and new data and will become available for use by other ERSP scientists. In the past year, geophysical characterization data, including 2-D resistivity and induced polarization (IP), collected at targeted locations across the 300 Area in general, and more densely (5 m spacing) over the IFC study area, have been added to the database. The resistivity data show subsurface transitions that are in good agreement with boundaries between major hydrostratigraphic units as inferred from geologist's logs. Thus, the geophysical measurements establish a basis for interpolating lithology between boreholes, down to depths of at least 40 m. In conjunction with information from a recent borehole, these data allowed the construction of an initial stratigraphic model and the assignment of associated effective hydrogeologic properties. This model will be refined and updated with the results from the IFC well array, which will be completed in the spring of 2008 and will be fully operational by the summer of 2008.

Data Assimilation for Stochastic Site Characterization and Conditional Simulation

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We propose a framework for characterizing the Hanford site hydrogeology (using hydraulic conductivity as the main example) by a spatial stochastic model, and calibrating this model by assimilating various types of data. The framework is general, in that it aims to incorporate a broad range of relevant data resources, including direct measurements, well tests, tracer tests, geophysical survey, etc. The calibration procedure is Bayesian, so that prior information is accounted for, data that become available sequentially are routinely accommodated, and thorough analysis of prediction uncertainties is possible. Overall, the framework can be characterized as general, unifying, yet practical; Bayesian; and computation intensive. As technical details and computational challenges are being addressed, historical data for the Hanford site are incorporated to establish a preliminary parameterization consistent with existing knowledge. The model formulation as well as parameter evaluation will be updated as new observations are conducted. Eventually, this framework attempts to support numerical predictions of physical, chemical, and biological processes at the Hanford site, by providing sound representations (in the form of conditional simulations) of the hydrogeological background, and by assisting with statistical evaluations of the predictions.

Oak Ridge IFC: Research Highlights and Future Directions at the Oak Ridge Integrated Field Research Challenge

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This project is a DOE-ERSD funded Integrated Field Challenge (IFC) 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 (FRC) is being used by the IFC 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 those 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 that 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 Oak Ridge IFC resides within a climate regime that receives ~1,400 mm rainfall/y, of which 50% contributes to groundwater and surface water recharge.

The Oak Ridge IFC 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 Oak Ridge IFC 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.

Oak Ridge IFC: Natural Attenuation and Recharge Studies

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The Oak Ridge Integrated Field-Research Challenge (ORIFC) Project is utilizing a series of novel tracer tests, isotopes, well sampling, hydraulic testing, and geophysical information to quantify the rates and mechanisms of coupled processes that control natural attenuation of U, Tc, and nitrate along contaminant pathways and transition zones at the Oak Ridge Field Research Center (FRC). It is hypothesized that microbial denitrification is the only geochemical mechanism for permanently decreasing NO_3^- flux, where the denitrification rate is governed by pH and electron donor concentration. The master variable for U, and possibly Tc, attenuation is pH. Aluminum hydrolysis and the presence of carbonates are the dominant pH buffering reaction impeding U and Tc precipitation and adsorption. Our studies also focus on the spatial extent and impact of episodic and seasonal precipitation events, in order to quantify the groundwater flux drivers at the FRC—and the effects of recharge on diffusion, advection, dilution, and delivery of oxygen, dissolved organic carbon (DOC) and higher pH water, which influence coupled geochemical and microbial processes and contaminant migration. In undeveloped areas of the groundwater plume, recharge is expected to be fairly uniform, but locally impacted by differences in the permeability of geologic layers that intersect the surface, because they dip at 45°. Primary sources of preferential recharge near the S-3 Ponds are the unlined drainage ditch that surrounds the Ponds and the more permeable fill material. A perched zone exists beneath the drainage ditch in fill 1–2 m thick that becomes saturated during most of the year. The groundwater in the perched zone has high dissolved oxygen (DO), a pH of 6.5, and contaminants that include U (1.0 ppm) and nitrate (<200 ppm). As this high DO and pH perched water migrates down, it mixes and reacts with the lower pH highly contaminated groundwater in the shale/saprolite pathway, impacting contaminant mobility.

Extensive sampling is being conducted across the Bear Creek Valley Watershed to assess natural attenuation processes. We are tracking changes in gas concentrations (e.g., N_2/Ar), aqueous phase conservative natural tracers (e.g., Cl from the waste source) and changes in the isotopic composition of water, nitrate, and organic carbon electron donors ($^{15}N/^{14}N$, $^{18}O/^{16}O$, $^2H/^1H$, and $^{13}C/^{12}C$) to help quantify the dominant natural attenuation processes active at the site. As expected, pH, contaminant concentration, and recharge/dilution impact these measurements. Contaminant levels appear to have a significant impact on microbial populations and diversity determined from samples taken concurrently with the geochemical and isotope samples. Genomic DNAs were isolated from groundwater, purified, amplified with primers 27F and 1492R, and cloned to produce 16S rRNA gene libraries. Over 4,000 clones were sequenced, and data was analyzed using DOTUR. GeoChip analysis has also been conducted to determine the impact of nitrate, metal and radionuclide contamination on functional gene diversity in FRC groundwater. Preliminary results show that T-RFLP fingerprints are highly reproducible in samples from the same core depth, while depth-related variability can be large, even in samples from different depths of the same core.

Oak Ridge IFC: Subsurface pH and Oleate Manipulation for the Immobilization of Uranium

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The source zone groundwater (Area 3) at the Oak Ridge IFC site is characterized by a low pH (~3.5), but extremely high contents of U (up to 60 mg/L), Al (~500 mg/L), nitrate (up to 10,000 mg/L), and other toxic metals and ions. Few treatment options currently exist to effectively remove or immobilize U(VI) and other toxic metals *in situ* at the site. This study was performed to evaluate (1) the subsurface pH manipulation for immobilizing U(VI) through precipitation or co-precipitation with Al and other metal ions and (2) biological reduction and precipitation of U(IV) through amendments of oleate at a less contaminated site (IFC Area 2) at about neutral pH conditions.

Initial laboratory investigations indicate that a controlled addition of strong base (KOH) provided a rapid yet effective means of sequestering U(VI), Tc(VII), and other toxic metals (Ni^{2+} and Co^{2+}) in soil and groundwater at the Area 3 site. Greater than 90% of soluble U(VI) and Tc(VII) could be precipitated or immobilized at pH >4.5 through the co-precipitation with or adsorption onto Al-oxyhydroxides and minerals. The wet-chemical and spectroscopic analyses (e.g., EXAFS and FTIR) provided evidence and mechanisms of the co-precipitation and adsorption processes. Small- to intermediate-scale column flow-through experiments were performed to determine the effectiveness of controlled base additions on U(VI)/Tc(VII) immobilization in the contaminated sediment and the impact of pH manipulation on hydrogeochemical properties and microbial activity. Results confirmed that more than 92% of U(VI), Tc(VII), Co(II), and Ni(II) could be retained through the surface adsorption and co-precipitation in sediments and be stabilized by maintaining a slightly acidic pH condition. No significant changes in hydraulic properties and no sign of denitrification were observed to date.

The present study provides a scientific basis and demonstrates the technical feasibility to stabilize contaminant U(VI) and Tc(VII) through the subsurface pH manipulation, which may lead to a significantly reduced U(VI)/Tc(VII) flux at this highly contaminated site. Biological reduction of U(VI) by Ca-oleate amendment was also evaluated in batch tests using Area 2 groundwater and sediments at circumneutral pH. Many current and recently tested strategies have involved the injection of soluble low molecular weight electron donors. In contrast, the sparingly soluble Ca-oleate is hypothesized to provide a long-term source of electrons for biological U(VI) reduction. The results of initial anaerobic microcosm tests appeared promising: Amended sediments showed nearly complete removal of U from the aqueous phase. The reduction of U(VI) to U(IV) was verified by XANES analysis, demonstrating the effectiveness of oleate as an electron donor for U reduction. Deltaproteobacteria populations increased significantly compared to unamended controls as revealed through 16s rDNA analysis. A clone type showing ~98% similarity to *Desulforegula conservatrix* constituted ~30% of recovered clones in libraries originating from the oleate-amended sediment. This δ-proteobacter oxidizes long chain fatty acids, produces acetate and propionate, and reduces sulfate to sulfide. However, this organism is not known to reduce Fe(III) or U(VI). Iron reducers such as *Geobacter* spp. were also present in enrichments, but in much lower abundances (~5%).

Characterization and Monitoring at the Oak Ridge IFC Using Geophysical Methods

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Geophysical approaches are being developed and utilized at the ORNL IFC to enable: (1) watershed characterization; (2) monitoring processes associated with recharge and natural attenuation; and (3) monitoring transformations associated with subsurface manipulations.

As of February 2008, we had collected 18 surface seismic and 14 surface resistivity regional lines for the *Watershed Characterization effort*, as well as shorter lines to infill and enhance the regional grid. A variety of methods have been used to ground-truth the geophysical measurements, including coring, driving in pushprobes, installing and sampling wells, and conducting flowmeter tests. Early results indicate that the total dissolved solids are high downgradient (nitrate ~4900 ppm), in a region that corresponds with a high electrical conductivity signature observed on the surface electrical lines. The surface seismic datasets also indicate the presence of a regionally extensive anomaly approximately parallel to geologic strike; drilling is in progress to explore the hydrogeological-geochemical properties associated with this anomaly and its implications for regional flow. We are developing a Bayesian approach to systematically integrate the multiscale wellbore, crosshole, and surface datasets to provide the spatial distribution of lithofacies important to flow and their associated uncertainty, where the refraction travel times and depth of refusal of push probes are used as initial input.

We are performing modeling and time-lapse surface refraction studies near the ORNL S-3 ponds to advance the *Monitoring of Processes Associated with Recharge and Natural Attenuation*, with intent to use geophysical methods to gain insight into recharge-TDS-pH variations over field-relevant scales. To better understand potential error associated with using time-lapse seismic surveys for recharge monitoring, we have investigated temporal variability in frequency and amplitude of the geophone responses, using multiple strategies in geophone placement over time, with an emphasis on periods immediately preceding and following both major and minor precipitation events. These studies indicate a correlation between precipitation events and variability in geophone response, as expected. We have also developed a coupled, local-scale hydrological-geophysical modeling framework for integrating disparate datasets and for exploring the impact of recharge on TDS. With the developed framework and the site-specific petrophysical models, we will synthetically explore the sensitivities of the electrical resistance tomographic datasets to variations in lithology versus recharge-associated TDS variations.

Finally, we are exploring the potential of geophysical methods for *Monitoring of Manipulation Transformations*, with a particular focus on exploring geophysical responses to the evolution of precipitates associated with pH adjustments aimed at immobilizing U. We have developed specialized flow-through experimental cells that permit synchronous measurement of seismic *P*-wave velocity, complex resistivity, and TDR measurements at the column scale, and hard x-ray microtomography imaging at the synchrotron scale. The synchrotron-to-column scale information will be used in conjunction with crosshole-scale datasets to explore precipitate evolution impacts on geophysical responses at a variety of scales. Ultimately, these studies will be used to address the potential of the geophysical methods for *in situ,* field scale, remote monitoring of precipitate evolution as an indicator of U immobilization associated with pH adjustment.

Reoxidation of Bioreduced Uranium by Dissolved Oxygen and Nitrate and Restoration of Reduced Conditions at Area 3, IFC Site, Oak Ridge, TN

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The research objective is to study enhanced contaminant stability strategies for source control. The work was performed in a bioreduced zone, Area 3, DOE ERSD IFC site, Oak Ridge, TN.

In situ bioremediation of a highly uranium-contaminated aquifer was conducted by controlling groundwater flow and stimulating microbial growth through weekly injection of ethanol. Aqueous U(VI) fell below the US EPA drinking water limit (<30 µg/L). The bioreduced U(IV) was stable under anaerobic conditions. The impact of dissolved oxygen (DO) and nitrate on stability of bioreduced U(IV) in the subsurface was examined *in situ*. Introduction of DO (4.0–5.5 mg/L) through injection of oxygenated water to the subsurface caused reoxidation and remobilization of uranium. After DO was introduced, sulfide and Fe (II) concentrations gradually decreased to near zero in the area close to injection wells with increased U concentration. The extent of remobilization of U was observed to be higher in wells that were closer to the injection well.

After exposure to DO for more than 60 days, resumption of ethanol injection restored Fe(III), sulfate, and U(VI) reduction within 1.5 days. As ethanol injection, continued U concentration again fell below 30 µg/L. When control of groundwater flow was suspended, nitrate-contaminated groundwater infiltrated to the reduced area and caused a rebound of U concentration. The penetration of outside contaminated groundwater was confirmed by tracer tests with bromide. Nitrate (>2 mM) was then injected to the bioreduced subsurface, together with a bromide tracer. This caused observation of nitrite and ammonia with a decrease in sulfide and Fe (II) to zero. Reoxidation and remobilization of uranium was observed. Resumption of ethanol injection after nitrate exposure for 6 days showed sequential restored reactions of denitrification, Fe (III)-, sulfate-, and U (VI) reduction. U (VI) reduction occurred as sulfide began accumulation. The reoxidized subsurface was restored to anaerobic conditions, with U below 30 µg/L through ethanol injection. Bacteria known to reduce U (VI)—including *Desulfovibrio* spp., *Desulfosporosinus* spp., *Anaeromyxobacter* spp., and *Geobacter* spp—were found. *Geothrix* spp. was a dominant Fe(III)-reducer, and Fe(II)-oxidizing *Thiobacillus* was also present in groundwater and sediments. XANES analysis of uranium speciation confirmed the changes in U(VI)/U(IV) ratio before and after nitrate reoxidation tests.

Multiprocess and Multiscale Modeling and Data Analysis
at the IFC Site, Oak Ridge, TN

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Saprolite cores collected from around the former S-3 Ponds waste disposal site on the Oak Ridge Reservation in east Tennessee, USA, exhibit low pH and high concentrations of Al, Ca, Mg, and Mn, as well as various trace metals (e.g., Ni and Co) and radionuclides (e.g., U and Tc). Because uranium is one of the major contaminants of concern at the site, its behavior was of particular interest. The mobility of uranium depends highly on pH. Groundwater titration experiments showed that when pH was increased from 3.87 to 5.45 with addition of dissolved sodium hydroxide, concentration of aqueous uranium decreased from 50 ppm to less than 5 ppm. Pretreatment of groundwater (such as with base titration) was also found to be necessary to increase the groundwater pH and reduce the levels of some toxic metals for bioremediation. However, base additions to the sediments to increase pH are strongly buffered by various precipitation and sorption reactions. Therefore, batch, column, and field experiments have been carried out to investigate the geochemical processes that control contaminant mobility and bioremediation. Multiprocess and multiscale modeling and data analysis has been performed to develop a practical model to predict uranium immobilization under conditions where pH is manipulated for remediation of geochemically complex sites.

The method of Spalding and Spalding was used to model soil buffer capacity by treating aquifer solids as an insoluble polyprotic acid. Aluminum precipitation and dissolution kinetics was included in HydroGeoChem v5.0 (HGC5) in addition to an equilibrium reaction model that considers aqueous complexation, precipitation, sorption, and soil buffering with pH-dependent ion exchange capacity. The model successfully simulated batch titration experiments, and comparison of reactive transport model results with pH manipulation column experiments were in close agreement. A travel-time-based reactive transport model was developed to simulate an *in situ* bioremediation experiment for demonstrating enhanced bioreduction of U(VI), considering microbial reduction of nitrate, sulfate, and U(VI). A local-scale model was developed for Area 3 to facilitate interpretation of flow and tracer studies in the vicinity of well FW106. Reaction models developed through simulation of batch, column, and field experiments will be incorporated into the local-area 3 model to help design future on site investigation. Work was undertaken to develop a site-wide model encompassing an area that includes Oak Ridge Field Research Center (FRC) Areas 1, 2 and 3, the former S3 Ponds and the Bear Creek watershed from its head waters to the tributary NT2. Ultimately, all the findings obtained through multiscale lab and modeling studies will be integrated into the FRC site-wide model for comprehensive and generic prediction.

The Integrated Field-Scale Subsurface Research Challenge Site (IFC) at Rifle, Colorado: Preliminary Results on Microbiological, Geochemical and Hydrologic Processes Controlling Iron Reduction and Uranium Mobility

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Research at the Integrated Field-Scale Subsurface Research Challenge Site (IFC) at Rifle, CO, addresses knowledge gaps in (a) geochemical and microbial controls on stimulated U(VI) bioreduction, (b) U(VI) sorption under reducing conditions, (c) post-biostimulation U(VI) stability and removal, and (d) rates of natural U(VI) bioreduction. The overall goal of the project is to develop a mechanistic understanding of the hydrological, microbiological, and geochemical processes controlling uranium mobility, targeting new knowledge that can be translated into scientifically defensible flow and reactive transport process models. Work at the Rifle IFC is focused on field and laboratory experiments that utilize novel proteogenomic, stable isotope probing, and biogeophysical monitoring techniques to link microbial metabolic status and biogeochemical processes controlling uranium removal during acetate amendment.

Fieldwork during the summer of 2007 validated sampling approaches for recovering sufficient biomass from acetate-amended groundwater to enable community genomic and proteomic analyses. The experiment replicated dominance by *Geobacter* sp. during acetate amendment, accompanied by significantly lowered uranium groundwater concentrations (<0.137 µM) below the regulatory standard. Additional research assessed the impact of electron donor limitation on the metabolic state of *Geobacter* sp. and tested biogeophysical approaches for monitoring their *in situ* activity. Proteomic analysis of the recovered biomass revealed dominance by members of the Geobacteraceae, with large numbers of detected proteins associated with cell-cycle metabolism, chemotaxis, and element cycling. Strain-resolved analysis suggested that strain-variant *Geobacter* species, closely related to *G.* M21 and *G. bemidjiensis*, were active during acetate amendment. Understanding the role that these strain-variant species play in the microbial community will assist with further interpretation of the community proteomic dataset.

The experiment also confirmed the importance of heterogeneities in controlling the flux of electron donor and the impact of naturally reduced zones on the duration of Fe reduction. The initial Rifle IFC experiment provides the basis for future experiments for the project, and suggests that we will successfully meet the project goal of predicting the response of redox-sensitive metal contaminants to *in situ* biostimulation of aquifer systems. In addition, we are using the microbiological, hydrogeophysical, geochemical, and mineralogical data obtained to develop numerical models that will enable accurate prediction of both natural attenuation and biostimulated conditions in alluvial aquifers.

Microbial, Geochemical, and Physical Responses to Biostimulation in Rifle IFC Soil Column Experiments

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During the biostimulation of microbial iron reduction for the purpose of U(VI) removal, it has been postulated that iron reduction proceeds so long as bioavailable iron remains, after which sulfate reduction dominates. Although field experiments at the Rifle Integrated Field Challenge (IFC) site have linked the onset of sulfate reduction to decreased rates of U(VI) removal from groundwater, column experiments using site sediments and synthetic groundwater (7mM sulfate) have shown sulfate reduction to have no adverse effect on U(VI) removal. Similar experiments using low sulfate (9 µM) groundwater indicate sufficient Fe(III) to sustain iron reduction in excess of 200 days. Prolonged iron reduction through electron donor limitation has also been demonstrated using a set of low (500µM) acetate column experiments. While effective for prolonging iron reduction (>110 days), this approach results in only limited decreases (~40%) in effluent U(VI). Concurrent sulfate reduction has also been documented during this time. These results suggest that acetate concentrations may play an important role in promoting significant U(VI) removal (<0.13µM) from groundwater.

To address the discrepancy between field and column experiments, we are assessing the activity of iron reducers before and after sulfate reduction using Mössbauer spectroscopy and columns packed with Rifle sediments supplemented with ^{57}Fe goethite. Site groundwater, amended with 3 mM acetate and 20 µM U(VI), is pumped through the columns at a rate of 0.035 mL/min, with effluent concentrations of acetate, Fe(II), U(VI), and sulfate monitored at regular intervals. Columns are sacrificed at 10-day intervals, and the sediment samples are analyzed for Fe(II), U(IV), and acid volatile sulfides using standard analytical procedures. Changes in ^{57}Fe goethite accompanying biostimulation and measured using Mössbauer spectroscopy at 10-day intervals have indicated measurable decreases in goethite concentration.

The columns begin with highly similar bacterial populations at three locations along their length (85% similarity). After 60 days, slight differences emerge between the locations, with the similarity dropping to 65%. The microbial population is dominated by six distinct TRFLP peaks [187, 208, 213, 235, 277, and 292 bp] obtained using the restriction enzyme *MnlI*. These peaks represent 70±7% of the overall community profile. Three of the peaks have been identified, a *Desulfovibrio*-like clone (208 bp), a *Rhodoferax*-like clone (213 bp), and a *Geobacter*-like clone (187 bp), totaling 14.5, 26, and 3.1% of the overall community profile, respectively. Fingerprinting of intact ribosomes indicated that the 208, 123, and 235 bp peaks have the highest amount of RNA, suggesting that these microorganisms grow more rapidly than others in the columns. Digestion with a second restriction enzyme (*AluI*) indicates that these peaks represent a single bacterial species. An ordination analysis is being performed to link changes in the microbiology to changes in the geochemistry.

Environmental Proteomic Analysis of Microbial Communities at the Rifle Integrated Field Challenge Site

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Strain-resolved meta-proteomic analysis of environmental microbial communities has the potential to offer unique insights into such communities' complex structure and function. The aim of this study was to use meta-proteomic techniques to gain a better understanding of the microbial community during the stimulated bioreduction of U(VI) in a subsurface aquifer at the Rifle Integrated Field Challenge (IFC) site near Rifle, CO. During the course of the bioreduction experiment, 500 L groundwater samples were passed through a tangential flow filtration system, before the biomass rich product was flash-frozen and sent to Oak Ridge National Laboratory and Pacific Northwest National Laboratory for analysis. Three samples were taken in total from two different wells, all during a two-week period of stimulated Fe(III)- and U(VI)-reduction in the subsurface.

LC-MS/MS data was initially searched against all sequenced bacteria, and revealed that members of the *Geobacteraceae* dominated all the samples, with between three- and four-thousand proteins identified per sample. Subsequent analysis was therefore performed against seven *Geobacter* genomes. A broad spectrum of proteins have been identified, including those involved in core-metabolic processes, such as the citric-acid cycle and amino-acid biosynthesis. Not surprisingly, proteins associated with motility (flagella, chemotaxis) are abundant; *Geobacter* species respire on insoluble Fe(III) minerals, leading to the dissolution of these phases. Thus, these species must move to fresh electron-accepting minerals to survive. Mechanisms for obtaining growth-limiting elements in the nutrient-poor subsurface can also be detected in the proteomic dataset. A number of proteins involved in nitrogen cycling and fixation are present in the samples, along with sulfur cycling proteins and phosphate uptake mechanisms.

While identifying proteins gives some insight into the subsurface *Geobacter* community, it is also important to understand how strain variation influences the interpretation of proteomic data. To achieve this, we are currently utilizing a novel "peptide mapping" approach. This technique allows more rigorous comparison of data between the three sample sets. Initial results suggest that while peptides matching *G. bemidjiensis* and *G. M21* are most abundant in the three samples, co-existing unique peptides matching *G. uraniumreducens*, *G. metallireducens* and *G. sulfurreducens* are present at lower quantities, indicating that at least two different *Geobacter* strains are present. By comparing the ratio of conserved, non-unique peptide regions between samples, protein abundance can be deduced. Conversely, the ratio of co-existing unique peptide sequences within a single sample can be used to identify the ratio of *Geobacter* strains present in a sample. By using these two sets of data, a more complete understanding of the *Geobacter* community function and structure during periods of optimal uranium bioremediation can be obtained.

Geophysical and Electrode-Based Approaches for Monitoring the *In situ* Activity of *Geobacter* Species at the Rifle Integrated Field Challenge Site

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Experiments at the Rifle Integrated Field Challenge (IFC) site near Rifle, CO, have demonstrated the ability to remove uranium from groundwater by stimulating the growth and activity of *Geobacter* species through acetate amendment. Prolonging the activity of these strains in order to optimize uranium bioremediation has prompted the development of monitoring methods diagnostic of their *in situ* activity. We have addressed this issue through the development of two novel methodological approaches.

The first is an electrode-based sensor patterned after microbial fuel cells. We hypothesized that subsurface electrodes could be used to monitor rates of acetate oxidation, as evidenced by electron transfer to electrodes by anodophilic microbes, such as *Geobacter* species. During a recent field experiment, graphite electrodes serving as anodes were installed in boreholes downgradient from a region of acetate injection. Cathodes consisted of air-coupled graphite electrodes embedded at the ground surface and connected to the borehole electrodes via 560-ohm resistors. Downgradient increases in current density tracked the delivery of acetate to the electrodes, whereas no change in current density was observed at an upgradient control; the increases were not correlated with the accumulation of Fe(II) or dissolved sulfide. Confocal laser microscopy of a subsurface electrode recovered during peak current density revealed a firmly attached biofilm; microbial community analysis of the same electrode detected sequences dominated by *Geobacter* strains (67%), along with a lower percentage mixture of gamma, beta, and alpha-proteobacteria and firmicutes.

The second approach extends our work in using spectral induced polarization (SIP) measurements to monitor geochemical and mineralogical changes accompanying stimulated iron and sulfate reduction. SIP monitoring during a recent field experiment targeting iron reduction revealed a spatiotemporally anomalous phase response, which correlated with increases in both Fe(II) and dominance by *Geobacter* strains (>90%) in filtered groundwater samples. The magnitude of the phase response was positively correlated with frequency, consistent with our previous predictions. The spectral response accompanying iron reduction was validated using a biostimulated column experiment containing Rifle sediments and groundwater. Analysis of both the field and laboratory datasets indicates that the accumulation of electroactive ions, specifically Fe(II), alters the ability of pore fluids to conduct electrical charge, thereby generating the phase effect. This mechanism likely underlies the anomalous baseline phase response observed in the vicinity of naturally occurring bioreduced sediments, suggesting a means for characterizing the ubiquity of such zones across the Rifle floodplain.

Scientific Focus Areas (SFA)

Subsurface Science Scientific Focus Area at Lawrence Berkeley National Laboratory

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A Subsurface Science Scientific Focus Area (Subsurface Science SFA) program has been developed at LBNL to address critical knowledge gaps associated with environmental remediation of metals and radionuclides in the subsurface. As described by the oral and poster presentations, we propose to conduct fundamental studies within the following two *Scientific Research Areas*, each of which addresses a grand challenge and has a unique objective:

- **Area 1: Sustainable Systems Biogeochemistry (SSB).** The objective of the research conducted in the SSB Area is to improve and quantify our understanding of biogeochemical processes as needed to design scientifically defensible and *sustainable* remediation strategies. Research in the SSB Area will be conducted under two theme-based nodes. The *Sustainable Remediation Strategies* node will focus on understanding processes and on developing specific approaches relevant to sustainable engineered remediation and natural attenuation of subsurface Cr-, U-, ^{129}I-, and ^{90}Sr-contaminants. The *Molecular Signatures* node aims to facilitate discovery of genes associated with bioremediation-relevant microbial activities, create a high-throughput approach to assess the *in situ* expression of these genes, and determine whether there are reliable quantitative correlations between key genes (i.e., their presence or expression) and bioremediation-relevant metabolic activity.

- **Area 2: Integrated Characterization, Modeling, and Monitoring (CMM).** The objective of the CMM Research Area is to improve characterization, modeling, and monitoring approaches as needed to quantify and predict subsurface processes associated with engineered remediation and natural attenuation strategies. Research in the CMM Area will be conducted under four approach-based nodes: *Synchrotron Science*, *Isotope Geochemistry*, *Environmental Geophysics*, and *Reactive Transport Modeling*. Projects within the CMM Area will tackle challenges unique to each approach, improving and advancing the individual techniques. Additionally, a major objective of CMM research is to test and document the synergies that come from integration of multiple approaches, which will lead to the development of protocols for optimized CMM.

We propose that much of the research conducted under the two *Scientific Research Areas* be integrated at three key *Field Study Sites*. This will enable: field-testing of developed methods; integration across research nodes; collaboration between LBNL researchers and those at other institutions; and leveraging with EM and ERSP investments. Since the environmental challenges and contaminants at each of the sites are distinct from one another, the integrated approach will enable investigation of a wide range of problems relevant to DOE.

- At the *Hanford 100 Area,* we propose to explore enhanced sustainability of Cr(VI) bioremediation by determining the biogeochemistry necessary to establish a resilient biomass that optimally recycles cells as they die, a strategy called "cryptic growth." The Hanford 100 Area is part of the Columbia River Corridor, which has been chosen by DOE Environmental Management as a designated field study site. Research at the Hanford 100 Area will be performed in close collaboration with Genomics: GTL, PNNL, and Fluor Hanford investigators.

- At the *Savannah River Site F-Area,* we propose to develop a scientific basis for monitored natural attenuation (MNA). We hypothesize that understanding the spatio-temporal evolution and migration of geochemical gradients associated with the acidic groundwater plume is key to whether MNA is a viable remediation strategy at the site. We propose to establish fundamental criteria for characterizing and predicting the spatiotemporal evolution of geochemical gradients, and to ascertain the impact of the gradients on ^{90}Sr, ^{129}I, and U mobility in the naturally heterogeneous subsurface. The F-Area is also a designated DOE Environmental Management field site and this work will be performed in close collaboration with EM-supported scientists at SRNL.

- At the *Rifle Site*, we propose to work in close collaboration with the ERSP-supported Rifle Integrated Field Challenge (IFC) team, who are exploring biogeochemical processes controlling uranium mobility. The LBNL effort will complement the IFC research by developing and testing characterization and reactive transport modeling approaches that will establish the impact of heterogeneity on critical processes, and the feedbacks between flow characteristics and biogeochemical transformations.

The proposed program is scientifically rich and has potential for broad impact at DOE sites undergoing cleanup, closure, and stewardship activities.

Oak Ridge Scientific Focus Area: Biogeochemical and Molecular Mechanisms Controlling Contaminant Transformation in the Environment

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The ORNL Science Focus Area (SFA) Program addresses the DOE's Environmental Remediation Science Program (ERSP) need for improved understanding of scientific issues that limit contaminant remediation at the Oak Ridge Reservation (ORR). Over the next 5–10 years, ORNL's SFA will address significant knowledge gaps regarding biogeochemical transformations that determine uranium (U) subsurface mobility and mercury (Hg) toxicity. The goal is to achieve detailed understanding of fundamental mechanisms with results that have general applications to DOE and other contaminated sites nationwide.

Microbial processes control biogeochemical cycling and are fundamental to reduction-oxidation transformations that determine the mobility and toxicity of U and Hg in ground and surface waters. Details of the electron transfer processes that determine U reduction-oxidation by microbes are poorly understood. The program will focus on identifying molecular mechanisms involved in biological metal reduction in general, and U in particular, by examination of the structures and interactions of key cytochrome proteins directly involved in the electron-transfer pathways. This study is designed to elucidate subcellular processes using new tools in genomics and proteomics developed in the GTL program, and to complement molecular-scale studies under way in other ERSD SFA programs (e.g., PNNL). In addition, the roles and mechanisms of metal oxidizing bacteria in U(IV) oxidation will be studied and aligned with the Integrated Field Challenge project at ORNL.

Methyl mercury (MeHg) production at contaminated sites presents a serious knowledge gap at Oak Ridge Reservation and across the DOE complex. ORR field data show that reduction in total dissolved Hg does not necessarily lead to a decrease in aqueous MeHg or to reduced bioaccumulation in fish, suggesting that factors other than total Hg in water are rate limiting. The ORNL SFA program will therefore focus on fundamental understanding of processes that control Hg speciation and methylation, and on determining the effects of environmental variables on rates of methylation and demethylation in controlled microcosms and laboratory experimentation, using advanced chemical, spectroscopic and stable isotope techniques. Mercury redox transformation, speciation, and bioavailability for Hg methylation will be examined. Functional genomics will be used to determine key microbial groups that influence MeHg production under varying geochemical conditions. Molecular simulation will be applied to known demethylation processes to provide an atomic-level understanding of key reaction pathways, and will be extended to elucidate methylation mechanisms based on recognition of field microbes using advanced genomic techniques. Both conceptual and numerical models will be used at appropriate scales to aid interpretation of reaction mechanisms.

This research, in collaboration with other ERSD investigators, is designed to make fundamental contributions to the prediction and mitigation of Hg at contaminated sites. The program will benefit from external expertise and other national user facilities (e.g., EMSL and APS) to complement existing personnel and capabilities.

SSRL Environmental Remediation Sciences Program

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The success of natural attenuation and *in situ* remediation strategies depends upon a complex interplay of dynamic molecular-scale biogeochemical processes in the subsurface. Our understanding of these processes is typically poor. Numerous knowledge gaps exist that present significant barriers to developing remediation approaches required for site closure.

The SSRL Environmental Remediation Science Program will investigate the structures and dynamics of important natural solids and the fundamental biogeochemical processes that control the stability and fate of priority metal contaminants in the subsurface. A central hypothesis that will guide this work is: *The chemical dynamics of complex environmental solids (e.g., UO_2, PuO_2, and $Fe(II,III)(hydr-)oxides$) and their capacities to perform as long-term sinks for priority contaminants are governed by their molecular- and nanoscale structures and compositions, which in turn are strongly influenced by reaction with groundwater solutes.* These complex environmental solids are often nanoparticulate, and surface structure/dynamics may profoundly influence their bulk energetics and adsorptive capacities. Natural organic matter and microbial biofilm coatings are common and are believed to mediate the reactivity of environmental particles. Therefore, the general hypothesis regarding structure/dynamics must be extended to account for nanoparticle surface structure and the effect of sorbed (organic or inorganic) molecules or surface coatings. Major gaps exist in our knowledge of these important natural materials.

This project will elucidate the fundamental relationships between the molecular-scale structures and dynamics of important environmental solids. In particular, we will focus on UO_2, because of its importance to natural attenuation and stimulated *in situ* subsurface remediation strategies, and on $Fe(II,III)(hydr-)oxides$, because of their importance as reactive and enigmatic facilitators of biogeochemical processes and as contaminant sinks. A combination of synchrotron, aqueous chemical, radioanalytical, and microscopic techniques will be used to provide insights into the environmental behavior of these materials—the mechanisms by which contaminants may be redistributed or immobilized within/onto these solids during redox cycling—and to improve their stability and yield in the subsurface during natural attenuation or stimulated bioreduction treatments. Other priority contaminants that will be investigated include: Pu, Cr, Sr, Pb, and As. This work will provide fundamental knowledge relevant to remediation and risk assessment at the major DOE contamination sites. The SSRL SFA will also support site-specific molecular-scale contaminant characterization, transport, and remediation research at the Hanford, Rifle, Yucca Mountain, and Fry Canyon, Utah sites. The long-term objective of this program is to contribute enduring qualitative and quantitative knowledge of key subsurface biogeochemical processes governing the subsurface behavior of these contaminants—knowledge that helps gain public and regulatory acceptance of nuclear legacy site remediation and closure.

Subsurface Immobilization of Metal Contaminants by Amendment-Driven Mineral Precipitation

George Redden (PI)

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Addressing subsurface radioactive or toxic metal contaminants is a challenge at sites across the Department of Energy (DOE) complex. One example is at the Idaho Nuclear Technology and Engineering Center (INTEC) facility at Idaho National Laboratory (INL), where ^{90}Sr has contaminated the underlying vadose zone and poses threats to the regional aquifer. Viable remediation methods are needed to immobilize the ^{90}Sr if transport exceeds current estimates, and to reduce programmatic risk and uncertainty. Formation of mineral phases *in situ* can immobilize metal contaminants through contaminant precipitation and co-precipitation or contaminant encapsulation and/or hydraulic isolation. The spatial and temporal distributions of mineral nucleation and growth in the subsurface, and the impacts of precipitates on the physical and chemical state of contaminant metals, depend on how multiple reactants are transported and mixed in complex heterogeneous systems. Field-scale applications also require the capability to accurately predict and sense the occurrence and distribution of the amendment-stimulated reactions.

The objective of the INL Subsurface Science Scientific Focus Area (SFA) research program is to advance the scientific understanding required for the development and application of field-scale *in situ* immobilization strategies for subsurface metal contaminants. Two principal hypotheses form the basis for the INL SFA research program plan:

- The formation and distribution of mineral precipitates in heterogeneous subsurface matrices, and the subsequent impacts on metal contaminant mobility, can be predicted from knowledge of the spatial and temporal structure of mixing zones between mineral-forming reactants.
- Changes in macroscopic properties of subsurface systems due to mineral precipitation can be detected and quantified by measuring changes in electrical properties of the media.

These and focused supporting hypotheses will be addressed using experimental campaigns integrated with research on basic biogeochemical processes, field-applicable sensing methods, and computer simulations and enhancements to existing numerical models. The ^{90}Sr contamination at INTEC will be used as the primary main-case study for the research program, although the results are expected to have general application to subsurface remediation needs throughout the DOE complex. Calcium carbonate and phosphate minerals will be the model-precipitated phases in the experiments, and ^{90}Sr will serve as the model target contaminant. The four research elements of the INL SFA plan are:

I. **Transport/Precipitation Coupling in Porous Media:** Spatial and temporal distributions of precipitates, and impacts on contaminant mobility, will be determined as functions of amendment delivery and media properties in physical experiments.
II. **Basic Processes and Parameters:** The focus will be on determining governing relationships and parameters for conditions relevant to the experiments and to the field.
III. **Model Application, Validation and Improvement:** Predictions for amendment transport, mixing, precipitation, and physical property changes will support experiment design.
IV. **Sensing Methods:** Methods to infer biogeochemical processes from geophysical signatures will be developed and tested using experiments and computer simulations.

Los Alamos Science Focus Area: Pu/Actinides in the Environment

Principal PIs: Amr Abdel-Fattah, Hakim Boukhalfa, Peter Lichtner, Donald Reed, Robert Roback, Paul Reimus, Michael H. Ebinger (POC).

LANL Collaborators: Lia Brodnax, David Clark, Mei Ding, Yixang, Duan, David Janecky, Ceryl Kuske, Wolfgang Runde, and Stephen Stout.

University and National Laboratory Collaborators: John Bargar (SSRL), Larry Hull, George Redden (INL), Bruce Rittman (Arizona State Univ.), Peter Santschi (Texas A&M), and Powell Weronski (Inst. of Catalysis and Surface Chemistry, Polish Academy of Science)

The stability and long-term behavior of subsurface actinide contaminants at DOE sites are determined by coupled physical, geochemical, and biological processes. The coupling of these processes is particularly important for plutonium, due to its complicated redox behavior and rich environmental chemistry. Multiple plutonium species can form inorganic and organic complexes of Pu(III, VI, V, and IV), suspended colloids, polymers and psuedocolloids, mineral adsorbates, and a wide variety of solubility-controlling solids. The overall objective of the Los Alamos team is to evaluate the processes, mechanisms, and geochemical conditions that control Pu/actinide transformation and movement in the subsurface, and apply this information to predict Pu/actinide transport under different remediation scenarios. Our approach is to evaluate the various processes, mechanisms, and geochemical conditions that control Pu/actinide speciation and mobility in the subsurface, then use this evaluation to inform a modeling approach for applying the biogeochemical information to predict Pu/actinide fate and transport. The proposed work will identify and fill significant knowledge gaps in the scientific basis for monitored natural attenuation and *in situ* stabilization of contaminants, and will provide immediately useful data to modeling and risk-assessment efforts. We propose a combination of aqueous chemical, radioanalytical, spectroscopic, microscopic, and x-ray-based analyses to characterize Pu speciation and solution/solid phase distributions. Colloid formation, characterization, and transport experiments will be conducted simultaneously. Geochemical models and semi-quantitative transport models will be used to help interpret experimental data. Experiments will be conducted with laboratory-produced materials to constrain experimental conditions, as well as with site-specific contaminated materials to provide complex and realistic biogeochemical conditions. Finally, field experiments will be proposed to test hypotheses and models under full-complexity, upscaled field conditions.

Subsurface Immobilization of Plutonium

Donald T. Reed (PI)

Los Alamos Science Focus Area, Los Alamos National Laboratory, Los Alamos, NM

A concurrent experimental and modeling study is being conducted that centers on key subsurface processes that lead to plutonium immobilization. Plutonium is the near-surface contaminant of concern at several DOE sites and continues to be the contaminant of concern for the permanent disposal of nuclear waste. Our project goal is to understand the long-term stability of environmentally precipitated "immobilized" plutonium phases under changing redox conditions in biologically active systems. This research is, in part, done in collaboration with Professor Bruce Rittmann at Arizona State University. Our hypothesis is that stable plutonium phases will prevail when anoxic and reducing subsurface conditions persist.

There are several factors, under DOE-relevant conditions, that contribute to establishing the overall stability and immobility of precipitated plutonium phases. The most important of these are microbial processes, the reactivity of the reduced metals/phases that are present, inorganic/organic complexation, and the tendencies of all of these processes towards the formation of aggregates/colloids. These key processes are usually coupled and site-specific, leading to a very complex environmental chemistry that is difficult to predict with our current level of understanding. Our experimental and modeling focus, in this context, reflects the coupling of microbial and geochemical processes under these DOE-relevant conditions.

Experimentally, we have made significant progress in establishing the key abiotic and biotic interactions that lead to the reduction of higher-valent plutonium. The reduction of plutonium (V/VI) in biologically active systems has been demonstrated (metal reducers, halophiles, and by analogy sulfate-reducing bacteria and methanogens). Higher-valent plutonium can only persist as the $Pu(V)O_2^+$ species in biologically active systems, due to the abiotic reduction of $Pu(VI)O_2^{2+}$ by organic species typically present as transient electron donors in these systems. PuO_2^+ forms weak aqueous complexes and is very bioavailable in these systems, leading to relatively high toxicity and relatively low (typically less than 10%) adsorption onto biomass. Both direct enzymatic and indirect reduction of PuO_2^+ was observed—in some cases leading to the prevalence of Pu(III) species. In parallel, abiotic reduction of higher valent plutonium by aqueous Fe^{2+} and Fe(II/0) phases was established in near-neutral systems, and we have started investigating the key role that phosphate plays in the formation of highly immobilized plutonium phases. This research will be expanded to explore the possible role of Pu(III) phases in these coupled subsurface immobilization processes.

Modeling activities have centered on upgrading the CCBATCH biogeochemical model to include Pu and U speciation data. We upgraded CCBATCH to allow selected complexes of Fe^{3+} as primary electron acceptors and completed the experiments with different ratios of Fe^{3+} and NTA. The bioavailable form of Fe^{3+}, as well as of U and Pu, will be determined by matching the simulated results to the experimental one. Future work will involve predicting the conditions under which Pu precipitate phases will prevail and their stability under a long-term, continuous transport system.

Student Presentations

Targeting the Metabolically Active Iron(III)- and Sulfate-Reducing Bacteria with a High Potential for U(VI) Bioimmobilization in Contaminated Subsurface Sediments

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Uranium bioremediation at the source zone of contamination is currently focused towards stimulating indigenous subsurface microbial communities to bioimmobilize U(VI) by reducing it to U(IV). Ethanol and acetate are the primary electron donors that have been shown in previous bioremediation studies at field sites to promote U(VI) reduction. The objective of this study was to elucidate the sulfate- and/or Fe(III)-reducing bacteria that mediate U(VI) reduction in the presence of commonly used electron donors in Area 2 sediments from the Oak Ridge Field Research Center (FRC) in Oak Ridge, TN. Sediment microcosms were amended with either ^{13}C-ethanol or ^{13}C-acetate and monitored for electron acceptor (SO_4^{2-}, Fe(III) and U(VI)) and donor utilization. At time points corresponding to phases of terminal electron accepting processes, *gltA* and *dsrA* transcripts were quantified and ^{13}C incorporation into community DNA was examined by density gradient centrifugation, along with PCR amplification and terminal restriction fragment length polymorphism (TRFLP) analysis. Iron(III) reduction rates were similar in all treatments. Sulfate reduction rates were most rapid in the ethanol-amended treatments, whereas little to no sulfate reduction occurred in the acetate or molybdate treatments. U(VI) concentrations initially decreased in all treatments, but increased with the onset of iron reduction. In ethanol-amended treatments U(VI) was reduced concurrently with sulfate, whereas U(VI) reduction occurred independently in acetate-amended microcosms. Levels of *dsrA* and *gltA* transcripts were indicative of sulfate and Fe(III) reduction activity, respectively, and may be responding to electron acceptor availability. Our results suggest that the activity of sulfate- and iron-reducing bacteria is important to the fate of U(VI), and that the role of these microbial groups in U(VI) bioimmobilization is electron-donor dependent. It is likely that sulfate-reducing bacteria play a more important role with ethanol as an electron donor, and microbial community analysis is ongoing to assess the composition of the metabolically active community.

Biomineralization of Uranium Phosphate from Contaminated Waste Sites

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Our research focuses on the remediation of uranium from contaminated waste sites, such as the DOE Oak Ridge Field Research Center (FRC), via the biomineralization of soluble U(VI) in aerobic conditions. Biomineralization, or the precipitation of U(VI) minerals facilitated by the enzymatic activities of micro-organisms in oxic conditions, offers an alternative to the more extensively studied bioreduction. Our previous work has identified two aerobic heterotrophic bacteria (*Rahnella* sp. Y9602 and *Bacillus* sp. Y92) isolated from a uranium-contaminated subsurface at the FRC with enhanced phosphatase activity. These bacterial strains were incubated in simulated groundwater with 15 mM nitrate in the presence of an organophosphate substrate (glycerol-3-phosphate or G3P) and 200 µM UO_2^{2+} in the pH range 4.5 to 7.0. Sufficient G3P was hydrolyzed to precipitate 73 to 95% total uranium. EXAFS spectra identified the solid phase formed as an autunite/meta-autunite group mineral. The results of our previous work have shown the potential for uranium biomineralization as a possible remediation strategy in contaminated areas where high concentrations of nitrate and calcium in the subsurface, along with aerobic and acidic conditions, can hinder the bioreduction of U(VI) to the insoluble uraninite.

The objective of our present work is to simulate uranium biomineralization in contaminated FRC soils to determine its potential application to in situ bioremediation. Plug flow reactors (PFR) loaded with FRC Area 3 soil were utilized to investigate if: (1) oxic conditions could be maintained in inoculated soil columns, (2) indigenous FRC soil bacteria exhibited phosphatase activity when stimulated with an organophosphate, and (3) sufficient phosphate was produced to precipitate substantial soluble U(VI). Triplicate PFRs (12 cm length) were filled with homogenized sediment from Area 3. Aerated simulated groundwater (pH 5.5) amended with 15 mM nitrate as the sole nitrogen source and 20 mM bromide (tracer) was pumped through the columns at a flow rate of 1.4 mL/hr for one week. G3P (10 mM) was then added to the influent as the sole carbon and phosphorus source to monitor phosphatase activity, while UO_2^{2+} (200 µM) was added on Day 10. Effluent oxygen and other reduced species involved in remineralization processes (i.e., Fe^{2+}, Mn^{2+}, H_2S) were monitored voltammetrically in line in a flow cell with Au/Hg microelectrodes. PFRs were run for 30 days with samples analyzed for pH, UO_2^{2+}, PO_4^{3-}, G3P, Br^-, Cl^-, NO_3^-, and SO_4^{2-}. The PFRs remained oxic throughout the experiment, as indicated by the presence of $O_2(aq)$ and the absence of any reduced species (Fe^{2+}, Mn^{2+}, H_2S). Microbial phosphatase activity began within 16 hours of G3P addition, as evidenced by a steady increase of phosphate in the effluent. Further study of the soil through EXAFS and other chemical extraction techniques is ongoing to characterize the uranyl solid phases.

These preliminary results suggest that the addition of an organophosphate substrate to contaminated soils may promote microbial phosphatase activity that can produce sufficient phosphate to precipitate uranium phosphate minerals.

Chromium(VI) Reduction Capacity within *Desulfovibrio vulgaris* Planktonic and Biofilm Cells

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Desulfovibrio vulgaris Hildenborough is a model organism for sulfate-reducers that can reduce heavy metals and radionuclides (e.g., Cr(VI) and U(VI)) and has shown great potential for the bioremediation of contaminated soils. *D. vulgaris* planktonic and biofilm cells were characterized for both reduction capabilities, physiological response, and survival during Cr(VI) exposure. Planktonic cells were washed to remove residual sulfide and inoculated into fresh medium to an OD_{600} of 0.1. A concentration of 0.05 mM Cr(VI) caused planktonic cells to lag for approximately 25 hours, even though Cr(VI) was not detectable 5 hours post-exposure. During this period, a small amount of lactate was utilized, even though there was no accumulation of acetate and sulfate was not reduced. Viability decreased 4-fold and 40-fold during the first 10 and 20 h post-Cr(VI) exposure, respectively. Cell growth effects were not dependent upon increased ORP, but could be restored upon a non-dose dependent addition of ascorbate at the time of Cr(VI) exposure. Transcriptomic analysis of the planktonic cells indicated the majority of differentially expressed genes included annotated genes involved in phage synthesis, particular reductases/dehydrogenases, heavy metal resistance, and protein turnover. Biofilm cells grown upon glass slides within a CDC reactor were placed into a separate reactor with freshly prepared LS4D medium to remove planktonic counterparts and exposed to 0.l mM Cr(VI). Additional Cr(VI) was flowed through the reactor at approximately 0.25 μmol/min, and the dilution rate was approximately 0.38 h^{-1} to minimize planktonic growth. Cr(VI) levels declined steadily over the first 10 hours while protein levels remained fairly constant throughout Cr(VI) exposure. Interestingly, cell viability did not decrease within the first 20 hours post-Cr(VI) exposure. Initial results suggested that planktonic cells have a higher rate of reduction per mg of protein compared to biofilm cells, but biofilm cells retained greater viability during Cr(VI) exposure.

Optimization of Flow-Field Flow Fractionation-Inductively Coupled Plasma Mass Spectrometry for U(VI) Characterization

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Flow-field flow fractionation (Fl-FFF) coupled with online detection is a powerful method for gaining understanding of the distribution of elements bound to colloids of varied diameters, such as natural organic matter and natural inorganic nanoparticles, which might exist in an intermediate or field scale sample. In Fl-FFF, an aqueous suspension is injected into a thin channel where a perpendicular cross flow pushes the constituent particles against a membrane. The particles then diffuse up into channel and elute at different times. The relative diffusion coefficients are directly related to hydrodynamic diameter through Stokes Law. Unlike other separation techniques, there is no interaction with a second, stationary phase. However, variables such as choice of a surfactant in carrier fluid, flow rates, and ionic strength all must be considered to minimize sample-membrane interaction and minimize change to the sample over the course of the run.

As a size fraction elutes, online elemental detection by inductively coupled plasma mass spectroscopy (ICP-MS) measures the concentrations of uranium associated with the varying size fractions. Uranium has a detection limit in the parts-per-trillion range, making it relevant to fractionated environmental samples. We will discuss optimization of the two instruments in the context of results to date of uranium sorption onto synthesized hematite nanoparticles.

Scaling of U(VI) Interactions with Synthetic Iron Oxide-Coated Sand: Experiments and Modeling

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In this study, iron oxide-coated sands (IOCS) were synthesized, using a homogeneous suspension method, and the interaction of uranium with these synthetic sorbents were assessed. The iron content of the synthesized sands was 0.04%, 0.18, and 0.31% respectively. The iron content and the surface area showed a positive and nonlinear relationship. The synthesis of IOCS with three different iron content and surface area was used to independently manipulate solid (Fe) loading and surface site (surface area) for studying the scaling of adsorption. The U(VI) adsorption isotherms of the three IOCS showed differences in adsorbing U(VI), though the experiments had similar solid (Fe) loading. However, when adsorption was scaled to their respective specific surface areas, the three sands exhibited similar adsorption isotherms. Furthermore, the pH edges on the three IOCS showed differences when normalized to iron content, but showed similar adsorption when scaled to surface area. Published literature data sets were used to support our results. The diffuse double layer model developed by Waite et al. (1994) for describing U(VI) interaction with HFO was used in a purely predictive mode to describe our data set. The model captured the differences in adsorption when adjusted for the surface area of the IOCS. By using a synthetic medium and an established model, we have clearly shown that the adsorption of U(VI) onto Fe-dominated material scales better to reactive surface areas than to reactive mass. These findings are significant in the understanding of U(VI) interactions to Fe-dominated subsurface media.

Effects of Chemical and Physical Heterogeneities on Uranium(VI) Transport at an intermediate Scale

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Long-term stewardship of sites contaminated with radioactive elements requires a modeling construct sufficiently robust to describe contaminant migration over large scales, and long periods of time. However, much of the data collected related to radionuclide migration has been at either the bench scale, to determine fundamental migration behavior, or at the field scale, in the course of a performance assessment. Currently, there is an informational gap between the bench and field scales; that is, there is little knowledge of how to defensibly upscale reactive transport models from the bench to the field scales. In this presentation, we report on experiments taking place at the intermediate scale (between lab and field scales) designed to elucidate methodologies to "link" pore- and field-scale processes.

Two intermediate scale tanks (2.44 m × 1.22 m × 7.6 cm, and 2.44 m × 0.61 m × 7.6 cm) have been constructed, and two separate packings of uranium contaminated sediment from the Naturita Uranium Mill Tailings Remedial Action (UMTRA) site have been completed. The first packing, in the larger of the two tanks, was physically homogenous, using only the <2 mm fraction of sediment. In the second, smaller tank, physical heterogeneity was introduced by splitting the <2 mm fraction into 0 to 0.250 mm and 0.250 mm to 2 mm fractions. These two size fractions were packed in alternating layers of different thicknesses, with a "block" of <0.250 mm fraction at the upgradient end. In both tanks, samples for uranium and water quality analysis, as well as measurements of pressure head, were taken through bulkhead fittings installed through the wall of each tank. In the larger tank, a bromide tracer test has been completed to determine hyrdrologic parameters for this sediment.

Uranium distribution within the tanks was found to vary with pH, alkalinity, dissolved calcium, and the rate of U release from the different particle-size categories and the nature of the heterogeneity distribution. In the larger of the two tanks (homogenously packed), effluent uranium concentrations ranged from 7.26 μM at early time points, and decreased to ~1.5 μM as the tank began to exhibit tailing behavior (a period of about 90 days). The spatial gradients of the major chemical constituents were generally smooth, but were variable both as a function of time and space. Kinetic hindrances to uranium desorption were exhibited during stop flow events; these hindrances may be either physical or chemical in nature.

Effluent concentrations in the heterogeneous packing started higher (~12 μM) and declined much faster (a period of about 30 days). In a 48-hour stop flow event, there was no evidence of uranium rebound. Visual interpretation of kriged data plots generally shows more uranium retention in the areas where the <0.250 mm sediment was packed, relative to the areas where the >0.250 sediment was packed.

Characterization of Protein-Protein Interactions Involved in Iron Reduction by *Shewanella oneidensis* MR-1

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The interaction of proteins implicated in dissimilatory metal reduction by *Shewanella oneidensis* MR-1 (outer membrane [OM] proteins OmcA, MtrB, and MtrC; OM-associated protein MtrA; periplasmic protein CctA; and cytoplasmic membrane protein CymA) were characterized by protein purification, analytical ultracentrifugation, and cross-linking methods. Five of these proteins are heme proteins—OmcA (83 kDa), MtrC (75 kDa), MtrA (32 kDa), CctA (19 kDa), and CymA (21 kDa)—and can be visualized after sodium dodecyl sulfate-polyacrylamide gel electrophoresis by heme staining. We show for the first time that MtrC, MtrA, and MtrB form a 198-kDa complex with a 1:1:1 stoichiometry. These proteins copurify through anion-exchange chromatography, and the purified complex has the ability to reduce multiple forms of Fe(III) and Mn(IV). Additionally, MtrA fractionates with the OM through sucrose density gradient ultracentrifugation, and MtrA comigrates with MtrB in native gels. Protein cross-linking of whole cells with 1% formaldehyde show new heme bands of 160, 151, 136, and 59 kDa. Using antibodies to detect each protein separately, we found that heme proteins OmcA and MtrC cross-link, yielding the 160 kDa band. Consistent with copurification results, MtrB cross-links with MtrA, forming high-molecular-mass bands of approximately 151 and 136 kDa.

Stability of Uranium Incorporated into Fe(hydr)oxide Structure upon Oxidation and Under Fluctuating Redox Conditions

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Owing to both ecosystem and human health consequences, understanding uranium's potential for mobility in surface and subsurface settings is important. In anaerobic soils and sediments, the more mobile, oxidized form of uranium (UO_2^{2+}) may be reduced through biological or chemical pathways to U(IV), forming sparingly soluble solids. However, various factors within the subsurface may limit reduction of the uranyl ion, including formation of ternary calcium-carbonato complexes of uranium. Additionally, authogenic UO_2 is potentially susceptible to reoxidation in the presence of commonly occurring oxidants including molecular oxygen. Therefore, determining reaction pathways of uranium that lead to solids stable (i.e., having limited solubility) under both aerobic and anaerobic conditions is critical for limiting dissolved concentrations and migration of uranium. We examined uranium incorporation into transforming Fe(hydr)oxides and secondary fate under cyclic oxidizing and reducing conditions.

In reducing environments containing ferrihydrite, 3 mM Fe(II), 4 mM Ca, and 3.8 mM HCO_3^-, aqueous uranium concentrations decrease from 0.16 mM to 0 mM in a period of 15 days. Increasing Fe(II) concentration to 10 mM results in aqueous uranium concentrations decreasing from 0.16 mM to 0 mM within 5 days. In identical systems without Fe(II), uranium decreases from 0.16 to 0.10 mM after 5 days of anoxic incubation. Changes in solid phase color during incubations suggest changes in iron mineralogy, as well as the potential for formation of reduced uranium solid phases. Uranium EXAFS and XANES analysis reveal oxidized uranium both adsorbed on and incorporated in the structure of goethite and magnetite. Additionally, uranium reduction and concomitant $UO_{2(s)}$ precipitation occurs within ferrihydrite systems reacted with 10 mM Fe(II). By contrast, U(VI) is retained strictly by adsorption in systems without Fe(II).

Uranium in different treatment systems maintained under reducing conditions for 15 days followed by 5 days of oxidation (1 cycle) for 3 cycles exhibited widely diverging fates. Aqueous uranium in systems with 10 mM Fe(II) initially decreased to 0 mM after the first reduction period, but eventually rebounded to 0.16 mM with subsequent oxidation and reduction stages. In systems with 3 mM Fe(II), dissolved uranium remained below detection until the second oxidation period, before gradually increasing to a final concentration of 0.11 mM.

Our results thus reveal that the fate of uranium is intimately tied not only to anaerobic/anaerobic conditions, but also to geochemical conditions controlling uranium speciation, as well as to the fate of iron.

GeoChip Analysis of the Impact of Nitrate and Heavy Metal Contamination on Functional Gene Diversity in FRC Groundwater

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The objective of this study is to examine the bacterial community structure in wells of varying heavy metal and acid contamination, to determine which contaminants have the greatest effect. Five monitoring wells and an uncontaminated background well from the Field Research Center (FRC) site of the U.S. DOE ERSP (Environmental Remediation Science Program) at Oak Ridge, Tennessee, were sampled to provide a gradient of groundwater nitrate, pH, and uranium concentrations. DNA from these samples was analyzed with a comprehensive functional gene array, containing 24,243 probes for >10,000 genes involved in carbon, sulfur, nitrogen, and metal cycling. Genes with the highest signal intensities from each sample were correlated with the groundwater geochemistry of that well. Wells with similar geochemical profiles had greater gene overlap than dissimilar wells. A higher percentage of nitrogen fixation genes were detected in groundwater with lower nitrate concentrations, while the percentage of nitrate reduction genes generally decreased with decreasing nitrate. Wells with elevated sulfate concentrations had a greater percentage of genes dedicated to sulfate reduction, and higher signal intensities for *dsrAB* genes than the background, indicating a greater abundance of those genes. Contaminated wells did not have a higher percentage of metal reduction and resistance genes than the background, but the total signal intensity of those genes was 1.4- to 2.3-fold greater than the background, indicating that metal-related genes were more prevalent in the contaminated wells. Uranium, nitrate, and sulfate were identified by CCA as important factors in determining community structure.

This study provides an overall view of the functional genes present in a highly contaminated environment, and shows the differences in functional populations between wells with varying contamination. As indicated by this work, contaminant level has significant effects on bacterial community structure, the knowledge of which may be important in planning and implementing successful bioremediation strategies in the future.