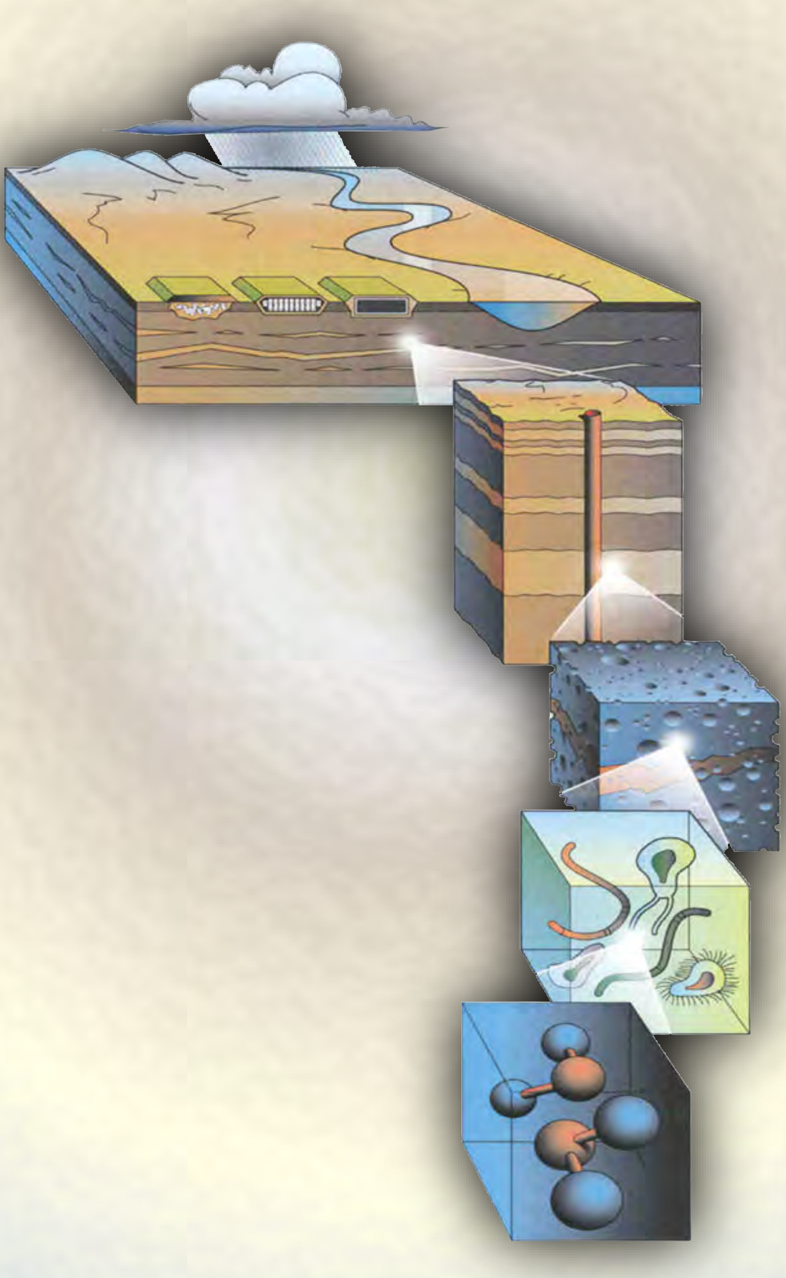
LBNL-43E-2011

Subsurface Biogeochemical Research (SBR) Contractor-Grantee Workshop

April 26 - 28, 2011



U.S. DEPARTMENT OF ENERGY

BERKELEY LAB

LBNL-43E-2011

U.S. Department of Energy (DOE)–
Subsurface Biogeochemical Research (SBR)
6th Annual PI Meeting

Abstracts

April 26–28, 2011

JW Marriott Hotel
Washington, DC

Subsurface Biogeochemical Research

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(Note: First author shown in parenthesis when not PI)

Subsurface Biogeochemical Research

2011 Principal Investigators Meeting

On behalf of the Subsurface Biogeochemical Research (SBR) program managers in the Climate and Environmental Sciences Division (CESD), Office of Biological and Environmental Research (BER), welcome to the 2011 SBR Principal Investigators meeting. Thank you in advance for your attendance and your presentations at this year's meeting. As the events in Japan continue to unfold, we are all reminded that the research we perform on radionuclide behavior in the environment has implications beyond legacy waste cleanup and in fact has its place in the discussion on the expanded use of nuclear power.

As in the past, there are three broad objectives to the Principal Investigators meeting: (1) to provide opportunities to share research results and promote interactions among the SBR scientists and other invited guests; (2) to evaluate the progress of each project within the program; and (3) to showcase the scientific expertise and research progress over the past year to senior managers within the DOE Office of Science, the technology offices within DOE, and other invited attendees from other Federal Agencies.

This past year has seen a few significant changes within BER and within the SBR program. In November, our Associate Director for BER, Anna Palmisano, retired from Federal service. Just this month, Dr. Sharlene Weatherwax (Division Director for Biological Systems Sciences) has been named as the new Associate Director for BER. In August, BER welcomed Dr. Gary Geernaert as the new Division Director for CESD. Gary joins the division from Los Alamos National Laboratory with a background in atmospheric science. Within the SBR program, a new Strategic Plan was completed last June (currently posted on the SBR and the Office of Science website). The new strategic plan is intended to foster integration within the Environmental Systems Science portion of the BER budget that includes both SBR and Terrestrial Ecosystem Sciences (TES). Both these programs share a goal of advancing a predictive understanding of environmental processes and utilizing iterative, multidisciplinary approaches to understand complex environmental systems of relevance to DOE. CESD in general is undergoing continued discussions on integration among its programs in an effort to develop a new strategic plan for the division. This effort also includes identifying opportunities for integration with BER's Biological Systems Science Division (BSSD).

The program this year includes three poster presentation sessions, six plenary sessions, and three breakout sessions. The plenary session on Tuesday morning will feature introductory presentations by BER program staff and three keynote addresses from Dr. Ken Bencala (USGS), Dr. Michael (Mick) Follows (MIT) and Dr. Sue Brantley (PSU) that will lead into three breakout sessions Tuesday afternoon. The breakout sessions are intended to highlight key developments in SBR research and foster a dialog among session participants on scientific paths forward in each particular area. The SBR program managers are asking for input from the SBR community at these sessions to help guide future efforts and/or identify areas of integration within BER programs. On Wednesday, plenary sessions will continue in the morning, followed by an early afternoon poster session. After an extended break for lunch, plenary sessions will continue in the afternoon, followed by an evening poster session. Thursday's plenary session will focus on selected highlights of research efforts at the IFRC sites and on a new potential TES field effort in the Arctic. This new field site is an obvious point of integration between the SBR and TES programs.

Once again, on behalf of the SBR program staff, we are excited to learn of your research progress and anticipate more important advances in our mission to support DOE's efforts in understanding contaminant fate and transport. We look forward to meeting with you and discussing your research plans and results.

Best Regards,

Todd Anderson

SBR Program Manager

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Subsurface Biogeochemical Research
Contractor-Grantee Workshop
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Monday, April 25

5:00–7:00 p.m. **Evening Registration**

Tuesday, April 26

7:00–8:00 a.m. **Registration**

8:00–9:30 a.m. **Welcome and Introductory Comments**

8:00–8:15 a.m. **Gary Geernaert**, Director, DOE Climate and Environmental Sciences Division (DOE-CESD)

8:15–8:45 a.m. **Todd Anderson** (DOE-CESD)
SBR Program Update

8:45–9:00 a.m. **Dan Drell,** DOE Biological Systems Science Division (BSSD)
Changes to the JGI Community Sequencing Program

9:00–9:15 a.m. **Joe Graber** (DOE-BSSD)
Carbon Cycle Research within the Biological Systems Sciences

9:15–9:30 a.m. **Mike Kuperberg** (DOE-CESD)
Terrestrial Ecosystem Science

9:30–12:15 p.m. **Invited Speakers**

9:30–10:15 a.m **Ken Bencala** (U.S. Geological Survey)
Basic Hydrology Challenges in Understanding Hyporheic Exchange at the Stream-Catchment Scale

10:15–10:45 a.m. ***Break***

10:45–11:30 a.m. **Michael (Mick) Follows** (MIT)
Modeling Marine Microbial Populations and Biogeochemical Cycles

11:30–12:15 p.m. **Sue Brantley** (Penn State University)
Extrapolating Rates of Critical Zone Processes Across Scales: Successes and Challenges

12:15–2:00 p.m. **Buffet Lunch (Research Team Meetings)**

2:00 p.m–5:00 p.m. **Breakout Sessions (*see following pages for details*)**

5:30–8:00 p.m. **Poster Session I –** *Hors d'oeuvres and Refreshments (Cash Bar)*

Breakout Session A: ***Contaminant Fate and Transport at the Groundwater-Surface Water Interface***

Organizers: **Joel E. Kostka** (FSU) and **Philippe Van Cappellen** (University of Waterloo)

Abstract

The success of subsurface remediation or monitored natural attenuation strategies, as well as public perception and acceptance of those strategies, will not only depend on what happens in the subsurface, but also on whether contaminants are discharged or released to surface waters. Because of the active and complex flow dynamics, the groundwater-surface water interface (GWSWI) exhibits unique geophysical and biogeochemical characteristics, including fluctuating hydraulic gradients, enhanced redox oscillations, and drying-rewetting cycles. The role of this unique and highly dynamic interface on the fate of subsurface contaminants at DOE sites must be further understood in order to develop adequate predictive models that guide remediation and natural attenuation efforts.

The GWSWI modulates the release or discharge of nutrients and contaminants to surface waters. Critical hotspots or hot moments in contaminant transformation have been associated with the GWSWI. However, the underlying biogeochemical mechanisms and reaction networks unique to the interface have yet to be completely unraveled. The prediction of discharge and the assessment of risk for contaminant release from the watershed to regional scale are currently limited by our lack of mechanistic understanding of the biogeochemical functioning of the GWSWI. This breakout session will provide a state-of-the-science review of the coupled physical, chemical, and biological processes that control the structure and function of the GWSWI, with a focus on the hyporheic and riparian zones in DOE-relevant systems. The goals of the session are to identify key processes that control contaminant transformation, to delineate knowledge gaps, and to highlight research directions that will lead to a more robust predictive understanding of contaminant flux and discharge at the GWSWI. Some of the specific questions that will be addressed during discipline-specific as well as general discussions are listed below.

A. Groundwater-Surface Water Interactions: Key Questions and Limitations

2:00 p.m. **Roy Haggerty** (Oregon State University)
2:30 p.m. **Philippe Van Cappellen** (University of Waterloo)
3:00 p.m. **Joel E. Kostka** (Florida State University)

B. Groundwater-Surface Water Interactions: Contaminant Transport at DOE Sites

3:30 p.m. **Lee Slater** (Rutgers University)
3:50 p.m. **Scott Brooks** (Oak Ridge National Laboratory)
4:10 p.m. General Discussion
5:00 p.m. Adjourn

Breakout Session B: ***Integrating Microbial Metabolism into Descriptions of Environmental Processes at a Variety of Observational Scales***

Organizer: **Todd Anderson (**DOE-CESD)

Abstract

SBR’s close association with the Genomic Sciences program, including some overlap of funded scientists and common field sites and materials, provides an opportunity to leverage advances in genome-enabled techniques to advance a more fundamental understanding of the metabolism of microorganisms and detection of the active members of microbial communities in the environment. Historically, research on cellular metabolism has tended to focus on organisms currently in culture, for obvious reasons. In a few cases, detailed metabolic modeling has enabled a translation of metabolic activity investigated in the laboratory to environments where the microorganism under study is found, thereby enabling understanding of that microorganism in its environment. This “bottom-up” approach to understanding microbial communities in the environment, one species at a time, shows some promise for predicting the activity of specific microorganisms in controlled environmental settings, but may be limited in describing the interactions occurring in natural microbial communities. Process-based models of microbial activity, a mainstay of environmental simulations, describe the collective activity of dominant microbial communities, but lack predictive power in explaining environmental impacts on microbial activity. Alternatively, a variety of metagenomic and proteomic techniques take a broader approach to describing the composition and genetic potential of entire microbial communities in the environment. These “top down” techniques have the potential to describe larger-scale microbial ecological phenomena, but their broad utility remains constrained. For SBR, it is also unclear how these broader approaches could be integrated with physical/chemical models of environmental processes. This session will highlight approaches to understanding and modeling microbial processes in the environment at a variety of scales. The intent of the session is to engage the audience in a conversation about these approaches and how best to integrate genome-enabled information on microbial activity/ecology into coupled models of environmental processes. A potential outcome of this session would be a series of recommendations that advance a predictive understanding of microbial activity in the environment.

2:00 p.m. **Todd Anderson** (DOE-CESD)
2:15 p.m. **Derek Lovley** (University of Massachusetts)
2:45 p.m. **Eric Roden** (University of Wisconsin)
3:15 p.m. **Lisa Stein** (University of Alberta)
3:45 p.m. **Eoin Brodie** (Lawrence Berkeley National Laboratory)
4:15–5 p.m. Open Discussion, Adjourn

Breakout Session C: ***Pore-Scale Processes—Matching Measurements to Models While Upscaling***.
Organizers: **Peter Nico**, **Jonathan Ajo-Franklin** (Lawrence Berkeley National Laboratory)

Abstract

It is taken as a given that precipitation and dissolution processing taking place at the pore scale as a result of remediation or natural perturbations of subsurface systems can have profound impacts on the larger scale behavior of the system. However, both understanding exactly how pore-scale processes occur and linking those changes to specific large-scale system behavior remain extremely challenging from both an experimental and computational perspective. In this session, we will explore new technological methods and intellectual approaches to image, quantify, and understand pore-scale changes while simultaneously evaluating which changes control the evolution of macroscale systems. One of the specific challenges in this process is having both models at the appropriate scale and measurements at a matching scale to inform and validate the models.

2:00 p.m. **Li Li (**Penn State U.), *Overview of Modeling Approaches and Challenges to Upscaling*
2:20 p.m. **W. Brent Lindquist (**Stony Brook U.), *Effects of Pore-Structure Change and Multiscale Heterogeneity on Contaminant Transport and Reaction-Rate Upscaling*
2:40 p.m. **Markus Berli** (Desert Research Inst.)**,** *Tomographic Imaging of Near Root Process*
3:00 p.m. **Bill Moses/Peter Nico** (LBNL), *Imaging Flow and Heterogeneity Development at Intermediate Scale*
3:20 p.m. **Jon Chorover (**U. of Arizona), *Measuring Field Processes Across Scales*
3:40 p.m. Open Discussion
5:00 p.m. Adjourn

Wednesday, April 27

8:00–9:30 a.m. **Plenary Session**

Pore Scale Processes

8:00–8:30 a.m. **Jenny Druhan** (Lawrence Berkeley National Laboratory)
Mechanisms for Stable Isotope Variation During Bioremediation

8:30–9:00 a.m. **Dorthe Wildenschild** (Oregon State University)
Current Status of Imaging Microbial Biofilms in Three-Dimensional Opaque Porous Media using X-Ray Microtomography

9:00–9:30 a.m. **George Redden** (Idaho National Laboratory)
Precipitation Reaction Fronts in Subsurface Environments: Insights from Experiments and Challenges for Modeling and Engineering.

9:30–10:00 a.m. ***Break***

10:00–12:30 p.m. **Poster Session II**

12:30–2:00 p.m. **Box Lunch (Research Team Meetings)**

2:00–5:30 p.m. **Plenary Session**

Microbiological/Biogeochemical Processes

2:00–2:30 p.m. **Carrie Miller** (Oak Ridge National Laboratory)
Biogeochemical Controls on Hg Transformations at a Contaminated Site: The Role of Dissolved Organic Matter and Redox Gradients

2:30–3:00 p.m. **Frank Loeffler** (University of Tennessee)
Microbial Uranium Reduction and Monitoring Tools

3:00–3:30 p.m. **Mike Wilkins** (Pacific Northwest National Laboratory)
Proteogenomic Insights from the Analysis of Biostimulated Microbial Communities

3:30–4:00 p.m. ***Break***

Molecular Scale Processes

4:00–4:30 p.m. **Brad Tebo** (Oregon Health & Science University)
Manganese Oxidation in the Subsurface and Its Impact on Uranium Immobilization: What Mn Can Do for U

4:30–5:00 p.m. **John Bargar** (SLAC National Accelerator Laboratory)
Progress in Understanding Uranium Speciation and Dynamics in Reduced Sediments: Research at Molecular to Centimeter Scales by the SLAC SFA Program

5:00–5:30 p.m. **Michelle Scherer** (University of Iowa)
Fe^{2+} *Sorption at the Fe Oxide-Water Interface: A Revised Conceptual Framework*

6:00–8:30 p.m. **Poster Session III—***Hors d'oeuvres and Refreshments (Cash Bar)*

Thursday April, 28

8:00–10:00 a.m. **Plenary Session**

8:00–8:30 a.m. **David Lesmes** (DOE-CESD)
Overview/Scope of CESD Programs

IFRC Highlight Presentations

8:30–9:00 a.m. **Chris Murray** (Pacific Northwest National Laboratory)
A Geochemical Heterogeneity Model for a Contaminated Vadose Zone-Aquifer System

9:00–9:30 a.m. **Chris Schadt** (Oak Ridge National Laboratory)
Microbial and Geochemical Dynamics During Bioreduction Stimulated by Emulsified Vegetable Oil

9:30–10:00 a.m. **Ken Williams** (Lawrence Berkeley National Laboratory)
Getting a Grip on Subsurface Complexity: Geochemical, Microbiological, and Hydrological Research at the Rifle IFRC

10:00–10:30 a.m. ***Break***

10:30–12 p.m. **Plenary Session**

10:30–11:00 a.m. **Ian Gorton** (Pacific Northwest National Laboratory)
Update on the Status of the ASCEM Project (Advanced Simulation Capability for Environmental Management)

11:00–11:30 a.m. **Stan Wulschleger** (Oak Ridge National Laboratory)
Introduction to the Next Generation Ecosystem Experiment (NGEE)

11:30–12:00 pm NGEE Discussion

12:00 pm **Close-Out Final Announcements and Adjourn**

Abstracts

University-Led Research

Three-Dimensional Reconstruction of Biological Organization and Mineralization in Sediment-Attached Biofilms During Uranium Bioremediation

University-Led Research

B. Luef, K.C. Wrighton, V. Shah—*U. of California-Berkeley*; C.J. Castelle, R. Csencsits, B. Gilbert,
S.C. Fakra, S.W. Singer, L.R. Comolli—*LBNL*; J.F. Banfield (PI), *U. of California-Berkeley*

Despite the well-established potential for subsurface uranium bioremediation, the role of planktonic organisms versus sediment-attached biofilm communities in uranium bioreduction remains unclear. We seek to understand how the physical, chemical, and biological heterogeneity associated with planktonic microbial communities and biofilm formation affect uranium reduction, as well as the mobility and chemical stability of the uraninite precipitates. Understanding how these parameters impact bioreduction and colloid immobilization may help identify organic-substrate amendment regimes consistent with sustained remediation,and enable prediction of long-term local redox conditions and uraninite stability.

Here, we characterize groundwater planktonic bacteria samples from the U-contaminated Rifle aquifer during *in situ* acetate amendment. This study is distinguished from prior microscope-based characterization efforts targeting subsurface consortia, in that the samples were cryo-plunged on site immediately after collection and examined using electron tomography and scanning transmission X-ray microscopy (STXM). Characterization tools include 2D and 3D cryogenic-transmission electron microscopy (cryo-TEM), conventional and cryogenic high resolution TEM (HRTEM and cryo-HRTEM), analytical transmission electron microscopy (energy dispersive spectroscopy, EDS) and STXM.

An intriguing finding is that essentially all of the ~200 cells examined via 2D and 3D cryo-TEM were decorated with ~10 to 200 nm clumps of nanoparticles. Aggregates are tightly adhered to the outer membrane. The aggregates are comprised of few nm diameter particles that were shown by HRTEM and selected area electron diffraction to be amorphous. EDS has established that the aggregates contain abundant iron, with smaller amounts of calcium, silica, chloride, magnesium and vanadium, and trace amounts of phosphorus, sulfur, and potassium. STXM-based Fe $L_{2,3}$ near edge X-ray absorption fine structure (NEXAFS) spectroscopy has determined the Fe valence state to be a range of mixtures of Fe(III) and Fe(II) on small clumps of nanoparticles on the cells, whereas primarily Fe(III) on extracellular larger nanoaggregates.

A key challenge for all TEM-based studies of multispecies consortia is the identification of cell types. We have recently completed the development of a correlative microscopy technique that uses rRNA-specific fluorescence *in situ* hybridization (FISH) probes applied to cryo-TEM grids after they have been imaged. Applying this method to groundwater samples has determined that the prevalent microorganisms are *Geobacter*. Accumulation of iron oxide particles on the outer membrane may provide a mechanism that allows *Geobacter*, an acetate-utilizing iron-reducing bacterium that requires direct contact with the electron-accepting mineral, to function in the planktonic state. Next, we plan to use antibodies to label multi-heme *c*-type cytochromes to determine their distribution and topological relationships with the cell-associated aggregates. The characterization of sediment-attached biofilms in 3D, determination of their membership, mineral associations, and biomass are also part of the next phase of our work.

Mercury Reduction by Denitrifying and Iron-Reducing Bacteria in Subsurface Enrichment Cultures

University-Led Research

N. Yee, T. Barkay (PI), C.-C. Lin, Y. Wang, M. Parikh, X. Zhao—*Rutgers-New Brunswick U.*;
R. Kukkadapu, *EMSL*

In this project, we conducted laboratory studies to investigate the microbial pathways for the reduction of Hg(II) to Hg(0) in the subsurface. Nitrate and iron-reducing enrichments were established using subsurface sediments taken from Oak Ridge, TN. An artificial groundwater medium was formulated with chemical components constrained by onsite monitoring wells to simulate *in situ* groundwater chemistry. In nitrate-reducing enrichments, we observed an inverse relationship between Hg concentrations and onset and rates of denitrification in cultures containing between 53 nM and 1.1 µM of inorganic Hg. At high Hg concentrations, denitrification was irreversibly inhibited. A decline in the number of species in the subsurface community, indicated by the number of peaks in tRFLP patterns, occurred with increasing Hg concentrations. A single tRFLP peak observed for the 312 nM Hg treatment consisted of the 16S rRNA gene of *Bradyrhizobium* spp. A *Bradyrhizobium* sp. isolate from a nitrate enrichment reduced Hg(II) to Hg(0), and the translation product of its *merA* gene had a 97% identity to that of *Proteobacteria* and *Firmicutes*. At low Hg concentrations, denitrification was initially inhibited, but was restored upon enrichment of Hg-resistant denitrifying bradyrhizobia possessing horizontally transferred *merA* genes.

Enrichment cultures established with Oak Ridge FRC subsurface sediments and poorly crystalline ferrihydrite [30 mM Fe(III)] as the electron acceptor resulted in the cultivation of a purified consortium populated by *Clostridia*. Robust iron-reducing activity was observed when the enrichment culture was provided with peptone as a carbon source. Approximately 83% of the ferric iron was reduced in 14 days, and the color of the mineral changed from reddish brown to black. PCR amplification of the 16S rRNA gene showed a single DNA sequence of a bacterium most similar (99% identity) to *Clostridium tunisiense* strain $E1^T$. We refer to this bacterium as *Clostridium sp.* strain FGH. The precipitates formed during iron reduction were analyzed using XRD to identify the secondary iron minerals phases. Broad X-ray diffraction peaks characteristic of poorly crystalline nanomagnetite were detected. Abiotic experiments showed that magnetite can rapidly reduce Hg(II) to Hg(0). The results of this study provide new insights into the ecological role of *Bradyrhizobium* and *Clostridium* in subsurface mercury transformation processes.

Understanding and Predicting the Reactive Transport of Transuranic Contaminants in Porous Media

University-Led Research

M. Barnett (PI), *Auburn U.*; T. Albrecht-Schmitt, *Notre Dame U.*;
J. Saiers, *Yale U.*; D. Shuh, *LBNL*

Our primary hypothesis is that Np and Pu can interact with surfaces in fundamentally different ways than other metals, metalloids, and oxyanions – namely by reductive surface precipitation from undersaturated solutions. We are testing our hypotheses by studying the sorption of Pu and Np and their analogues to representative heterogeneous subsurface materials from SRNL.

We are investigating the role of dissolved and colloidal organic matter in binding and co-transporting U(VI) (an analogue of Pu(VI)) within granular porous media. Three humic acids (HAs) were tested separately for their effects on the transport of uranium through water-saturated columns. U exhibited very low mobility in experiments conducted without the HAs. In the experiments with HAs, U mobility was comparatively high, with maximum relative breakthrough concentration ranging from 16% to 55%. The strength of the HA-effect on U mobility is positively correlated with the NMR-detected content of paraffinic C and the hydrophobicity of NOM, which is inconsistent with the more widely recognized effects of carboxyl and phenolic functional groups. To help interpret the differences in U transport between the column experiments, we developed a continuous stirring flow system to quantify U adsorption and desorption. The time-series data on desorption can be closely approximated by a coupled desorption-diffusion model and suggests that the small molecular-weight fraction HAs are most important in facilitating U transport.

Th(IV) (as a analogue for Pu(IV)) sorption experiments are also being conducted with SRNL geomedia and representative minerals goethite and kaolinite. Our results indicate that strong Th(IV) sorption occurs at pH>4, even in the presence of strong ligands like carbonate. These sorption experiments will be quantified using a surface complexation model normalized to surface area. Complementary experiments will also be conducted to examine the effects of goethite and kaolinite on Th(IV) transport.

The sorption of Np in its most environmentally prevalent oxidation state of +V onto SRBL geomedia was examined to determine the mobility and transport of Np in the subsurface. The studies demonstrated that Np(V) is not sorbed onto the sandy material under any environmentally relevant conditions. In contrast, Np(V) begins to sorb onto the clay material under slightly basic conditions. According to hydrolysis constants, the neutral $NpO_2(OH)$ species is likely the form that is sorbed. At very high pH values, the Np(V) can be directly precipitated, but these conditions are not environmentally relevant.

Complementary investigations of column soil materials by use of the Advanced Light Source-Molecular Environmental Science (ALS-MES) Beamline scanning transmission X-ray microscope (STXM) successfully imaged and conducted near-edge X-ray absorption fine structure (XAFS) spectroscopy studies with soft X-rays. The ALS-MES STXM will next be used to map the light element associations on the 50 nm length scale and characterize the speciation of the constituents in column materials. Further STXM experiments will be performed with existing Th, Np, and Pu column/batch materials. The ALS-MES STXM studies will be complemented by judicious spatially resolved XAFS studies at the micron scale using ALS X-ray beamlines. The hard X-ray experiments for the radioactive batch/column materials will then be conducted at the SSRL.

GPR Imaging of Channeled Flow in Fractured Bedrock

University-Led Research

G. P. Tsoflias, *U. of Kansas*; M.W. Becker (PI), *Cal State U. Long Beach*

The highly non-uniform flow of water through fractured bedrock complicates remediation and prediction of groundwater contamination. In particular, channeled flow obscures estimates of surface reactions and matrix diffusion. Our exploratory project is to develop field methods capable of estimating complex flow “connectivity” among remediation and monitoring wells, and apply these methods to constrain reactive transport parameters. Our two primary tools are harmonic interference hydraulic tests and multitracer breakthrough tests. These tools are tested at our bedrock research site using surface ground penetrating radar (GPR) to image the fracture aperture distribution and the path of tracer transport.

GPR experiments were conducted at the Altona Flat Rock fractured sandstone field site using surface GPR reflection to image a water-saturated subhorizontal bedrock fracture 7.6 m below surface. Three-dimensional GPR data were acquired at 50 MHz and 100 MHz frequencies covering approximately a 65 m^2 area at a 0.25 m x 0.5 m grid. Fracture water salinity was controlled by re-circulation of saline traced formation water in a dipole flow setup encompassing the region monitored by GPR. Comparison of GPR reflection amplitudes between background clean water (9.3 mS/m), and traced water of 200 mS/m and 600 mS/m fluid electrical conductivity showed that regions of low reflection amplitude remain mostly unchanged when water salinity changes. In contrast, regions of high reflection amplitude show a large increase in response to fluid salinity increase. This result is in agreement with the expectation that fracture regions with greater aperture, and therefore greater clean water reflection amplitude, conduct most of the saline tracer. GPR amplitude reveals 1 m to 1.5 m wide flow channels trending across the survey area and the flow dipole field. The spatial scale of these channels corresponds roughly to hydrodynamic dispersivity measured from interwell saline tracer breakthrough. GPR imaging offers the capability to define the geometry of flow channeling and reduce the uncertainty of transport predictive modeling in bedrock groundwater systems.

The 2011 summer field season will complete the GPR measurements of fracture aperture and tracer migration, using a series of dipole tracer tests in order to calibrate GPR amplitude and phase response to fracture aperture and tracer concentration.

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

University-Led Research

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B. Cao, *Washington State U.*; P.D. Majors, *PNNL*

Collaborators: K.M. Kemner, B. Mishra, and M.I. Boyanov—*Argonne National Laboratory*;
M.J Marshall, L. Shi, D.W. Kennedy, R. Brown, Y. Xiong, M.F. Romine, M.S. Lipton, N.G. Isern—*PNNL*

In this project, novel microscale experimental approaches are being applied to understand how microscale biotic and abiotic reactions and their combined interactions control uranium mobility in the subsurface at the Hanford 300 Area IFRC. Microscale geochemical gradients (dissolved O_2 (DO) and H_2 concentrations, redox potential, and pH) were measured in Hanford 300A subsurface sediment biofilms and correlated with the average U speciation. These measurements revealed significant differences in geochemical parameters across a sediment biofilm/water interface in the presence and absence of U(VI) under oxic and anoxic conditions. While the pH was relatively constant within the biofilm, the redox potential, DO, and H_2 concentrations were heterogeneous at the microscale (<500–1000 μm). Under bulk aerobic conditions, the addition of U(VI) resulted in the development of microenvironments with high DO levels (DO hotspots) that were not observed in the absence of U(VI).

The presence of U(VI) under anoxic conditions resulted in a decrease in H_2 concentrations. U LIII-edge X-ray absorption spectroscopy (XANES and EXAFS) showed that 80-85% of the U was present as U(IV). Our results demonstrate the importance of microscale geochemical gradients for understanding U mobility in the subsurface. Extracellular polymeric substances (EPS) in the subsurface sediment biofilms are hypothesized to play important roles in U biotransformations and mobility. To investigate the roles of EPS in U immobilization, we used *Shewanella* HRCR-1, an isolate from the Hanford Reach of the Columbia River, as a model, and characterized the composition of EPS using FTIR and proteomics to provide insight into the potential interactions of EPS with uranium. Redox proteins including the homologues of two S. oneidensis MR-1 outer membrane c-type cytochromes, MtrC, and OmcA, which have been implicated in extracellular electron-transfer reactions, were identified in the EPS fractions. The presence of these proteins was also confirmed by immunoblot analysis. We further quantified the contribution of EPS to U(VI) immobilization via sorption and reduction by EPS fractions. We found that, in reduced form, the isolated cell-free EPS fractions could reduce U(VI). Polysaccharides in the EPS likely contributed to U(VI) sorption and dominated the reactivity of loosely associated EPS, while redox active components (e.g., outer membrane c-type cytochromes), especially in bound EPS, likely facilitated U(VI) reduction. As a novel noninvasive tool in microscale studies, NMR was used to determine material-transport rates within live biofilms.

In situ two-dimensional effective diffusion coefficient maps of water and surface-averaged relative effective diffusion coefficient profiles in *S. oneidensis* biofilms were obtained. Accurate spatially resolved diffusion coefficients are critical for modeling uranium diffusion in the subsurface sediment biofilms. We also applied NMR to monitor temporal and spatial concentration profiles of metabolites in biofilms. Using an in-house designed NMR-compatible flow cell (EMSL), the metabolism of *Shewanella oneidensis* biofilms and the metabolic responses to the influx of U(VI) were noninvasively monitored.

Reactivity of Iron-bearing Phyllosilicates with Uranium and Chromium through Redox Transition Zones

University-Led Research

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This project will perform thermodynamic, kinetic, and mineral structural studies on the reactivity of phyllosilicate Fe(II/III) with metal-reducing bacteria, and with two important poly-valent DOE contaminants (Cr and U) that show high mobility in their oxidized state. Both of these contaminants are important from a risk perspective at the Hanford site, while U is ubiquitous throughout the DOE legacy complex. We focus on Fe-bearing phyllosilicates because these are important components of the reactive fines fraction of Hanford, ORNL, and INL sediments. Moreover, their complex redox properties and reactivity are not well understood, and relatively little SBR research has targeted their biogeochemical behavior. Consequently, there are no credible reaction-based models that can be used to describe the biogeochemical contributions of phyllosilicate redox reactions to the subsurface mobility of U, Cr, or other polyvalent contaminants (including Tc) as needed to successfully meet SBR's long-term measure.

This project was begun in September 2010, and to date we have prepared oxide-free, clay-sized fractions (<2.0 µm and <0.5 µm) from Hanford sediments collected from Well C6209 59-60 ft b.g.s. and from the Mercer Clay formation in Cambria County, Pennsylvania. Both these sediments contain high Fe concentrations in their clay fractions and are located adjacent to steep redox gradients. Elemental and iron contents were determined by complete dissolution in HF/H_2SO_4, and Fe(II) content was determined by an HF/H_2SO_4-phenanthroline assay. For the Hanford clay fractions, we measured an iron content of 11.2% (9.98 mmol Fe/g) and an Fe(II) content of 1.4%. The unaltered clay fractions were then completely reduced using the citrate-bicarbonate-dithionite (CBD) method. The unaltered and fully reduced clay fractions were then characterized by a suite of complementary techniques, including synchrotron radiation x-ray diffraction (SR-XRD), Fe K-edge x-ray absorption spectroscopy (including EXAFS and XANES), and transmission electron microscopy with electron energy loss spectroscopy (TEM-EELS). In the near future, these samples will also be analyzed with Mössbauer spectroscopy. Combined with aqueous and solid-phase elemental analyses and Fe(II/III) speciation measurements, we plan to synthesize all these data together to better define the structural coordination of Fe in natural phyllosilicates. Currently, the speciation of Fe in these materials is difficult to assign because of the multiple possible forms of Fe, such as "structural Fe(III)" (likely in the octahedral sheet of the TOT layer), "structural Fe(II)" (originally present in the unaltered material and newly formed by CDB reduction), "surface-complexed Fe(II)" (to edge sites), and "interlayer-exchanged Fe(II)" (to basal plane).

We also measured the reactivity of U(VI) with these clay fractions. Specifically, we reacted U(VI) with the unaltered materials to measure U sorption and used U L_{III}-edge EXAFS spectroscopy to model the coordination of sorbed U(VI). We reacted U(VI) with the CBD-reduced materials to measure U reduction and used U L_{III}-edge EXAFS and XANES to confirm U(VI) reduction extent and identify U reaction products. For these experiments, we found that phyllosilicate-Fe(II) did not reduce U(VI) to a great extent– similar to previous results we obtained with the specimen clay minerals nontronite and chlorite. In the near future, we plan to conduct similar experiments with Cr(VI). We are also about to begin a series of experiments with CBD-reduced clay fractions and colorimetric redox-active probes and nitroaromatic compounds, in an attempt to directly measure the standard-state reduction potentials (E^0) of these phyllosilicates.

Inorganic Controls on Neptunium Mobility in the Subsurface

University-Led Research

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Neptunium-237 has a long half-life (2.14 million years) and is highly soluble in near-surface groundwater in the pentavalent oxidation state. It is identified as a subsurface contaminant of concern by the DOE. Co-precipitation of Np into minerals may be important in determining the long-term behavior of this radionuclide in the subsurface. We have undertaken a detailed experiment-based study of the co-precipitation of the Np(V) neptunyl ion into common rock-forming minerals, and are characterizing the details of the structural incorporation and the impact of this on the mineral stability. The specific objectives of this project are to develop a detailed understanding of the factors that impact co-precipitation of Np(V) into minerals that may either naturally attenuate it, or may be used in a designed remediation strategy. We are growing single crystals of the target minerals in the presence of aqueous solutions containing neptunyl under closely controlled conditions. The concentration and distribution of Np in the resulting crystals will be chemically characterized, together with spectroscopic characterization of the oxidation state and coordination geometries for Np(V) in the synthetic crystals. We will examine the impact of Np speciation in solution, as well as other factors such as crystal growth rate on Np co-precipitation, and correlate Np uptake with structural details of the minerals to produce predictive models.

This project is currently in Year 1. We have optimized synthesis methods for calcite ($CaCO_3$), gypsum ($CaSO_4.2H_2O$), borax ($Na_2B_4O_5(OH)_4.8H_2O$), and arcanite (K_2SO_4) that provide crystals of suitable size for detailed characterization. These minerals present several possible incorporation sites for the neptunyl ion, including interstitial substitution and substitution for monovalent or divalent cations. Following optimization of synthesis conditions, each mineral has been re-grown in aqueous systems containing hundreds to thousands of ppm Np(V). Analyses of these minerals are under way using laser-ablation and solution-mode inductively-coupled-plasma mass-spectroscopy (ICP-MS).

Preliminary results indicate that both gypsum and calcite readily incorporate significant Np, but acranite excludes Np even when it was present in solution at over 1000 ppm. Whereas this may indicate incorporation of the neptunyl ion is more readily achieved in the smaller Ca sites than in K sites, further studies are planned to determine the details of the coordination environments about Np in the studied phases.

Chemical analyses of synthesized Np-doped phases will be continued using ICP-MS. Detailed characterization of thin slices of Np-bearing crystals will be done using electron energy loss spectroscopy (EELS) in a TEM. X-ray absorption spectroscopy is planned to confirm the oxidation state of the Np in the minerals, and to probe the local environment about the Np cations. The synthesis program will be expanded to several more minerals that provide a range of potential incorporation sites for Np(V), with an emphasis on developing a model for Np(V) incorporation at different structural sites.

Field-Deployable Nanosensing Approach for Real-time Detection of Free Mercury Speciation and Quantification in Surface Stream Waters and Groundwater Samples at the U.S. DOE Contaminated Sites

University-Led Research

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Our proposition targets a critical element of the Environmental Remediation Science Program (ERSP) research portfolio, which is the development of enabling scientific tools for characterizing the spatial and temporal evolution of complex subsurface systems. We propose a field-portable, on-site sensing device for real-time speciation of elemental and inorganic mercury in surface stream waters, groundwater, and sediment samples. The originality of our proposition relies on the combination of two well-known phenomena, i.e., the amalgamation of metallic mercury (Hg(0)) and gold (Au) and the Surface Plasmon Resonance (SPR) of gold nanorods (Au NR). When Au NR are exposed to the presence of Hg(0), its amalgamation to Au causes a reduction of the effective aspect ratio of NR and a blue shift of the maximum absorption wavelength of the longitudinal SPR mode band. The linear correlation that exists between the aspect ratio of Au NR and the position of the maximum wavelength of the longitudinal SPR mode makes quantitative analysis possible. The limit of detection depends on the reproducibility of the maximum absorption wavelength of the reference signal. Because the intensity of the reference signal provides signal-to-noise ratios with negligible contribution from instrumental and environmental noise, outstanding limits of detection (10^{-13} g.L^{-1}) are possible with no need for sample pre-concentration steps.

During the first year of the project, a theoretical model was developed to explain the mechanism of mercury sensing with Au NR. The model—which we have coined the slicing pocket approach (SPA)—predicts the largest spectral shifts for Au NR with low AR. This hypothesis was tested with Au NR of AR 1.8 and 2.6 using portable spectrometers for prototype sensor design. The spectral radiance of their excitation sources, the blaze wavelengths of the diffraction gratings, and the spectral responses of their detector units were appropriately selected to provide the best performance within the spectral region of interest. The analytical figures of merit were the following: *Au NR AR 1.8:* Correlation Coefficient = 0.9953; Sensitivity (nm.M^{-1}) = 1.39×10^{7}; Limit of Detection (M) = 3×10^{-8}; *Au NR AR 2.6:* Correlation Coefficient = 0.9841; Sensitivity (nm.M^{-1}) = 1.04×10^{6}; Limit of Detection (M) = 2×10^{-7}. The better sensitivity and LOD of Au NR with AR 1.8 correlate well to our theoretical predictions and make the SPA model a valuable guide for future optimization of Au NR substrates.

Future studies include immobilization of Au NR on solid substrates, AFOM under flow conditions, interference studies with synthetic mixtures of increasing complexity, and further development of prototype sensing instrumentation.

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

University-Led Research

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Our research seeks to resolve the coupling between silicate weathering and radionuclide sorption-desorption during disequilibria that ensue when high-level radioactive waste (HLRW) is introduced to circumneutral soil, sediment, or rock. Focus is on the near-field region of caustic liquid waste release to the Hanford vadose zone, where hyperalkaline solutions (pH $11\text{-}13^+$) accelerate incongruent aluminosilicate weathering, leading to likely neoformation of zeolites, feldspathoids and carbonates (latter depending on $P\mathrm{CO}_2$) that can sequester radionuclides into bound form during nucleation and crystal growth. Whereas cesium-137 and strontium-90 and nitrate are incorporated into aging-induced recalcitrant forms within product solids, iodine-129 (as iodide) is very weakly retained under such conditions. Even for more strongly sorbed/precipitated Cs and Sr, however, the thermodynamic stability of the product is challenged when the caustic source is removed and replaced with more dilute background pore water (BPW), characteristic of groundwater recharge.

Therefore, a specific goal of the current project is to probe the kinetic stability of contaminant-containing neo-precipitates formed by waste-sediment reaction. This is done through saturated, unsaturated and dry/wet cycle BPW leaching experiments on well-characterized HLRW-weathered sediment columns. Column solids are sampled for quantitiative XRD, Sr K-edge EXAFS, ^{27}Al and ^{29}Si NMR, and TEM-EDX before, during, and after introduction of BPW solutions. This approach facilitates establishing quantitative relations among neo-precipitate transformation, contaminant speciation, and solute transport for various waste-weathering scenarios. The complete effluent solution data set is being modeled using the reactive transport code CrunchFlow, which is parameterized through separate quantitative chemical analyses, as well the XRD, XAS, and NMR time series. In this way, we are working to develop a predictive mechanistic understanding of contaminant dissolution and transport during BPW infusion.

Neo-precipitates range from 3-10% of the reacted sediment mass, depending on treatment regime (6 or 12 mo, with or without CO_2, high or low contaminant concentration). The principal controls on contaminant effluent release – and that are now included in the model – are (i) generalized Sr and Cs cation exchange on both native sediment and neo-precipitate surfaces, (ii) frayed edge site (FES) Cs exchange, and (iii) neo-precipitate dissolution/ transformation. These controls are reflected in various components of the data set, including (1) column experiments conducted with aluminosilicate precipitates in isolation revealed nonstoichiometric release of Cs and Sr relative to Al and Si; (2) small loss of sodalite or cancrinite was observed over hundreds of pore volumes BPW, while effluent solutions were characterized by a low and nonstoichiometric molar ratio of neo-phase Si to $\mathrm{NO}_{3;}$ (3) growth of Ca-Willhendersonite occurred at the expense of Ca-Chabazite, and was observed to contain both Sr and Cs after extensive leaching. The associated slight shortening of aluminosilicate bond distances due to the structural transformation, along with the near perfect Si-Al ordering, renders extra framework cations less exchangeable.

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

University-Led Research

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Transport and mixing of reactive compounds enhance biological and chemical processes in the subsurface. Micro- and molecular-scale phenomena related to mixing translate to larger, system-level scales and influence the degree of success or failure of a desired engineering result in porous media. *Our objective is to experimentally characterize and computationally describe the growth and distribution of microbes and their activity related to mineral formation in porous media that receive two or more reactive amendments.*

Our model system links microbially induced urea hydrolysis with calcium carbonate precipitation to study the spatial relationships between amendment transport, consumption, and progressive changes of porous media permeability and heterogeneity as biomass and minerals develop. To study the physiology of microbes that precipitate calcium carbonate, we have added urease genes to the plasmid vector PJN105 and inserted the plasmid into *Pseudomonas aeruginosa* AH298, which possesses a chromosomal green fluorescent protein (gfp). This organism expresses gfp and hydrolyses urea. Using small (2 x 2 cm) and intermediate scale (4 x 8 cm) two-dimensional porous media reactors, we have controlled biofilm growth and mineral precipitation in flow systems. Interrogation of the microbes and minerals in the reactors includes noninvasive tracer and confocal image analysis, influent and effluent chemistry, and destructive analysis of the reactor contents at the end of each experiment. Relying on x-ray tomographic imaging, we can differentiate biofilm, aqueous and solid phases in simulated porous media. A reactive transport model capable of simulating transient phenomena in systems with a large network of chemical or biogeochemical processes, and the tight coupling between the chemical processes and changes in media properties that govern transport, is being adapted for simulations of flow-cell experiments.

The research will expand our current understanding of delivery and mixing of nutrients in biogeochemically dynamic porous-media systems and help identify key physical, chemical, and biological processes that influence the mobility of DOE priority contaminants (e.g., ^{60}Co, ^{90}Sr, U) in the subsurface. Current and future work is focusing on applying the developed tools to assess the effect of microbial growth and calcium carbonate precipitation in small and intermediate scale porous media reactors. Different spatial and temporal injection strategies for the control of permeability as well as distribution of biomass and minerals will be compared. These studies will develop the basis for large (e.g., field) scale application of microbially controlled mineral precipitation strategies, which minimize biomass growth, mineral precipitation, and permeability reduction around injection wells while ensuring homogeneous (or, if desired, localized) mineral precipitation elsewhere.

Geoelectrical Measurement of Multiscale Mass Transfer Parameters

University-Led Research

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Evidence of multiscale mass transfer at contaminated sites includes long “tailing” behavior and concentration rebound observed during remediation of groundwater. The lack of experimental methods to verify and measure mass transfer *in situ* or independently of tracer breakthrough results in significant uncertainties in estimates of controlling parameters. Our research objectives are to develop and demonstrate geophysical strategies to measure mass-transfer parameters over a range of spatial scales; and to provide geophysical estimates of mass-transfer parameters for Hanford 300 Area materials. Here, we investigate the utility of two geophysical methods--time-lapse resistivity (ER) and complex resistivity (CR)--to improve the basic and site-specific understanding of mass transfer.

During our first year of funding, we have focused on (1) training, whereby students from Pennsylvania State U. and Lancaster U. studied at Oregon State U.; (2) selection of materials for controlled laboratory experiments; (3) design and testing of laboratory setups for time-lapse ER and static CR measurements; and (4) development of codes for data modeling and analysis. To date, we have constructed the column ER/tracer-test apparatus and performed testing using samples of the porous zeolite clinoptilolite, which has well defined mass-transfer properties. Time-lapse ER data were inverted using the Lancaster U. code R3t*, and parametric sweeps were performed in COMSOL to identify transport parameters. CR spectra measured on sieved fractions of the same zeolite show different relaxation-time distributions. Calibration of a multiple Debye model to the CR spectra is ongoing. Pore-scale models are being developed to simulate both hysteresis observed in time-lapse ER and induced-polarization response. The focus of our experimental work will soon transition to cores. Our work dovetails with CR analyses being performed by Rutgers U. for the Hanford Integrated Field Research Challenge (IFRC). Additional core material is being sourced.

Additional laboratory-based CR measurements will focus on understanding the influence of tracer distribution on CR spectra in an attempt to relate electrical spectra to mass-transfer properties. Our original field plan focused on ER monitoring of push-pull tracer experiments at the Hanford 300 Area IFRC site; however, vertical in-well flows and the dynamic nature of the water table at the site render push-pull experiments problematic. We have proposed instead to focus on installation of vertical ER/temperature/fluid-sampling arrays adjacent to the Columbia River. This revised plan will investigate whether time-lapse variation in ER observed in research under a separate grant (DEAI02 08ER64565, PI L. Slater) may be explained by mass transfer concomitant with the exchange of groundwater and surface water with different total dissolved solids. Field experiments are planned for summer 2011.

*Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

The Natural Enrichment of Stable Cesium and Related Alkali Metals in the Savannah River Site (SRS) Soils

University-Led Research

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In this research, we are testing two interrelated hypotheses about Cs sorbed by weathered micaceous materials in subsurface materials from the SRS: (1) that stable Cs has become significantly enriched relative to K in subsurface micaceous particles as a result of chemical weathering processes, and (2) that the Cs so present is sufficient to be a major factor determining the ability of the subsurface materials to take up and hold ^{137}Cs. Quantification of the amounts of stable Cs concentrated in various sites within 2:1 phyllosilicates by natural processes is highly relevant toward understanding the future sorption of ^{137}Cs by the mica, illite, vermiculite, and HIV (Al-hydroxy interstratified vermiculite)phases present in the subsurface at and in proximity to SRS. Such knowledge will help in the development of ^{137}Cs remediation strategies for the long-term, which is a critical aspect of the SBR goals.

The clay mineralogy of the near-surface soil samples collected from SRS consists of kaolinite and an HIV. The amounts of these phases are quantified through the use of powder synchrotron radiation. Experimental study of acid extractions across a range of temperature (room temperature to 75°C), supplemented by a long-term (8 months) study of Rb isotope exchange at room temperature, indicates three distinct populations of Cs, Rb, and K in five near-surface SRS soil samples:

(1) Exchangeable ions that reach exchange equilibrium within a few days at room temperature. This population accounts for about 2% of the K and about 5% each of the Rb and Cs in the soil samples.

(2) Ions that undergo restricted exchange, at rates that are very low at room temperature. Acid-extraction rates increase nearly tenfold when temperature is increased to 50°C and again by a somewhat smaller factor when temperature is increased to 75°C. This population accounts for about 25% of the K, roughly 60% of the Rb, and nearly 90% of the Cs in the soil samples. At room temperature and low acidity, however, only 12% to 20% of the Rb in five samples equilibrated with added isotopically enriched Rb over 8 months, which indicates that most of the Rb in this population exchanges so slowly that it is effectively fixed in the soil.

(3) Ions extractable by acid only very slowly at 75°C. We consider these ions to be bound in silicate structures—most likely in remnants of primary muscovite within HIV particles—that dissolve only very slowly at 75°C. Most of the K, but not most of the Cs, and Rb, is in the silicate structures.

In these five soil samples, the amount of Cs is closely correlated with the amount of material finer than sand, corresponding to roughly 15 mg of Cs per kg of fine material. The average mass fraction of Cs in typical fine material of the earth's upper crust (shale) is also 15 mg/kg. The relatively large amounts of Cs in these SRS soil samples imply that the soils have effectively retained Cs over thousands of years of pedogenesis, while most of the original K (from the parent sediments) was leached away. Our results, coupled with measurements of Cs in muscovite from the Coastal Plain, indicate that very little of the natural Cs resides in primary silicate minerals. Rather, nearly all of the Cs has apparently become fixed in sites where it is slowly removable by acid extraction. These sites are likely to be near the apices of interlayer wedges within HIV, where steric hindrance to ion exchange could account for the slowness of extraction of the Cs by acid. Further study with chemical extractions, isotope exchange with radiocesium, and *ab initio* modelling will be useful to elucidate the sorption and retention mechanisms of Cs in the SRS soils and in DOE complexes elsewhere.

Variation of Cesium Concentration, Clay Content, and Mineralogy with Depth of Three Savannah River Site Soils

University-Led Research

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Three uncontaminated soil cores collected from the Savannah River Site (SRS) were used to study the uptake of Cs and other alkali metals in the soils at SRS. Each core presents unique soil forming processes: Core 1–"Fuquay," a thick soil with developed horizons formed on a nearly level plateau in the Upland soil region of SRS, Core 2–"FQTRS," a young soil formed on the SRS parent bedrock Tobacco Road Sand, and Core 3–"FQQAL," an azonal soil formed on quaternary alluvium deposits.

By comparing average upper continental crust concentrations, previous results showed that stable Cs in the Fuquay core is significantly enriched with respect to K. This enrichment in stable (natural) Cs, far greater than that in the other two cores, is suspected to affect the soils' ability to sorb and retain radiocesium in contaminated areas at SRS. This enrichment may be explained by variations in the mineralogy among the cores. In order to test that hypothesis, each section of the cores were examined for (1) natural cesium concentration, (2) variations of the clay content, and (3) mineralogical characterization of the clay fraction.

The trends in Cs concentration with depth vary in these three soils. In the FQTRS core, the peak Cs concentration is correlated with a peak in percentage of clay content, ~25%. In the other two cores, the Cs concentration is not correlated with the percent clay. The peak Cs concentration in the Fuquay core is 121 cm below the surface, where the clay percentage is only ~10%. The Cs concentrations in the alluvium soil, FQQAL, increase to a peak at a depth of 51 cm, then decrease, while the highest clay content is detected 86 cm from the surface.

Characterization and quantification of the mineralogy of the cores using synchrotron powder x-ray diffraction techniques revealed that significant amounts of Al-hydroxy interlayered vermiculite (HIV), with as much as 27% of 14 Å phase quantified in the clay fraction of the Fuquay core. The other minerals in the clay fraction are kaolinite and quartz, with trace amounts of gibbsite. The clay fractions of cores 2 and 3 are composed mostly of kaolinite and quartz. Only small amounts of the HIV phase are seen in the XRD analyses. The low retention of Cs in these soils versus the Fuqauy, Core 1, is likely a function of the low cation exchange capacity of kaolinite and the small amounts of HIV.

Among the three cores, the apparent correlation between the amounts of Cs sorbed and the clay mineralogy revealed that HIV exerts a strong control on Cs sorption capacity. Further study by chemical extractions, quantification of HIV in both clay and whole rock fractions, and *ab initio* molecular modeling studies will elucidate further the sorption and retention mechanisms of Cs in the SRS soils. Such knowledge will help in the development of ^{137}Cs remediation strategies for the long term, which is a critical aspect of the SBR goals.

Fungi in U-Polluted Subsurface Groundwater: Autochthonous or Transient Members of Microbial Community?

University-Led Research

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The major goal of this project is to study biotic interactions in polluted subsoil by using *in situ* cultivation devices. Our intent is to build a representative library of microbial isolates serving as a gene pool for various bioremediation applications. Earlier we reported on isolation from FRC of 71 bacterial strains representing 58 species, about a third of which were previously known only from their phylogenetic signatures. Here we report on investigation of microbial composition in the borehole FW106, which exhibits the highest degree of contamination, and a site of earlier metagenomic analyses. Unexpectedly, we found abundant populations of micromycetes. We present below a summary of taxonomic and physiological data, with special emphasis on adaptation of isolated microorganisms to toxicity stress.

Samples of groundwater were taken from the boreholes FW215 (Nov-2009) and FW106 (Jul-, Sep-, Oct-2010) by the ORNL personnel, shipped on ice to our laboratory and kept under anoxic conditions at 2-4 ^{o}C before use for isolation, typically within 2-3 d of sampling. Isolation was done under aerobic and anaerobic conditions by using (1) plating, (2) enrichment anaerobic culture, (3) serial dilutions in microtiter plates and (4) ichip technique. As nutrient media, we used R2/R2A under aerobic conditions and various combinations of electron donors/acceptors for anaerobic cultivation to encourage development of the metal-reducing organisms. After isolation and purification, the identity of isolates was established by sequencing their ribosomal genes (16S, 18S, 25S, ITS). The obtained isolates were tested for their resistance to major toxic compounds detected in FW106 at concentrations ranging from zero to up to 10-100 times above the ambient. Growth was followed by OD_{600} in plate readers. In parallel microcosm experiments, groundwater samples were amended with various C-sources and incubated under oxic or anoxic conditions at 20^{o}C; growth was recorded from the rate of CO_2 production with membrane-inlet mass-spectrometry and residual dynamics of added and endogenous organic compounds (GC-MS).

In laboratory incubations under anoxic conditions, consumption rates of the added substrates, and concomitant metal (Fe, U) reduction, were relatively low, confirming published FRC data. However, under oxic and microaerophilic (~1 % of DO) conditions, microbial growth initiated immediately even without added C-sources. The bulk of visually observed cell mass was formed by various fungi, which we subsequently isolated and purified. Employing extended incubation times, we observed development of bacteria in close association with fungal mycelium as “primary colonizers.” Each sample of groundwater was characterized by unique combinations of micromycetes with none displaying a global dominance. Based on a combination of morphological, physiological, and phylogenetic data, they were identified as *Pseudeurotium zonatum, Arthrinium sp., Penicillium glabrum, Rhizopus sp. Exophiala oligosperma* and *Mucor hiemalis*. The bacterial library contained 20 strains representing mainly firmicutes and actinobacteria, some of which were related to uncultivated species found in groundwater. The isolated microorganisms proved more resistant to all major toxic factors (nitrate, Ni, Al, benzoic acid, acetone, butanol, tetrachlor- and dichloethene) than taxonomically similar organisms obtained from culture collections and isolated from the nonextreme habitats. Kinetic analyses of growth dynamics in the presence of toxic compounds provided insights into the mechanisms of inhibition and resistance. In particular, we were able to discriminate reversible and irreversible, continuous and threshold inhibition, inhibitors reducing metabolic activity and growth rate as well as toxic compounds reducing cell yield due to uncoupling action.

Based on obtained results, we tentatively conclude that fungi are a genuine component of the FRC subsurface microbial communities rather than “accidental visitors.” They evolved under unique combinations of environmental conditions, such as high toxicity, acidity, low to zero oxygen availability with organic pollutants as abundant source of potentially utilizable carbon. None of them was able to metabolize under strictly anaerobic conditions. Their survival strategy appears to be an explosive growth on organic pollutants during temporal aeration of the otherwise mostly anoxic subsurface environment.

U(VI) Reduction in Microfluidic Networks and Batch Cultures by Biotic and Abiotic Processes

University-Led Research

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The objective of this work has been to characterize the combined effects of hydrology, geochemistry, and biology on the bioremediation of U(VI). Our underlying hypothesis was *bioremediation of U(VI) in groundwater is controlled by transverse mixing with an electron donor along plume margins, and that* iron *bioavailability in these zones critically affects U(VI) reduction kinetics and U(IV) re-oxidation*. Our specific objectives are to (a) quantify reaction kinetics mediated by biological versus geochemical reactions leading to U(VI) reduction and U(IV) re-oxidation, (b) understand the influence of bioavailable iron on U(VI) reduction and U(IV) re-oxidation along the transverse mixing zones, (c) determine how transverse mixing limitations and the presence of biomass in pores affects these reactions, and (d) identify microbial populations that develop along transverse mixing zones and how the community development is influenced by the presence of iron and the concentration of electron donor.

The experimental tasks have utilized etched silicon microfluidic pore networks (micromodels) to simulate microscale hydraulic mixing zones within aquifer material. These micromodels have been used previously to understand the influence of transverse mixing (of electron donor and electron acceptor), biomass architecture, and biogeochemical reactions on contaminant fate and transport. We have used micromodels in the presence and absence of ferric iron, to assess the critical role that total iron plays in uranium bioremediation. Electron donors including acetate and lactate, U(VI), and Fe(III)-reducing cells have been introduced to the micromodels in a controlled manner to characterize the mechanisms and kinetics of both U(VI) reduction and U(IV) reoxidation, and to correlate this with biofilm structure along the transverse mixing zone(s). We have also used strictly abiotic experimental systems to determine the influence of abiotic reductants such as sulfides and hydroquinones on U(VI) reduction in the presence and absence of Fe(III).

During the past year, micromodel development has focused on two interdependent themes: establishing an Fe(III)- or U(VI)-reducing microbial community in the microfluidic network and quantifying changes in biomass, ferrous iron (effluent), and uranyl carbonate (effluent) during micromodel operation. We tested several inoculation strategies, but ultimately selected adding cells by pumping cells alone in bicarbonate buffer, from a washed cell suspension, into the micromodel while pumping medium that contained both electron donor and electron acceptor via the alternate syringe. This allowed cells to mix along the transverse zone with donor plus acceptor, without the risk of cells growing in the syringe or the pump lines, which minimized clogging or fouling. Cells did establish distinct biofilms along the transverse mixing zones, with biomass growth on the downgradient edges of the posts within the pore network. Biomass loads were very low, and changes in iron and uranium effluent chemistry were difficult to monitor. We continue to investigate the best method for establishing robust biofilms along the transverse mixing zone.

Additional experiments with batch cultures (to support the micromodel development) were run with *Geobacter metallireducens* and uranyl adsorbed to the surfaces of either ferrihydrite or aluminum oxide. U(VI) was reduced by hydroquinones (as a model reductant) irrespective of whether the system contained aluminum or iron, suggesting that adsorbed U(VI) is available even when iron is not present. However, the buffer selection and concentration of bicarbonate was critical to reduction in the presence or absence of iron. Increasing bicarbonate decreases reduction of U(VI) in both iron and aluminum suspensions. In addition, aqueous uranyl is not reduced in bicarbonate buffer, where it is immediately reduced in HEPES buffer. This has implications for past experiments in which HEPES buffered suspensions were used to establish rates and mechanisms of adsorbed uranyl reduction, as HEPES seems to increase the capacity for reduction, while bicarbonate (which is the environmentally relevant buffer) decreases uranyl reduction in the presence of solids.

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

University-Led Research

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Remediation of subsurface metal contaminants at DOE sites involves microbial mechanisms of oxidation/reduction or complexation; which are controlled in large part by the ecology of the microbial community. Recognizing and quantifying the relationships between community structure, function, and key environmental factors may yield quantitative understanding that can inform future decisions on remediation strategies.

The objectives of our project are to: (1) determine if the trajectories of microbial community structure, composition, and function following organic carbon amendment can be related to, and predicted by, key environmental determinants; (2) assess the relative importance of the characteristics of the indigenous microbial community, sediment, groundwater, and OC supply rate as the major determinants of microbial community functional response and bioremediation capacity. We are using sediments from three DOE sites: Oak Ridge, TN; Rifle, CO; Ringold formation, Hanford, WA. In order to provide a solid foundation for this experiment, we extensively characterized the three subsurface bacterial assemblages using G3 16S rRNA gene PhyloChips, G4 GeoChips, and analyzed the physical-chemical properties of the three sediments. Standard physical-chemical analysis of sediment material was performed on the bulk sediments (< 2.0 mm) as well as three particle size fractions (PSF) (< 20 µm, 20-200 µm, 0.2-2.0 mm).

The three sediments have markedly different particle-size distributions; which may affect the assemblage structure and composition between sediments and thus the trajectories of the transplanted assemblages. The majority of microbial cells are located on the smallest PSF in all sediments. Sediment type and PSF are significant determinants of detected richness at the subfamily level by PhyloChip. However, sediment type is more important than PSF for explaining bacterial assemblage composition overall.

The degree of compositional overlap at the operational taxonomic unit (OTU) level was low between the sediments, with 44.4% of 16,584 detected OTUs common to the three sediments. The mean detected (OTU) richness differed by site (Hanford: 5,145 ± 663; Oak Ridge: 8,390 ± 604; Rifle: 11,508 ± 747) (mean ± 1 s.d.) by PhyloChip analysis. Strikingly, the opposite pattern was observed for detected functional gene richness (Hanford: 16,953 ± 1,486; Oak Ridge: 12,880 ± 1,309; Rifle: 10,380 ± 956) (mean ± 1 s.d.) by GeoChip analysis. Ordination using presence/absence data at different taxonomic ranks (by PhyloChip) showed that the assemblages in each sediment are significantly different and distinguishable at the phylum level (Anosim $R=0.786$, $P < 0.001$).

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

University-Led Research

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Our objectives are (1) to determine the mechanisms of colloid mobilization and colloid-facilitated radionuclide transport in undisturbed Hanford sediments under unsaturated flow, (2) to quantify *in situ* colloid mobilization and colloid-facilitated radionuclide transport from Hanford sediments under field conditions, and (3) to develop a field-scale conceptual and numerical model for colloid mobilization and transport at the Hanford vadose zone.

We used fluorescent microspheres to study pore-scale colloid mobilization by moving liquid-gas interfaces. A cylindrical glass channel was used as a model for a soil pore. Colloids were deposited to the inside of the pore, and a series of air-bubbles were passed through the capillary at different velocities. We visualized colloid removal *in situ* and real time by confocal microscopy. The receding interface did not remove significant amounts of colloids from the solid-liquid interface, but the advancing interface removed a significant amount. Colloids were being attached to the moving air-water interface and translocated along the pore, trapped at the air-water interface. Increasing interface velocity led to decreased colloid removal. These results indicate that colloid mobilization will be most pronounced during infiltration, and less dominant during drainage events in porous media. Direct force measurements using a process tensiometer allowed us to quantify capillary forces for model particles and natural subsurface minerals. For particles in the size range of fine sands, silt, and clay, the repulsive capillary force exceeds attractive DLVO and gravity forces. The water-solid-air contact angle is a fundamental thermodynamic property controlling the strength of capillary forces acting at the air-water interface. We developed a method to experimentally determine the contact angle for subsurface clay minerals.

We further studied the interaction of Hanford colloids with fulvic and humic acids, and we found that the colloids form more stable suspensions in the presence of fulvic and humic acids. We monitored colloid transport in the field lysimeters at the Hanford 300N Lysimeter Site. The facility consists of six lysimeters, each 7.6 m deep. Fiberglass wicks were installed at 1, 2, 4, and 7 ft below surface to collect vadose zone water. We applied Eu-hydroxycarbonate and carboxylate-modified polystyrene colloids to the lysimeters. The lysimeters were irrigated with different flow rates. (Two lysimeters were exposed to natural rainfall, while the others were being irrigated regularly with multiples of the average recharge--124 mm/year, 248 mm/year, and 248 plus a 100 mm Chinook event.) Wick outflow has been analyzed for pH, electrical conductivity, colloid concentrations, electrophoretic mobility, and particle size.

Soil cores were taken in August 2010 to determine depth profiles of colloid concentrations. Colloids have been detected in the outflow, but most of the colloids still remain in the top 30 cm of the soil. We instrumented one additional lysimeter in the Hanford 200 Area (Field Lysimeter Test Facility) with fiberglass wick samplers in late February 2010. No colloids were applied to this lysimeter, which we used to monitor *in situ* colloid mobilization.

We developed an experimental approach and apparatus to study solute and colloid transport in porous media using the 2 m radius geocentrifuge at the Idaho National Laboratory. The experimental techniques include water flux scaling with applied acceleration at the top of a soil or sediment column and subatmospheric pressure control at the column base. We successfully tested the experimental techniques and equipment through a series of unsaturated flow-transport experiments using a conservative tracer and a sand column, with different centrifugal acceleration up to 40 g. Undisturbed Hanford sediments cores have been sampled for use in the geocentrifuge studies. The model PFLOTRAN is being adapted to simulate colloid transport through the Hanford Vadose Zone at the 200 area. Vadose zone sediments are represented as heterogeneous, layered sediments. Long-term, daily precipitation records from the Hanford Meteorological Station are being used to represent the time period from 1945 to present.

Uranyl Speciation at Oxide/Aqueous Interfaces at pH 4 and 7 and 1-10 mM NaCl Studied by Second Harmonic Generation

University-Led Research

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Resonantly enhanced second harmonic generation (SHG) is used to study uranyl adsorption at a buried mineral oxide/water interface in real-time, under flow conditions, and in the presence of environmentally relevant screening electrolyte concentrations for pH 4 and 7. The *in situ* SHG spectrum of surface-bound uranyl reveals well-defined electronic resonances in the UV, which are used to collect uranyl adsorption isotherms at the fused silica/water interface at pH 4 and 7, and in the presence of aqueous carbonate. The adsorption free energies determined by the Langmuir isotherm are consistent with physisorption via hydrogen bonding and invariant with electrolyte concentration for both pH conditions. The results showed that binding at pH 4 was 10 kJ/mol less favorable that at pH 7, indicating that the uranyl/surface site interaction varies with bulk pH. This finding suggests that the uranyl adsorbates are either neutral or univalent cationic species. Complementary surface charge density data, obtained using the Eisenthal chi(3) technique, reveals that the charge on the ionic uranyl species adsorbing under the experimental conditions are positive. It is proposed that a mixture of neutral and univalent, cationic uranyl species is surface active at pH 4 and 7. Insofar as the experimental conditions model those found in natural groundwater systems, the results of this work are valuable for the prediction and assessment of uranium pollution transport in groundwater and soils. Our thermodynamic results can also serve as important benchmarks for computer simulations of U(VI) transport in heterogeneous geochemical environments.

Bacterial Nanowires and Extracellular Electron Transfer to Heavy Metals and Radionuclides by Bacterial Isolates from DOE Field Research Centers

University-Led Research

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Electrically conductive extracellular appendages known as bacterial nanowires have been confirmed in organisms ranging from oxygenic, photosynthetic cyanobacteria to methanogenic co-cultures. Nanowires produced by metal-reducing bacteria *Geobacter sulfurrreducens* and *Shewanella oneidensis* MR-1 coordinate extracellular electron transfer to solid-phase electron acceptors such as iron and manganese oxides and electrodes in micorbial fuel cells at distances reaching 10s of microns away from cell surfaces. Reduction of U(VI) and precipitation of reduced uranium minerals has been observed in suspensions of metal-reducing and sulfate-reducing bacterial. Hence, bacterial nanowires provide significant potential for influencing biogeochemical processes important for the fate and transport of heavy metals and radionuclides in saturated subsurface sediments.The primary objective of this proposal is to investigate the composition, conductivity, and enzymatic activity of bacterial nanowires from organisms isolated from uranium-contaminated environments and to evaluate their role in mitigating the transport of uranium in saturated porous media.

Research has commenced toward the completion of targeted goals.

- Conductivity along the length of a bacterial nanowire has finally passed peer review and recently published in PNAS, with support from DOE gratefully acknowledged.
- Conductivity along the length of a *Desulfovibrio* nanowire was recently confirmed in a manuscript nearing submission.
- Innovative cultivation chambers have been designed to investigate '"long range" electron transfer to heavy metals and radionuclides at distances exceeding 1000 microns. These efforts were led by Jeff McLean with assistance from Greg Wanger (DOE-funded post doc).

A portion of the research conducted during the first 9 months of the project diverged from stated objectives while maintaining scientific relevance for DOE missions. Research conducted in collaboration with Andre Revil (Colorado School of Mines) and researchers from the University of Aarhus (Denmark) has tested and confirmed the hypothesis that bacterial nanowires contribute to the electronic properties of redox transition zones within anoxic sediments. This research has also culminated in the development and application of microsensors to evaluate the contribution of nanowires and associated biogenic minerals to the biogeophysical phenomenon referred to as "self potential." Instrumentation and approaches being developed through this ad hoc collaboration are expected to contribute to a more complete understanding of the fundamental processes at work within the remediation strategies being tested at various DOE Integrated Field Research Centers, including Oak Ridge, Rifle, Colorado, and Hanford. The results of this exciting research will soon be submitted for publication.

A Universal Parameter and Framework for Predicting Distribution and Transport of Microorganisms in the Subsurface Environment

University-Led Research

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This project aims to explore and develop new enabling techniques for micro-/nanoscale characterization of microbe cell surface properties and interactions--techniques that allow for identification and quantification of indicative properties related to microorganism migration and transport behavior in porous media and in subsurface environments. Nanoscale cellular surface properties that govern the macroscale cell-cell aggregation and cell-surface attachment tendencies for a number of IFRC-relevant microorganisms were identified, and new theoretical and mathematical framework for microbe transport model in porous media is proposed.

Comprehensive characterization of DOE-relevant microorganisms was conducted including hydrophobicity, surface roughness, zeta potential, cell membrane stiffness, extracellular surface molecular brush, and aggregation behavior. In addition, the nature, magnitude and range of intersurface forces and surface potential at cell-cell and cell-substrate interfaces are quantified using Atomic Force Microscopy (AFM). These microscopic properties are correlated with the macroscopic breakthrough curves and attachment efficiencies measured by flow-through column experiments. A theoretical model based on the elastic deformation of microorganisms under the influence of DLVO-electrostatic double layers is constructed, aiming to generalize the transport behavior of a wide spectrum of microbes in water-saturated porous media, especially the dual fast-slow deposition rates.

The sample microorganisms exhibited distinctive aggregation tendencies and attachment efficiency via transport through porous media, indicating microbial bio-originated factors that influence the microbe movement. The attachment efficiency (h) ranged widely from 0.93 for strong microbe-substrate attachment to 0.06 for weak attachment, depending on the bacteria strain as well as the ionic strength of the aqueous environment. The electrolyte concentration was found to have influence on the cell stiffness and thus adhesion-detachment. No consistent correlation was found between h and any individual conventional microscale physicochemical properties such as the electrophoretic mobility and elastic modulus of a single cell. Mathematical calculation of interaction energy based on DLVO theory failed to predict the deposition rates observed, indicating that the current DLVO theory is not sufficient for predict microbial transport, especially for those strains with higher aggregation index. A universal Tabor parameter N_{Tabor} was proposed, which incorporates microscopic cell behavior and interfacial properties into macroscopic attachment efficiency and cell adhesion-aggregation-transport. A remarkably strong correlation with preliminary data was shown.

For large and soft microbes under strong intersurface forces, the inevitable elastic deformation couples drastically altered the adhesion-detachment mechanism under fluid flow, especially in the presence of a repulsive energy barrier and an attractive secondary minimum in the DLVO surface potential. We derive the theoretical but experimentally verifiable "pull-off" force (F^*) needed to detach a spherical/cylindrical shell of bacteria from an adhering substrate, which depends on the exact surface potential as a function of intersurface separation and elastic modulus of the cell wall. Combined with the flow characteristics via the Peclet number that governs the advection-diffusion, F^* holds the key to microbe transport in porous media and also cell-cell aggregation. In the long run, once a microbe strain is identified and its interfacial properties fully characterized, the new Tabor parameter can reliably predict the fate and transport of microorganisms in granular porous media.

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

University-Led Research

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

During the third year, efforts focused on completing sensor validation studies in laboratory-scale box aquifers, field testing in both the La Quinta gallery and the Super 8 gallery at the Rifle IFRC site, and the development of a new field method for measuring microbial biomass fluxes using PFM measures of water fluxes. Field tests in the La Quinta gallery at the Rifle IFRC site were conducted to assess ambient groundwater, uranium, and microbial biomass fluxes. The latter were determined using a newly designed Baffled Multilevel Sampling (BMLS) device installed in typical screened monitoring wells to provide aqueous concentrations of dissolved or suspended constituents over multiple isolated vertical sections of the well. Biomass mass fluxes were calculated from the product of BMLS data on microbial cell counts from PCR analyses and PFM water fluxes collected from coincident well sections. The methodology for generating flux measurements from BMLS data and independent measures of water flux was validated against independent direct flux measures of sulfate and uranium fluxes using the PFM in wells located in La Quinta and Super 8 galleries. Expected microbial discharge for *Eubacteria* in the La Quinta gallery was estimated to be 1.7×10^{12} cells per day. The biomass discharges for *Geobacter*, *Methanogens*, and *Anaeromyxobacter* remain to be determined. Expected uranium discharges predicted from stochastic simulations using PFM measures of flux over the La Quinta gallery transect and the injection-well transect of the Super 8 gallery were 26 mg/d and 183 mg/d, respectively.

In the last year, advances were made with the use of natural tracer to measure water flux by PFMs and the use of PFMs to measure aquifer conductivities. Sulfate concentrations in undisturbed areas are surprisingly constant over time. A test was conducted in the Super 8 gallery showing that PFM-measured sulfate fluxes and a single sulfate concentration from a grab sample were sufficient to quantify depth average water flux in a well. In a separate test, it was demonstrated that PFM-measured water fluxes can be used with measured water-table gradients to quantify depth variations in aquifer conductivity. The depth-averaged conductivity from these measures compared closely with multiple slug tests results.

Future analyses of field data in the La Quinta and Super 8 galleries will be closely coordinated with existing ERSP projects to complement data analyses. For these studies, the uranium flux measured by the PFM sensor and biomass fluxes estimated from BMLS data and PFM water fluxes will be used in conjunction with molecular-biological tools to characterize microbial community and active biomass at synonymous wells. These field efforts will be designed to quantify spatial changes in uranium flux and field-scale rates of uranium attenuation (ambient and stimulated), uranium stability, and to quantitatively assess how fluxes and effective reaction rates are coupled to spatial variations in mineralogy, the microbial composition, and active biomass. Analyses of data will lead to estimates of the uranium discharge that can be used to estimate kinetic parameters that are “effective” in nature and optimal for modeling uranium fate and transport at the field scale. Proper development of these models is critical for long-term stewardship of contaminated sites in the context of predicting uranium source behavior, remediation performance, and off-site risk.

Assessing the Role of Iron Sulfides in the Long-Term Sequestration of Uranium by Sulfate-Reducing Bacteria (SRB)

University-Led Research

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This UM/ASU research seeks to identify the potential for reduced iron sulfide minerals to inhibit the rate of oxidation of reduced U solid phases formed by sulfate-reducing bacteria (SRB). SRB utilize sulfate as a terminal electron acceptor and produce sulfide. When iron is also present, iron sulfide solids are produced. At U-contaminated DOE sites, aqueous-phase U concentrations can be effectively lowered by reducing dissolved U(VI) species to insoluble U(IV) solids. SRB can accomplish this reduction step directly by enzymatic electron transfer processes, or indirectly, through chemical reduction by the sulfides species produced. *The working hypothesis of this study is that iron sulfides will inhibit the kinetics of re-oxidation of U(IV) to U(VI) when oxidants such as oxygen, nitrite, or Fe(III) are introduced.*

The experimental plan for the current period included: (1) biogenic (by SRB in presence of Fe(II) or U(VI)) and chemogenic (by NaS in the presence of Fe(II) or U(VI)) production of iron sulfides and uraninite; (2) evaluating the impact of a range of sulfate-reducing conditions on the solid phases formed by a SRB*;* (3) batch and column reactor studies of oxidation rates of FeS and uraninite by oxygen; and (4) solid and solution U, Fe, and S speciation characterization to establish oxidation mechanisms.

Biogenic and chemogenic iron sulfide solid and reduced U solid were successfully produced and scaled-up for the kinetic studies. From XRD, the generated iron sulfide and U(IV) solid phases were confirmed to be mackinawite (FeS) and uraninite (UO_2(s)), respectively. Interestingly, Mössbauer characterization of FeS solids gave a different pattern for biogenic vs. chemogenic FeS. The presence of structural Fe(III) or am-FeS are suspected in the biogenic samples. XAS, TEM, and Mössbauer spectroscopy data analyses are ongoing to confirm S, Fe, and U structural properties and to explain possible differences in reactivity of biogenic and chemogenic samples.

Biogenic solids were produced by *Desulfovibrio vulgaris*. Over a range of pH (6.5 to 8.6) and ratios of lactate:SO_4 (0.5:1 to 1.9:1) and SO_4:Fe (as dissolved Fe(II)),1:1 to 1:0.11), *D. vulgaris* primarily produced mackinawite (verified by XRD). Higher lactate:SO_4, lower SO_4:Fe, or weakly acidic pH enhanced mackinawite crystallization. Hydrogen and pyruvate as alternate electron donors showed enhanced FeS production compared to lactate. A study of U reduction by *D. vulgaris* was also inititated. *D. vulgaris* enzymatically reduced U(VI) to UO_2(s) when U(VI) was the only electron acceptor, with 90% conversion within 10 hours. U(VI) reduction was faster in the presence of sulfate, implicating abiotic reduction of U(VI) by biogenic H_2S. XAS data of of biogenic UO_2(s) gave first shell O atoms at 2.33Å and second shell U neighbors at 3.84 Å, consistent with previously reported XAS values.

Batch oxidation-kinetic studies were performed using chemogenic FeS(s) and UO_2(s) to identify the mechanism and rates of oxidation in the presence of oxygen under typical groundwater conditions. FeS (5 g/L) inhibited the rate of oxidation of UO_2 (1 g/L) compared to a control experiment without FeS, with elemental S implicated. When all elemental S was depleted, the Fe(III) oixde solids produced from FeS oxidation accelerated UO_2 oxidation to U(VI). Experiments with biogenic FeS and UO_2 are planned to confirm whether similar reaction products and oxidation trends will be observed.

Two UO_2(s) column experiments were prepared for oxidation studies using: (1) chemogenic FeS (2.75g FeS(s)/kg) and UO_2(s) (0.5 g UO_2(s)/kg), or (2) UO_2(s) alone (0.5 g UO_2(s)/kg sand): packed in mixture with RABS sediments from the Rifle CO site. U effluent data suggest FeS improves the retention of U during oxygen-saturated groundwater flow compared to the column with no FeS. Solid phases will eventually be characterized to assess solid-phase changes responsible for the U, Fe, and S effluent behavior. Future studies are planned for introducing biogenic FeS and UO_2 into the columns.

A Spectroelectrochemical Sensor for Pertechnetate

University-Led Research

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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 groundwater. The sensor will have the capability for on-site monitoring, either by immersion in subsurface water for continuous monitoring or for the immediate analysis of collected samples. The project builds on the substantial progress of a well-established UC-PNNL collaboration that 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 consists 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:

- Synthesized a range of low oxidation state dioxo Re-complexes of formula $[ReO_2(py)_4]^+$, $[ReO_2(CN)_4]^-$, $[ReO_2(P\cap P)_2]^+$ and $[ReO_2(S\cap S)_2]^+$. These complexes can be readily synthesized from ReO_4^- at ambient temperatures and display fluorescence in fluid solution.
- In an extension of the chemistry to analogous Tc complexes, $[TcO_2(py)_4]^+$ and $[TcO_2(im)_4]^+$ (im = imines ; e.g.,- 4-picoline, 4-dimethylamino pyridine, 4-cyanopyridine, 4-methoxypyridine, 4-nitropyridine) have been similarly synthesized. The spectroscopy of these complexes both in solid state and in fluid solution (H_2O and MeOH) has been monitored. These complexes exhibit luminescence in fluid solution (weak at room temperature, strong at lower temperatures), which is the first reported solution emission from a readily synthesizable Tc(V) complex.
- Synthesized lower oxidation-state Tc-complex $[Tc(dmpe)_3]^+$ to use as a model compound for developing Tc sensors. It exhibits the important properties of solution fluorescence at ambient temperatures and reversible electrochemistry.
- Luminescence spectroelectrochemistry for $[Tc(dmpe)_3]^+$ preconcentrated within a charge–selective polymer matrix (partially sulfonated triblock copolymer SSEBS) with electrochemical modulation of emission signal demonstrated a linear response over the concentration range of 0.16-340.0 *m*M of $[Tc(dmpe)_3]^+$ in aqueous phase with a detection limit of 24 nM.
- TcO_4^- shown to preconcentrate in sensor films containing anion exchange polymers and to undergo electrochemical reduction.
- Dual analyte model system developed: preconcentrated Pb^{2+} and Fe^{2+} detected by optical stripping voltammetry and *in-situ* ligand complexation followed by redox modulation of absorbance.
- An exhaustive study of the spectroscopy and electrochemistry of $Re(diimine)(CO)_3(halide)$ complexes. These will serve as models for the $Tc(diimine)(CO)_3(halide)$ that can readily be formed from $Tc(CO)_x(halides)_{6-x}$ which are constituents of tank waste samples from Hanford.
- Portable instrumentation for fluorescence spectroelectrochemical sensor fabricated and tested.
- Study of effects of components in Hanford subsurface water on sensor performance initiated with evaluation of effect of total ionic strength on sensitivity.

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Monitoring Spatio-Temporal Soil Moisture Variation in the Shallow Subsurface through Bayesian Inversion of Tomographic GPR Data

University-Led Research

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In this study, we adopt a minimum-relative-entropy (MRE) based Bayesian inversion framework to monitor the spatio-temporal distribution of dielectric permittivity, using tomographic radar first-arrival time information. First, we apply the approach using data from a synthetic, transient infiltration experiment in the shallow subsurface. In this experiment, a spatially correlated random saturated hydraulic conductivity field is generated and used as input to a numerical flow simulator (i.e., TOUGH2), which provides various yet realistic distributions of soil moisture and dielectric permittivity on a fine grid at different temporal snapshots. For each snapshot, a tomographic GPR simulator is utilized to produce first-arrival travel times. We then test the performance of our inverse modeling framework by inverting and monitoring dielectric permittivity variations using these traveltime data. The inverse modeling domain is divided into coarse gridblocks compared to a flow simulation grid, in order to reduce the dimension of unknowns. Each unknown parameter is first assigned a minimally subjective probability distribution function (pdf) using the MRE method, then pseudorandom dielectric constant parameter sets are drawn from these pdfs using a quasi-Monte Carlo sampling technique. We then compute first-arrival GPR travel times for each parameter set and the corresponding weight based upon the misfit between the calculated model responses and observations.

This inversion approach can deal with nonlinearity and nonuniqueness between data and model parameters and can quantify uncertainty in the parameter estimates, by treating those estimates as probability distributions rather than deterministic estimates. Moreover, the intermediate inversion results can be used as “memory functions” in the form of MRE pdfs for step-wise inversion. Testing results show that this framework is capable of reproducing estimates of saturation at gridblocks throughout the study area, and that accuracy and precision are improved as more data become available. We also studied the accuracy of inverse results with respect to data quality (e.g., noises in radar traveltime data). These outcomes spur ongoing efforts to validate the approach using field desiccation monitoring time-lapse radar data collected at the BC Cribs desiccation field study site. We are also comparing the inversion results using different geophysical forward-modeling approaches (i.e., straight ray-tracing vs. eikonal solver for radar traveltime calculations). We will also evaluate the spatio-temporal correlations between state variables, which can provide informative and supplementary constraints for radar data inversion.

Occurrence and Stabilization of Colloidal and Nanoparticulate Mercury Sulfides

University-Led Research

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Mercury (Hg) contaminates the soils and sediments at several Department of Energy facilities, such as the Y-12 site and adjacent East Fork Poplar Creek (EFPC) in Oak Ridge, TN. Historical and ongoing releases of Hg to streams pose great human and ecological health risks, particularly if the metal is converted by anaerobic bacteria to methylmercury, the highly bioaccumulative form of Hg. Thus, in anaerobic sediments and soils, the chemical speciation of Hg (e.g., formation of Hg sulfides) is directly linked to mobility at the sediment-water interface and subsequent bioavailability to methylating bacteria. Our previous work has shown that during the precipitation of $HgS_{(s)}$, nanoparticulate phases of Hg sulfides are formed as intermediates of precipitation reactions. These nanoparticles are stabilized in solution by dissolved organic matter (DOM) and are important precursors of Hg that may be labile to anaerobic bacteria in porewater. The overall objectives of this exploratory project are to evaluate the potential for the formation of mercury sulfide nanoparticles in the sediments of the EFPC and to identify the geochemical factors that control the production and persistence of nanoparticulate HgS in sediment porewater.

In our initial work on this project, we investigated the mechanisms of Hg-sulfide-DOM interactions under conditions of HgS supersaturation. This work is relevant to the Oak Ridge site where effluent from Y-12 contains relative high concentrations of mercury (>100 ng/L). When this effluent is released to the EFPC, the mercury binds to DOM in the stream, and mercury gradually accumulates in the sediments of downstream rivers, including anaerobic regions that are relevant for methylmercury production. We used a combination of dynamic light scattering, X-ray diffraction (XRD), and transmission electron microscopy (TEM) to show that humic acid was capable of stabilizing aggregates of HgS nanoparticles (~5 nm). These particles appeared mostly amorphous in TEM images; however, XRD data demonstrated metacinnabar-like structure.

We performed additional experiments with zinc-sulfide-DOM nanoparticles, which behave similarly to mercury sulfides. In these experiments, zinc sulfide was precipitated in the presence of dissolved cysteine, an amino acid that was used as an analogue for thiol functional groups in DOM that bind metals such as Zn^{2+} and Hg^{2+}. We used small angle X-ray scattering (SAXS) and X-ray absorption spectroscopy (XAS) to probe the cluster size, aggregate structure, and coordination environment of Zn-S-cysteine nanoclusters that formed during the early stages of precipitation. Our results demonstrated that cysteine slowed aggregation rates of ZnS clusters by coordinating to Zn atoms on the edges of clusters. Furthermore, in solutions with molar ratios of 2:1 cysteine:ZnS, we observed larger cluster subunits compared to solutions with 1:1 cysteine:ZnS, indicating that the cysteine did not block growth sites on the surface of monomer subunits.

We hypothesize that the mechanisms of ZnS-cysteine polymerization are analogous to heterogeneous metal-sulfide-DOM precipitation (including HgS). Therefore, the next steps are to apply this same approach to study HgS precipitation kinetics with humic substances. In our related work with ZnS, we used nine DOM fractions obtained from several different aquatic environments and representing a range of DOM structure and composition. These results indicated that growth and aggregation rates of ZnS were strongly influenced by the aromatic carbon content and other macromolecular components of humic substances, rather than interactions of metal-binding functional groups. Ultimately this work will help to identify and characterize the geochemical forms of mercury that are being exposed to methylating anaerobic bacteria.

Effects of Pore-Scale Physics on Uranium Geochemistry in Hanford Sediments

University-Led Research

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The Hanford 300 Area sediments mainly contain coarse river cobble and gravel, which frequently have clay "skins", with sands and fines (silt + clay) variably filling the voids in between. The fines comprise only 1.78% of the total sediment mass, but hold a disproportionately large fraction of the total U. On the other hand, the larger-sized sediments (i.e., >2 mm) with their larger mass fraction may serve as a long-term U release pool, even though they have contain a much smaller U concentration. We hypothesized that the mass-transfer limitation of U release at the 300 Area is partly due to the low connectivity of intragranular pores in the sediments. Low pore connectivity results in porosity decreasing, and tortuosity increasing, as functions of distance from a grain's surface; these produce an anomalous pattern of contaminant release over time. The objective of this project is to determine whether, and to what extent, low pore connectivity affects U distribution and release in Hanford 300 Area sediments of various size classes. Our approach integrates laboratory experiments (stirred flow cell, state-of-the-art ICP-MS instrumentation to measure elemental concentration in liquid samples, laser ablation (LA)-ICP-MS for elemental mapping of 2-8 mm sized solid samples) with pore-scale network modeling to achieve the research objectives.

To date, we have performed the following tasks with the associated results:

1) We gathered 300 Area sediment samples (< 8 cm fraction) in the smear zone of the Integrated Field Research Challenge site (the same samples have been used in other SBR projects), separated the samples into four size fractions of <75 µm (4.4%), 75-500 µm (20.3%), 500-2,000 µm (33.4%), and 2-8 mm (41.8%), and determined their mass fraction percentage; the 2-8 mm fraction samples were classified by mineralogy (basalt comprised 83% of the weight, while breccia, calcite, chert, conglomerate, feldspar, and serpentine were 2-3% each);
2) We conducted 3-D elemental mapping on a 2-8 mm basalt grain to assess U concentration distribution as a function of distance from the grain's surface--we are not aware of any other work to produce such 3-D maps in geological media;
3) We set up and initiated stirred flow cell experiments to study the U release behavior from each of the sediment particle size fractions; we expect that the fine-sized fraction (<75 µm) will have a quicker and higher U release than the coarse-sized fraction (e.g., 2-8 mm);
4) We developed a pore-scale network model to examine pore connectivity issues related to contaminant retention and release. The model supports our hypotheses about porosity, tortuosity, and anomalous patterns of contaminant release, and predicts U concentration distributions consistent with those observed in (2).

Hg Stable Isotope Measurements as Indicators of Biogeochemical Cycling of Hg in East Fork Poplar Creek

University-Led Research

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Mercury (Hg) is a highly toxic contaminant, with complex biogeochemical cycling that complicates efforts to predict its environmental impact. Improved scientific understanding of the processes involved and the operation of those processes at contaminated sites is needed to improve remediation efforts and long-term stewardship at DOE sites. This has prompted the formation of the Oak Ridge National Laboratory Remediation Science Focus Area (ORNL-SFA), a research effort that includes basic research in Hg biogeochemistry and an initial focus on East Fork Poplar Creek (EFPC) on and near the Oak Ridge Reservation. The general goal of the research proposed here is to develop and apply a promising new geochemical tool, Hg stable isotope analysis, to enhance ORNL-SFA efforts by improving detection and quantification of key processes and fluxes.

Hg stable isotope studies have unique potential to distinguish different sources of Hg, to detect and quantify certain biogeochemical reactions, such as breakdown of methylmercury (Me-Hg), and Hg(II) reduction, and to reveal mechanisms of those reactions. Hg has seven stable isotopes (^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{204}Hg). Two distinct mechanisms alter the relative abundance of the isotopes. The first mechanism is the kinetic isotope effect, whereby lighter isotopes react more readily during reduction of Hg(II) to Hg(0), methylation, reductive demethylation and certain other processes. The resulting shifts in isotopic abundances are quantified by measuring the ^{202}Hg/^{198}Hg ratio relative to that of the NIST SRM standard. The second mechanism is the magnetic isotope effect—a mass-independent effect—whereby the reaction rates of odd isotopes depart from consistency with the even isotopes because of coupling of electron spins with nuclear spins, which exist in odd nuclei only. This effect occurs in photochemical reactions involving radical pairs in aqueous solutions and causes anomalies in ^{201}Hg/^{198}Hg and ^{199}Hg/^{198}Hg ratios.

Our results from recent (2010) sediments from the lower reach of the EFPC suggest a shift in the geochemical processing of Hg in the system. Older sediment samples exhibit a negative trend in ^{202}Hg/^{198}Hg from the contamination source to the lower part of the creek, but recently this trend has become nonexistent within the measurement uncertainty (~0.11 per mil). This sudden (over ~3 years) change in the isotopic ratios ($\delta^{202}\text{Hg}_{\text{SRM-3133}}$ changed from -1.24‰ to 0.11‰, in the downstream part of the EFPC) suggests the biogeochemical cycling or sourcing of Hg changed in the EFPC, and that this change happened rapidly and recently. Analysis of samples to be collected this spring will examine this issue in more detail.

The ^{202}Hg/^{198}Hg ratios measured in fish tissues taken from the EFPC show a weak positive fractionation trend with increasing distance downstream (from an average -0.57‰ ± 0.08 (n=4) upstream to -0.29‰ ± 0.15 (n=4) downstream). Fish act as integrators of Hg in the water, and probably do not alter the mass independent fractionation of the isotopes. The odd isotope anomalies in the fish tissue have a positive trend that suggests photo-degradation of Me-Hg occurs in the water.

Our next task is to measure the isotopic composition of dissolved Hg. We have implemented a published method for measuring Hg isotope ratios of total Hg in low concentration samples. This method appears to work well for several stream waters from Illinois; analyses of EFPC waters are set to begin this spring. We also have designed a method to extract and perform isotopic analysis on dissolved methyl-Hg. The method involves complexation of Hg with thiourea, collection of the Hg from a large volume of water onto a thiol-functionalized resin, and selective elution of different species via pH manipulation. Selective analysis of methyl-Hg is challenging because concentrations are very low (much lower than total Hg), requiring large-volume samples, but we expect this method can achieve that goal.

Determination of Hexavalent Chromium Reduction Using Cr Stable Isotopes: Isotopic Fractionation Factors for Permeable Reactive Barriers (PRBs) and Microbial Cr Reduction

University-Led Research

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Chromuim (Cr) stable isotope measurements in groundwater can provide improved estimates of the extent of Cr(VI) reduction, which immobilizes the Cr in a less toxic form. Cr isotope fractionation occurs during Cr(VI) reduction. As reduction proceeds, the remaining Cr(VI) becomes progressively enriched in heavier isotopes. This isotopic fractionation is quantified by measuring the $^{53}Cr/^{52}Cr$ ratio. The magnitude of fractionation is expressed as a fractionation factor, α, defined as $\alpha = R_{reactant}/R_{product}$, where $R_{reactant}$ and $R_{product}$ are the $^{53}Cr/^{52}Cr$ ratios of the reactant and the reaction product flux, respectively. α is often expressed in per mil units using the symbol ε where $\varepsilon = (\alpha - 1)$. The relationship between observed $^{53}Cr/^{52}Cr$ ratio shifts and the extent of reduction must be calibrated by determining ε for each particular site or geochemical regime.

Permeable reactive barriers (PRB) made of Fe^0 and *in situ* redox manipulation (ISRM) zones, created by injecting a reductant (e.g., sodium dithionite) into the subsurface, effectively remediate Cr contaminated aquifers. These reactive barriers, placed across the flow path of the plume, reduce Cr(VI) as it reacts with the corrosion products of Fe^0 or reduced sediments in ISRM zones. The dominant reducing phases in both PRBs and ISRM barriers include FeS, $FeCO_3$, Green rusts and Fe(II) sorbed onto/into Fe-oxyhydroxides. The Cr isotope method should prove useful in monitoring the performance of reactive barriers, for example to determine if Cr breaking through a barrier passes through zones of substantial but incomplete reduction or merely bypasses the barrier completely.

Here, we determine fractionation factors for Cr(VI) reduction by Fe(II) phases likely to occur in reactive barriers. Experiments include laboratory batch studies with pure mineral phases and microcosms with reduced sediments from two depths from the ISRM zone at the 100D area of the U.S. DOE Hanford site. The ε's for FeS, $FeCO_3$, green rust, Fe(II) sorbed onto goethite, and ISRM zone sediments are 2.12‰ (n=2), 2.68‰ (n=2), 2.65‰ (n=2), 3.92‰ (n=1), and 3.38‰ (n=2), respectively. Our results indicate that fractionation factors for Cr(VI) reduction by likely reactive-barrier phases vary substantially, but fall within a workable range. This aids in interpretation of Cr isotope data from the reactive barriers, by providing a better calibration of the relationship between Cr isotope ratios and the extent of Cr(VI) reduction.

A detailed understanding of the factors influencing isotopic fractionation of Cr during microbial reduction is required in order to extend the Cr stable isotope approach to field conditions involving microbial reduction. We are focusing on the basic relationship between bacterial metabolism type and fractionation factor by experimentally determining ε for contrasting metabolic reduction pathways. We determined the ε for Cr(VI) reduction by *Desulfovibrio vulgaris* in continuously fed batch reactors supplied with pyruvate as electron donor. Results indicate ε was 3.09‰ (n = 2). Future work will determine fractionation factors under controlled electron donor conditions in continuously fed batch reactors by anerobic *Geobacter sulfurreducens* and aerobic *Pseudomonas putida* cultures, each with a very different Cr(VI) reduction pathway. We aim to reveal fundamental relationships controlling the fractionation factor as a function of metabolism type and other variables, such as electron donor concentration. This basic information will improve quantification of Cr(VI) reduction using Cr stable isotope measurements in the sites undergoing active bioremediation.

Development of Enabling Scientific Tools to Characterize the Geologic Subsurface at Hanford

University-Led Research

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Petrophysical models that relate borehole neutron and gamma ray data to reservoir properties, including clay content, porosity, permeability, and water saturation, have been successfully employed in the petroleum industry, but remain relatively undeveloped in the area of subsurface remediation. The objectives of this research are to: (1) analyze core and outcrop samples from representative facies for a variety of mineralogical, chemical and physical properties, (2) predict the response of a variety of neutron and gamma logging tools based on these measurements, (3) develop algorithms to translate log responses into reservoir properties useful for input in flow and reactive transport models such as matrix density, lithology, porosity, and permeability.

Our analysis of selected core samples from Hanford well 399-3-18 (C4999) reveals significant correlations between K, Th, and U concentrations and both matrix density and total clay, as determined by pycmometry and Dual Range Fourier Transform Infrared spectroscopy, respectively. Based on these relationships, we developed algorithms to predict total clay and matrix density from existing spectral gamma logs. The availability of laboratory bulk density data allows us to compute porosity estimates for our sample set. The comparison of clay content and porosity reveals a relationship that is consistent with the relationship observed by Marion et al. (1992) and others, suggesting the existence of both clay-supported and framework (or grain) supported domains and the ability to estimate porosity from clay content. Further, the availability of total clay, matrix density, and porosity permit application of the k-Lambda model as a means to estimate permeability. These results, although limited, suggest the path towards developing additional algorithms to predict these important properties from existing logs. Analysis of selected samples from Hanford well 399-2-26 (C6210), located within the Hanford 300 Area Integrated Field Research Center (IFRC), indicates that the relationships between K, Th, and U concentrations and both matrix density and total clay closely match those observed in C4999, suggesting that the algorithms developed will be generally applicable to the large number of logs collected in the Hanford and Ringold formations. We are in the process of analyzing additional core samples from wells distributed within the Hanford 300 Area for which both geophysical logs and bulk density measurements are available.

Development of Surface Complexation Models of Cr(VI) Adsorption on Soils, Sediments and Model Mineral Mixtures

University-Led Research

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Cr(VI) is a highly toxic contaminant that has been introduced into many shallow subsurface environments. Although Cr(VI) is typically quite mobile, it can be retarded via adsorption and reduction reactions. The primary aim of this exploratory research is to develop methods for applying surface complexation models (SCMs) to complex mineral mixtures, including natural sediments. To achieve this goal, the following tasks are planned: (1) measurement of Cr(VI) sorption on kaolinite, montmorillonite, hydrous manganese oxide (HMO) and g-alumina as a function of ionic strength, pH, pCO_2 and sorbate/sorbent ratio, (2) development of SCMs describing Cr(VI) adsorption on kaolinite, montmorillonite, HMO and g-alumina, (3) measurements of Cr(VI) sorption on model mixtures of kaolinite, montmorillonite, HMO, g-alumina, goethite and hydrous ferric oxide (HFO), (4) testing of component additivity (CA) SCM predictions of Cr(VI) sorption on the model mixtures, (5) measurements of Cr(VI) sorption on bulk natural sediments with differing quantities of organic carbon and HMO/HFO before and after step-wise extractions, and (6) development of CA methods to describe Cr(VI) sorption on natural sediments.

Cr(VI) adsorption has been measured on g-alumina (5 g/L) as a function of pCO_2 (0, atmospheric, 2.5%), pH (4-10), ionic strength (.001, .01 or 0.1 M $NaNO_3$) and Cr(VI) concentration (10^{-4} to 10^{-6} M). Adsorption decreases with increasing pH, varying little with ionic strength or pCO_2, except that adsorption is inhibited at low pH under high ionic strength and high pCO_2. The data have been used to develop constant capacitance, double layer, and triple layer models (CCM, DLM, TLM) of Cr(VI) adsorption on g-alumina. Of these, the ionic-strength-dependent parameters of the CCM are best able to reproduce the observed adsorption behavior over broad ranges in solution condition. Kinetics experiments to test Cr(VI) uptake and release have been initiated with montmorillonite, kaolinte and HMO. Initial data suggests that Cr(VI) uptake at pH 3 is slower on HMO (days, rather than hours) than on g-alumina, with sorbed Cr(VI) released quickly and completely at pH 10. In contrast, very slow sorption/desorption kinetics are observed on kaolinite; at pH 3, sorption continues to increase slowly for at least two weeks. Uptake of Cr(VI) is very rapid at pH 3 (minutes to hours) on untreated montmorillonite, but is irreversible at pH 10 over several days, most likely due to reduction of the Cr(III) by either organic matter or Fe(III) in the clay lattice.

Planned experiments will use kaolinite and montmorillonite after extraction of Fe and/or organic matter (e.g., using peroxide, oxalate, dithionite) to test whether Cr(VI) sorption on these clays is reversible in the absence of reduction. Work planned for the remainder of Year 1 will include measurement of Cr(VI) adsorption on montmorillonite, kaolinite, and HMO as a function of pH, ionic strength and pCO_2, with development and comparison of SCMs for these sorbents. Work in Year 2 will focus on measurement of Cr(VI) sorption on model mixtures of minerals and on natural sediments. These data will be used to test SCM component additivity predictions of Cr(VI) sorption.

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

University-Led Research

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The function and regulation of most genes elucidated from genomic investigations of subsurface microorganisms is not yet known. In order to design effective approaches for engineered bioremediation and to accurately predict natural attenuation processes in subsurface environments, it remains crucial to: (1) recover relevant organisms into pure culture, and (2) interrogate the physiology and genetics of pure cultures under environmental conditions relevant to the subsurface of DOE sites. Thus, the overall goal of this research is to apply a combination of cultivation, genomic, and metagenomic approaches to elucidate the microbial groups mediating U(VI) reduction and nitrate attenuation in subsurface sediments co-contaminated with U(VI) and nitrate. In this project, a phylogenetically diverse group of over 30 strains of metal- and nitrate-reducing bacteria were recovered from contaminated groundwaters and sediments of the Oak Ridge IFRC (OR-IFRC), and environmental sequence data indicate that these species are abundant in the contaminated subsurface of DOE sites.

The bacterial community in the acidic, contaminated OR-IFRC source zone is predominantly composed of denitrifying microorganisms from the bacterial genus *Rhodanobacter*. These organisms are shown to be metabolically active in source zone groundwater, and in some cases, are essentially the only active organisms detected (comprising 50% to 100% of rRNA detected). In more moderately contaminated areas of the OR-IFRC, a diversity of metal- and sulfate-reducing bacterial groups were detected and pure cultures of U(VI)-reducing members were obtained. *Rhodanobacter* strains 2APBS1 and 116-2 were isolated from uranium-contaminated OR-IFRC subsurface sediments. Whereas organisms within this genus are not known to denitrify, phenotypic and genotypic analyses confirm that strains 2APBS1 and 116-2 contain a complete denitrification pathway. Consistent with their source zone habitat, strains 2APBS1 and 116-2 were shown to be highly tolerant of key stressors, showing growth at pH 4 and at salt concentrations of 3%. Furthermore, growth was observed at concentrations equal to or exceeding 100 mM nitrite and 400 mM nitrate. Fatty acid methyl ester data show that strains 2APBS1 and 116-2 contain high levels of 15:0 iso and 17:1 iso w9c; otherwise their fatty acid profiles are congruent with described *Rhodanobacter* species. Carbon utilization and enzyme assays indicate that these strains differ substantially from previously published species of *Rhodanobacter*. Based on phylogenetic, chemotaxonomic and physiological differences, these strains represent novel species of *Rhodanobacter*, and the names *Rhodanobacter denitrificans* strain 2APBS1 and *Rhodanobacter whiteii* strain 116-2 are proposed.

Groundwaters at over half of DOE sites contain organic in addition to radionuclide contaminants, and compounds such as monoaromatic hydrocarbons could provide an important source of carbon and electrons for biotransformation processes in the oligotrophic subsurface, thereby limiting the fate and transport of inorganic contaminants under natural attenuation conditions. *Geobacter daltonii* was isolated from uranium- and hydrocarbon-contaminated subsurface sediments of the OR-IFRC, and is the first organism from the subsurface clade of the genus *Geobacter* that is capable of growth on aromatic hydrocarbons. BLAST analysis was performed on the *G. daltonii* genome and compared to the *G. metallireducens* genome, the only other *Geobacter* species capable of aromatic contaminant degradation. As in *G. metallireducens*, genes predicted to play a role in aromatics degradation were identified and shown to cluster into large and small genomic islands. Of note, *G. daltonii* has uniquely two copies of the benzylsuccinate synthase (*bssA*) gene, which is involved in the first step of toluene oxidation. The a subunits have 76% identity to one another. Also interesting, these copies lie within completely independent operons, each with its own transcriptional regulator and accessory genes downstream. RT-PCR analysis revealed that the expression level of *bssAs* vary when *G. daltonii* is grown on toluene vs. benzene; however, expression analysis suggests that benzene oxidation proceeds through use of the toluene oxidation pathway in *G. daltonii*. Iterative cultivation and genomic approaches need to be integrated to understand the growth and physiological controls of microbially catalyzed contaminant transformation, as well as to develop metabolic models for key organisms in the subsurface.

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

University-Led Research

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This project addresses the scaling of geochemical reactions to core and field scales, and the interrelationship between reaction rates and flow in porous media. We are targeting the reaction of highly caustic, radioactive waste solutions with subsurface sediments, and the immobilization of Sr-90 and I-129 through mineral incorporation and passive flow blockage, respectively. We are addressing the correlation of results for pore-scale fluid-soil interaction with field-scale fluid flow, with the specific goals of: (1) predicting attenuation of radionuclide concentration; (2) estimating changes in flow rates through changes of soil permeabilities; and (3) estimating effective reaction rates. We have organized this project into three thrust areas.

(1) Reactive flow experiments. Mineral dissolution and precipitation alters pore network structure and subsequent flow velocities, creating a complex interaction between reaction and transport. To examine this and measure the magnitude of its impact, we are conducting controlled laboratory experimentation using reactive flow-through columns. Four reactive column experiments have been completed in which simulated tank waste leakage (STWL) is reacted with pure quartz sand, with and without Al. In columns experiments without Al, there was a substantial reduction in volume of the solid matrix (quartz dissolution). In experiments with Al, there was a net increase in the volume of the solid matrix (cancrinite precipitation). The rate and extent of reaction was found to increase with temperature.

(2) Multiscale Imaging and Analysis. Mineral dissolution and precipitation rates within a porous medium will vary spatially due to natural and reaction-created heterogeneities. We are using a combination of X-ray computed microtomography, backscattered electron and energy-dispersive X-ray imagery combined with computational analysis of these images to quantify pore structure, mineral distribution, structural changes and fluid-air and fluid-grain interfaces. One of the columns from the reactive flow experiments at PNNL was imaged using 3D X-ray computed microtomography at BNL and analyzed using 3DMA-rock at SUNY Stony Brook. The imaging results support the mass-balance findings reported by Dr. Um's group regarding the substantial dissolution of quartz in column S1. An interesting new finding is grain movement between runs due to the increase in void space. The other columns have been imaged and are currently being analyzed. At Princeton, we have successful developed a method to use 2D SEM imaging to analyze sections of reacted columns at the end of the flow experiments. The method was applied to a column reacted in the previous year. We are able to detect mineral precipitation at high resolution and infer changes in pore structure.

(3) Multiscale Modeling and Up-Scaling. We seek to account for pore-scale variation in physical and mineralogical properties, flow velocities, and (for unsaturated conditions) wetting fluid/grain surface areas, in reactive transport models. To accurately upscale reaction rates, we are using a combination of lattice Boltzmann modeling, reactive-transport-network flow modeling, and continuum-scale finite difference models. A specific application is to understand how carbonate rocks in contact with CO_2-rich brines change due to the precipitation or dissolution of fast-reacting minerals such as calcite and dolomite. In preliminary results from our geochemical model alone (no transport), we were able to identify the conditions that lead to significant changes to the pore volume, due to the precipitation of calcite and dolomite. The transport part of the model is being developed. We are fortunate to have established a collaboration with Prof. Hilfer of the University of Stuttgart, who has provided us with a carbonate rock image in the form of a voxelized cube.

Microbial Uranium Reduction and Monitoring Tools

University-Led Research

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This collaborative, multi-investigator research project aims to enhance understanding of the microbiology controlling U mobility, and to develop molecular and biogeochemical tools with which the fate of U can be monitored and predicted better than with available technologies.

Diverse groups of microorganisms affect the oxidation state of metals and, therefore, control the mobility of toxic radionuclides in subsurface environments. Relevant to uranium (U) redox speciation are bacteria that reduce predominantly water-soluble and mobile hexavalent U, U(VI), to U(IV), which has lower solubility and typically forms the uraninite mineral UO_2. Recent studies with *Desulfitobacterium* spp. suggested that the ability to reduce U(VI) to U(IV) is a shared feature among members of this group. Interestingly, extended X-ray absorption fine structure (EXAFS) analysis demonstrated that U associated with the solid phase in the *Desulfitobacterium* cultures was a mononuclear U(IV) species instead of the typical nanoparticulate uraninite with U-U coordination. More detailed studies indicated that solution conditions affected the solid phase U, and in the presence of phosphate, non-uraninite U(IV) was formed both in cultures of Gram-negative (i.e., *Anaeromyxobacter*) and Gram-positive (*Desulfitobacterium*) bacteria, and also in abiotic systems that used 9,10-anthrahydroquinone-2,6-disulfonate (AQH_2DS) as the reductant. *Desulfitobacterium* cultures and abiotic systems with AQH_2DS produced mononuclear U(IV) also in the absence of phosphate whereas *Anaeromyxobacter dehalogenans* strain 2CP-C reduced U(VI) to U(IV)-uraninite. These findings suggest that distinct biomolecular U(VI) reduction mechanism(s) operate in Gram-positive *Desulfitobacterium* spp. and Gram-negative *Anaeromyxobacter* spp.

Whether present as uraninite or mononuclear U(IV), reduced U is susceptible to reoxidation by oxidants (e.g., oxygen) and oxic/anoxic transition zones are hot spots that control U mobility. Hence, understanding, and possibly manipulating, the microbial communities and their activities at oxic/anoxic interfaces is key for controlling the long-term stability of the precipitated material. *Anaeromyxobacter* spp. are common subsurface bacteria, and recent work suggested that members of this group are uniquely adapted to life at oxic-anoxic transition zones where they consume oxygen and take advantage of oxidized metal species, including U(IV), as electron acceptors. Detailed physiological studies with *Anaeromyxobacter dehalogenans* strain 2CP-C demonstrated rapid consumption of atmospheric oxygen and microaerophilic growth at oxygen partial pressures at and below 0.18 atm. In the absence of oxygen, *Anaeromyxobacter* spp. use U(VI) as an electron acceptor and gain energy for growth from U(VI) to U(IV) reduction. Interestingly, laboratory studies revealed about 10-fold lower growth yields with U(VI) as electron acceptor than predicted from thermodynamic calculations. Because the number of cells (i.e., the amount of catalyst) directly affects the rates of U(VI) reduction, such detailed physiological information is required for the development of models that accurately predict U fate.

For novel tool development, the foci are on identifying biomarkers that correlate with U(VI) reduction activity and quantifying U isotopic fractionation with high precision mass spectrometry. The latter tool identifies specific isotope enrichments associated with microbial and abiotic U(VI) reduction or U(IV) oxidation reactions that occur in oxic/anoxic transition zones. Our analysis of samples from the Rifle IFRC site indicated that U(VI) becomes isotopically lighter as it becomes depleted due to reduction. Current efforts evaluate U isotope fractionation in biotic and abiotic systems and different bacterial pure cultures under controlled laboratory conditions to explore the nature of the isotopic effect(s) and determine if enrichment factors (ε values) can be obtained. Application of the new tools at DOE IFRC sites will demonstrate the value of the tools to assess, monitor, and predict *in situ* reductive processes. The comprehensive understanding of the mechanisms and pathways affecting U precipitation and mobilization, combined with field measurements, will contribute to the development of models that predict the long-term fate of toxic U in contaminated subsurface environments.

Advances in the Molecular Analysis of Communities Associated with Uranium Bioremediation

University-Led Research

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Optimization of strategy design for *in situ* bioremediation of uranium-contaminated groundwater requires an understanding of the physiological status of the microorganisms that are involved in the bioremediation. Previous studies within this project have demonstrated the value of evaluating physiological status by quantifying the abundance of gene transcripts or proteins that are diagnostic of rates of metabolism or limitation for key nutrients. A recently recognized stress to the subsurface microbial community during uranium bioremediation is the release of arsenic into the groundwater. Analysis of the expression of arsB, which encodes for an As(III) resistance membrane pump, recovered gene transcript sequences closely related to members of the family *Geobacteraceae* and *Rhodoferax ferrireducens*. These sequences accounted for more than 50% of expressed arsB sequences recovered in clone libraries. The gene arrA, which encodes a reductase involved in respiratory As(V) reduction, was also expressed during acetate amendment. Sequences most closely related to *Geobacter uraniireducens* Rf4 and *Geobacter lovleyi* SZ predominated. More quantitative assessment of arsB and arrA expression during the acetate amendment is expected to provide further insight into how arsenic is released into groundwater and how microorganisms cope with this toxic metalloid. Molecular diagnostic tools developed to date have focused on well-annotated sequences of highly conserved function. However, many of the most abundant gene transcript and protein sequences in the subsurface during *in situ* uranium bioremediation are of unknown function. For example, we found that c-type heme-containing proteins were prevalent in the groundwater during effective U(VI) removal, but their function was unknown. Mass spectrometry analysis identified one of the proteins as a putative c-type cytochrome encoded within the genome of *Geobacter sp.* strain M18, which was isolated from the Rifle site. Homologs of this cytochrome termed GscA (*Geobacter* subsurface c-type cytochrome A) were also identified in the genomes of *Geobacter* isolates in the phylogenetic cluster known as subsurface clade 1, which predominates in a diversity of Fe(III)-reducing subsurface environments. An antibody-based quantification approach for GscA verified its presence in the groundwater and demonstrated that its abundance positively correlated with the removal of U(VI) from the groundwater. Although the genetically tractable *Geobacter sulfurreducens* does not contain a GscA homolog, expression of the GscA gene restored function of a mutant missing the gene for OmcS, a c-type cytochrome associated with microbial nanowires and necessary for extracellular electron transfer to metals. These results suggest that GscA is a functional counterpart of OmcS in subsurface *Geobacter* species. A similar approach of mutant complementation is expected to aid in the elucidation of the function of other proteins of unknown function that are abundant during bioremediation.

Metallic-Like Long-Range Electron Conduction along PilA Pili of *Geobacter sulfurreducens*

University-Led Research

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The mechanisms by which *Geobacte*r species transfer electrons onto Fe(III) oxides is of interest, because *Geobacter* species are often associated with effective removal of uranium from contaminated groundwater during *in situ* uranium bioremediation, and Fe(III) oxides are the primary electron acceptor supporting the growth of the *Geobacter* in the subsurface. Previous studies have suggest that pili comprised of PilA filaments are important for Fe(III) oxide reduction, but there has been significant skepticism over whether these pili can in fact act as “microbial nanowires” for long-range electron conduction from the cell to Fe(III) oxides, as well as questions about potential mechanisms for electron conduction.

Conduction along the length of *Geobacter* pili was evaluated with a device previously employed to document the conductivity of Geobacter sulfurreducens biofilms and electron conductance through *Geobacter* co-culture aggregates. Filaments were sheared from the surface of *G. sulfurreducens* and purified. Filament aggregates were positioned to span two gold electrodes, which were separated by a 50 µm non-conducting gap. The conductivity of pili preparations was 6 µS/cm, whereas a preparation from a mutant, which could not produce the PilA protein that comprises the pilin, was only 0.5 µS/cm. The conductance of the preparation of containing PilA filaments was comparable to that of *G. sulfurreducens* biofilms that were deposited on the measurement device. Previous immunogold studies noted that the multi-heme c-type cytochrome, OmcS, is specifically associated with *G. sulfurreducens* pili, but suggested that OmcS spacing is too great for OmcS to facilitate electron conductance along the length of the pili. Rather, OmcS is hypothesized to facilitate electron transfer between the pili and Fe(III) oxides.

Additional examination of pili with atomic force microscopy confirmed the broad spacing of protein associated with the pili. Purification of filament preparations to remove associated cytochromes, or treatment of filament preparations with cytochrome denaturant, did not affect conductance. Measurements of conductivity as a function of temperature revealed that the conductivity of pili increased exponentially upon cooling, which is a characteristic of organic metals and ruled out: (1) metallic conduction by inorganic metals, which would increase linearly with cooling; (2) hopping conduction by metalloproteins, which would decrease upon cooling; and (3) tunneling conduction via cytochromes, which is independent of temperature. Studies of magnetoconductance provided further evidence for long-range conduction along pilin filaments.

The metallic-like conductivity of *G. sulfurreducens* pili is a property that has not been previously observed in any other natural protein. These results demonstrate that electrons can be conducted along the length of the PilA pili of *G. sulfurreducens* and that electron transfer between cytochromes is not the mechanism for conductance. Ongoing studies in which individual amino acids in PilA are being altered are expected to provide further insight into the mechanisms of conduction along the pilin length.

Detailed Modeling of Subsurface Microbes and Interaction between Microbial and Hydrogeochemical Process for Sustained Uranium Removal

University-Led Research

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Microbially mediated reductive immobilization of uranium in the natural environment comprises a complex set of coupled microbial, geochemical, and hydrologic processes. This project aims to significantly improve predictive capabilities of numerical models by integrating advanced models of microbial processes with state-of-the-art reactive transport simulators. We have developed new understanding of the role of outer surface cytochromes in U(VI) reduction by evaluating rates of U(VI) reduction in a suite of mutants in which multiple cytochrome genes have been deleted in the same strain. A similar genetic approach has demonstrated that elemental sulfur, which is expected to be an important electron acceptor for *Geobacter* species during the transition from Fe(III) reduction to sulfate redution, is reduced by cytochromes at the outer cell surface. We have developed a novel multiscale model by integrating our *Geobacter* electron capacitance model with a comprehensive simulator of coupled fluid flow, hydrologic transport, heat transfer, and biogeochemical reactions. This mechanistic reactive transport model accurately reproduces available experimental data describing bioremediation of uranium under a wide range of conditions. Model-based sensitivity analysis was used to elucidate the primary factors controlling uranium removal across time and space, facilitating improved structure and parameterization of the integrated field-scale reactive transport model and optimization of bioremediation design. We expanded the existing community metabolic model of *Geobacter* and sulfate reducers to incorporate the uranium reduction pathway for *Geobacter* based on the electron capacitance model and uptake kinetics associated with the multiple Fe(III) sources in the subsurface. This allows us to study simultaneous amendments of electron acceptors and donors (Fe(III) and acetate) as a sustainable bioremediation strategy; simulations suggest that this strategy can be optimized to maintain uranium concentrations below safety limits over long time frames. To enhance our understanding of microbial community interactions, we have developed a new kinetic model for *Anaeromyxobacter dehalogenans* (a uranium reducer with diverse electron acceptor utilization capability) and are integrating this with the previously developed kinetic model of *Desulfobacter*, the genus of sulfate reducers that most actively competes with *Geobacter* species for added acetate. To facilitate efficient simulations of these multi-organism systems, we developed and tested an approach for directly coupling genome-scale microbial models and reactive transport simulators. There remains a physical scale discrepancy between genome-scale metabolic models and field-scale reactive transport simulators; to inform this issue, we have developed new pore-scale simulations of iron reduction based on the *Geobacter* genome-scale model and are using them to test hypotheses regarding model scale effects. Our ultimate goal is to integrate multiscale microbial community structures with reactive transport models to accurately describe and predict the fate of uranium contamination, and provide an optimal control strategy for variable environmental conditions. We are collaborating with investigators from the Rifle IFRC project to integrate our methodologies into highly resolved simulations of field-scale experiments using high-performance parallel codes, and to evaluate the simulation results using advanced proteomic information as well as traditional hydrogeochemical observations.

Development and On-Site Testing of Catalytic DNA Biosensors for Radionuclides and Metal Ions

University-Led Research

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We are developing novel field-portable catalytic DNA biosensors to detect and quantify bioavailable radionuclides such as uranium, technetium, strontium, and plutonium, as well as metal contaminants such as mercury and chromium. The sensors will be highly sensitive and selective, not only for different metal ions, but also for different oxidation states of the same metal ion (such as U(IV) vs. U(VI), or Cr(III) vs. Cr(VI)). To achieve the goals, we are using the combinatorial biological technique called *in vitro* selection to obtain catalytic DNAs that are highly specific for a given radionuclide or metal ion. We also use state-of-the-art biochemical and biophysical techniques to elucidate the structural elements responsible for high selectivity. These DNA have been transformed into fluorescent or colorimetric sensors by labeling the DNA with fluorophores or gold nanoparticles through catalytic beacon and nanoparticle assembly technologies developed in the Lu's group, with detection limit down to 11 ppt and over millions-of-fold selectivity of uranyl over other radionuclides and metal ions.These sensors have been developed into commercially available sensor products that allow detection and quantification of radionuclides and metal ions in less than 2 minutes.

The portable fluorescent sensors have now been tested at two DOE SBR's Integrated Field-Research Challenge (IFRC) sites: Oak Ridge National Lab (ORNL) in Oak Ridge, Tennessee, and the Rifle Site in Colorado. Eleven groundwater samples were collected from ORNL wells adjacent to the former S-3 ponds and analyzed. There was a high correlation between the uranium concentrations obtained by this method, and the results obtained by inductively coupled plasma mass spectroscopy (ICP-MS). Four groundwater samples from the Rifle site were also analyzed and added to those derived by both ICP-MS and kinetic phosphorescence analysis (KPA). The most challenging aspect of analyzing these samples is the complex sample matrix and the wide range of concentrations to be analyzed in samples from various DOE sites, or different wells from the same site. Two approaches that can address these challenges are sample dilution and sample acid pre-treatment. Adding a single acid addition pre-treatment step was enough to make the uranium in the sample more accessible for our analysis, and the results after this pre-treatment test more closely correlated with the results from the "gold standard" of ICP-MS. Thus, this technique has shown its efficacy at two different DOE IFRC sites and can be adjusted to meet the individual needs of a given DOE site.

Detecting and quantifying radionuclides and metal contaminants onsite and in real-time in a simple and cost-effective way will impact many other areas of research under the SBR program. For example, it will enhance geochemistry/biogeochemistry research by lowering the costs of characterization, expediting sample analysis (i.e., enable in-field analysis), and providing more accurate information about the radionuclides and metal contaminants at the DOE sites. This information will strengthen the correlations between results obtained from microbial ecology and community dynamic analyses and DOE site properties, improve the understanding of the mechanisms of biotransformation, and provide deeper insight into biomolecular science and engineering. It will help numerical modeling by providing more accurate data and thus improving the understanding of how the coupled physical, chemical, and biological processes affect contaminant mobility, reactivity, and stability in subsurface environments across multiple temporal and spatial scales. Practical applications of these sensors will not only help assess the effectiveness of science-based solutions for remediation performed by researchers and engineers, but will also contribute to the long-term monitoring of DOE contaminated sites by DOE staff members, state and local regulation agents, and concerned citizens around the sites.

Radiochemically Supported Microbial Communities

University-Led Research

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This new project focuses on the hypothesis that H^2 and possibly other radiogenic substrates at DOE sites may support the growth of microorganisms, and thus indirectly influence the mobility of redox-sensitive radionuclides. Consistent with this hypothesis are initial results from our earlier ERSP Exploratory project indicating the presence of both H_2 and SSU rRNA library clones closely related to candidatus *Desulforudis audaxviator*, in underground nuclear test cavity fluids at the Nevada National Security Site (NNSS). In deep South African mines, *D. audaxviator* was shown to dominate fracture fluids of the Witwatersrand Basin, where it appears to persist by utilizing H_2 and SO_4^{2-} derived from radiochemical reactions in U-rich host rock. Until recently, these mines were thought to define the geographic limit of this genus and species; thus, the detection of *D. audaxviator* in radioactive subsurface water at the NNSS supports earlier assertions concerning the radiochemical lifestyle of *D. audaxviator*. In our preliminary study, full-length rRNA gene clones with up to about 98 percent sequence identity to *D. audaxviator* were obtained. The current project builds upon these initial observations with the sampling of new sites, more detailed microbial community and chemistry assessments, and radiochemical modeling.

Although no new hotwells have been available for sampling since our project was funded, we are scheduled to sample at least four sites in March and April of 2011, including the tunnel where *D. audaxviator* was previously detected. However, one site was sampled in anticipation of the new project in the late summer of 2010. ER-EC-11 is a new 1,264 m deep hole drilled into volcanic rock slightly off the NNSS where low levels of tritium have been detected. SSU rRNA gene clones from this site revealed a diverse microbial community composed principally of *Alpha*- and *Betaproteobacteria*, *Deinococcus-Thermus*, and *Firmicutes*. The most abundant group (17/81 full length clones) was from the *Deinococcus-Thermus* group. *Firmicute* clones were related to *Symbiobacterium*, *Geobacillus*, and *D. audaxviator,* one of which bore ~99% identity to *D. audaxviator*, possibly the closest match outside of South Africa to date. Future efforts will involve the analysis of new samples as these become available, including an attempt to amplify a North American *D. audaxviator* genome via single cell genomics. The cultivation of *D. audaxviator* from the NNSS will also be a project objective, as will compositional and stable isotopic characterization of radiogenic gases at the NNSS.

Electrical Responses of Abiotically Modified Grain Surfaces as Observed with Spectral Induced Polarization and Atomic Force Microscope Measurements

University-Led Research

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The main objective of this project is to investigate the influence of biogeochemical processes on the electrical properties of porous media using a combination of spectral induced polarization (SIP) and atomic force microscopy (AFM). These data are complimentary, as SIP measurements integrate properties of the fluids and grains to generate an electrical response at the column scale, whereas the AFM measurements allow us to directly probe the electrical characteristics of grain surfaces at the submicron scale. Our goal is to evaluate whether AFM data are useful for determining how specific biogeochemical modifications of grain surfaces might affect observed changes in the macroscopic SIP measurements. We have performed SIP experiments using glass beads for four different experimental conditions: (1) unaltered glass beads, (2) etched beads, (3) calcite precipitation on the beads, and (4) coating the bead surface with iron oxide. Images of the beads obtained with a scanning electron microscope confirmed qualitatively that each of the treatments was successful in altering the surface of the glass beads. SIP measurements were successful in differentiating between the treated and untreated beads. Topography measurements made via the AFM showed that the calcite precipitation approximately doubled the roughness of the bead surfaces, whereas the etching only increased the surface roughness by about 20%. Charge maps obtained with the AFM confirmed a generally low degree of charge on the glass surfaces, with little change in surface charge for the etched beads and an apparent decrease in charge for the calcite treated beads. AFM measurements for the iron oxide coated beads are currently under way. The next phase of this research will investigate whether bacteria or bacterially mediated changes of the bead surfaces create a significantly different type of SIP and AFM response compared to those observed here for abiotic transformations. The bacterium *Sporosarcina pasteurii* has been selected for these experiments, because it has been shown to be capable of biotically mediate calcite precipitation through ureolysis.

Role of Sulfhydryl Sites on Bacterial Cell Walls in the Biosorption, Mobility, and the Bioavailability of Mercury

University-Led Research

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Bacteria are ubiquitous in a wide-range of low-temperature aqueous systems, and can strongly affect the distribution and transport of metals and radionuclides in the environment. However, the role of metal adsorption onto bacteria, via the reactive cell wall functional groups, has been largely overlooked. Previous macroscale metal sorption, and XAS studies have shown that carboxyl and phosphoryl functional groups are the important metal binding groups on bacterial cell walls. However, our preliminary XAS studies indicated that Hg^{2+} binds to sulfhydryl groups in preference to the more abundant carboxyl and phosphoryl groups on cell walls when Hg concentration is submicromolar. The stoichiometry of these Hg-cysteine bacterial cell wall complexes also change as a function of aqueous Hg concentration, and the structures of such complexes can have a significant impact on the solubility and bioavailability of Hg. The overall goal of our study is to provide a quantitative and mechanistic understanding of the impact of bacterial sulfhydryl groups on the uptake, speciation, transport, and bioavailability of Hg in the environment.

We examined Hg adsorption on *Bacillus subtilis*, *Shewanella oneidensis*, and *Geobacter sulfurreducens* as a function of pH, and Hg and Cl^- concentration. Our studies indicate that Hg adsorbs strongly on all examined bacterial surfaces, with minor variation between different bacterial species. The Hg-XANES spectra indicate that the electronic state and the coordination environment of Hg complexes on bacterial cell walls change significantly as a function of Hg concentration above 0.5 mM, with minimal changes below this concentration. The Hg-EXAFS spectra indicate that Hg complexes entirely with thiols at the nanomolar and low micromolar concentrations, and with carboxyl sites at high micromolar concentrations. In addition, the structure of the cysteine complex changes from primarily HgS_3 to HgS_2 and HgS (S = cysteine), with increasing Hg concentrations in the submicromolar range. The presence of Cl^- reduced Hg adsorption on cells significantly because of the formation of an $HgCl_2$ aqueous complex. The EXAFS studies indicate that the structure of the Hg surface complex on the cell surfaces is not affected by the presence of Cl^-.

Our current studies are in progress: (1) to evaluate the site density of thiols on cell surfaces, and how they vary between different organisms using fluorescence spectroscopy, and (2) the role of aquatic natural organic molecules in Hg interactions with different bacteria under different pH and NOM concentrations. Detailed discussion of these results will be presented. Mercury is a common contaminant at several DOE sites, and our study provides important clues on the understanding of the ultimate fate and biological toxicity of Hg at these sites.

Mercury Release from Organic Matter (OM) and OM-Coated Mineral Surfaces

University-Led Research

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During the Cold War, 212,000 kg of liquid mercury (Hg) was released to the environment at the U.S. Department of Energy's Oak Ridge Y-12 Site. The Hg is now distributed downstream in the floodplain of East Fork Poplar Creek as Hg(II) bound to natural organic matter, mercury sulfide minerals, and methylmercury. We are investigating how Hg is released from organic matter and minerals in environments characteristic of the floodplain. Quantifying these processes is necessary to evaluate the dispersal of Hg(II) in soil-sediment-water systems and remediation approaches. Our hypotheses are that (1) Release of Hg(II) bound to reduced sulfur groups in soil organic matter, clay minerals coated with organic matter, and sulfide minerals requires biogeochemical agents that reduce the Hg(II) or oxidize the sulfur, whereas release of Hg(II) bound to oxygen-bearing functional groups does not require redox-sensitive agents; (2) Mercury bound to organic matter on clay mineral surfaces is immobilized more (i.e., is potentially less reactive to methylation) than Hg(II) adsorbed to uncoated clays; and, (3) Release rates are proportional to the quantity and type of binding site, each of which has a different molecular configuration and therefore different binding strength.

To test these hypotheses, we are investigating adsorption and desorption of mercury(II) in controlled laboratory experiments using prepared minerals and natural organic matter. We are evaluating the effects of dissolved organic matter isolates on Hg uptake on vermiculite under oxidizing conditions, and the effects of quinone molecules (hydroquinone, anthraquinone-2,6-disulfonic acid, and juglone) on reductive dissolution of mercuric sulfide solids under deoxygenated conditions. We are characterizing, using EXAFS spectroscopy, the molecular speciation of mercury in soil samples taken from the top 2 to 12 cm at a streambank and floodplain site along the East Fork Poplar Creek downstream from the Oak Ridge site. We are also characterizing mercury release from soil cores collected at the same two sites. Mercury concentration, mercury speciation via selective sequential extraction, organic matter content, elemental composition, cation exchange capacity, and soil mineralogy were determined in cores collected from the O/A (0–30 cm) and subsurface B (35–65 cm) soil horizons. To simulate the effects of a fluctuating water table, cores were flooded with water for 21 d, drained, dried for 7 d, and subjected to second and third flooding events. Pore water was sampled during flooding and analyzed for pH, total dissolved mercury (0.2 µm filtered), total dissolved iron, dissolved iron(II), dissolved sulfate and sulfide, dissolved organic carbon (DOC), DOC specific ultraviolet absorbance, and colloid characterization. Under reducing conditions, we observed a relationship between changes in soil geochemistry and their subsequent effect on porewater composition and mercury release. Data from second and third flooding events showed evidence of mercury desorption, diffusion, and precipitation dynamics driven by a fluctuating water table.

Molecular Mechanisms and Kinetics of Microbial Anaerobic Nitrate-Dependent U(IV) and Fe(II) Oxidation

University-Led Research

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The goal of this project is to use molecular genetic, spectroscopic, and microscopic techniques, together with kinetic and reactive transport studies, to describe and quantify the biotic and abiotic mechanisms underlying the related processes of anaerobic, nitrate-dependent U(IV) and Fe(II) oxidation, which influences the long-term efficacy of *in situ* reductive immobilization of uranium at DOE sites.

Research is focused on identifying the primary redox proteins that catalyze metal oxidation, environmental factors that influence protein expression, and molecular-scale geochemical factors that control the rates of biotic and abiotic interactions among U(IV) minerals, Fe(III) phases, and nitrogen oxide species formed during nitrate reduction. In these studies, we are using the widespread *Thiobacillus denitrificans*, the first autotrophic bacterium reported to catalyze anaerobic U(IV) oxidation, which is also capable of Fe(II) oxidation. A key part of the research program is the identification and characterization of uranium and iron species and phases produced during coupled oxidation-reduction processes under either biotic (enzymatic) or abiotic conditions in batch and column experiments with model solids and aquifer sediments. Reactive transport modeling will be used to synthesize molecular-scale reactions and bench-scale kinetic studies to develop a mechanistic basis for rate expressions. For oxidation experiments, biogenic UO_2(s) was synthesized under anaerobic conditions (using *Shewanella oneidensis* strain MR-1) following previously published methods. Precipitated solids were washed (1 M NaOH) and characterized by synchrotron X-ray absorption spectroscopy (collected at the Stanford Synchrotron Radiation Lightsource) at the U L-III edge. Spectroscopic results indicated poorly crystalline products of U(IV) with the local atomic structure of UO_2(s) that were stable in the X-ray beam with samples held at 77 K. After additional characterization, biogenic UO_2(s) will be slurry-loaded with ground quartz into small-volume columns to investigate rates of abiotic and biotic oxidative dissolution in anaerobic, flow-through experiments.

Current work with *T. denitrificans* focuses on determining whether the enzymes that we demonstrated to be involved in anaerobic, nitrate-dependent U(IV) oxidation are also involved in nitrate-dependent Fe(II) oxidation. We previously developed a genetic system in *T. denitrificans* that enables us to create insertion mutants and complement them in trans. We found that two membrane-associated, diheme, c-type cytochromes (a c4 cytochrome, Tbd_0187, and a c5 cytochrome, Tbd_0146) were involved in nitrate-dependent U(IV) oxidation. Notably, we have determined that these two mutants (Tbd_0146 and Tbd_0187) are *not* defective in nitrate-dependent Fe(II) oxidation, based on anaerobic cell suspension experiments. We are testing the Fe(II) oxidation capabilities of other mutant strains, including knockouts of other genes encoding membrane-associated c-type cytochromes (a group of proteins that we hypothesize to be catalyzing Fe(II) oxidation). The finding that different enzymes are associated with nitrate-dependent Fe(II) and U(IV) oxidation should make it easier to experimentally discriminate between enzymatic U(IV) oxidation and abiotic U(IV) oxidation mediated by biogenic Fe(III) oxides or oxyhydroxides.

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

University-Led Research

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Subsurface bacteria, including sulfate-reducing bacteria (SRB) reduce soluble U(VI) to insoluble U(IV) with subsequent precipitation of UO_2. We have shown that SRB reduce U(VI) to nanometer-sized UO_2 particles (1-5 nm), which are both intra- and extracellular, with UO_2 inside the cell likely physically shielded from subsequent oxidation processes. We evaluated the UO_2 nanoparticles produced by *Desulfovibrio desulfuricans* G20 under growth and nongrowth conditions in the presence of lactate or pyruvate and sulfate, thiosulfate, or fumarate, using ultrafiltration and HR-TEM. Results showed that a significant mass fraction of bioreduced U (35-60%) existed as a mobile phase when the initial concentration of U(VI) was 160 µM. Further experiments with different initial U(VI) concentrations (25-900 mM) in MTM with PIPES or bicarbonate buffers indicated that aggregation of uraninite depended on the initial concentrations of U(VI) and type of buffer.

It is known that under some conditions, SRB-mediated UO_2 nanocrystals can be reoxidized (and thus remobilized) by Fe(III)-(hydr)oxides, common constituents of soils and sediments. To elucidate the mechanism of UO_2 reoxidation by Fe(III) (hydr)oxides, we studied the impact of Fe and U chelating compounds (citrate, NTA, and EDTA) on reoxidation rates. Experiments were conducted in anaerobic batch systems in PIPES buffer. Results showed EDTA significantly accelerated UO_2 reoxidation with an initial rate of 9.5 mM day^{-1} for ferrihydrite. In all cases, bicarbonate increased the rate and extent of UO_2 reoxidation with ferrihydrite. The highest rate of UO_2 reoxidation occurred when the chelator promoted UO_2 *and* Fe(III) (hydr)oxide dissolution as demonstrated with EDTA. When UO_2 dissolution did not occur, UO_2 reoxidation likely proceeded through an aqueous Fe(III) intermediate as observed for both NTA and citrate. To complement these laboratory studies, we collected U-bearing samples from a surface seep at the Rifle field site and measured elevated U concentrations in oxic iron-rich sediments.

To translate experimental results into numerical analysis of U fate and transport, we developed a reaction network based on Sani et al. (2004) to simulate U(VI) bioreduction with concomitant UO_2 reoxidation in the presence of hematite or ferrihydrite. The reduction phase considers SRB reduction (using lactate) with the reductive dissolution of Fe(III) solids, which is set to be microbially mediated as well as abiotically driven by sulfide. Model results show the oxidation of HS^- by Fe(III) directly competes with UO_2 reoxidation as Fe(III) oxidizes HS^- preferentially over UO_2. The majority of Fe reduction is predicted to be abiotic, with ferrihydrite becoming fully consumed by reaction with sulfide. Predicted total dissolved carbonate concentrations from the degradation of lactate are elevated ($\log(p_{CO2}) \sim -1$) and, in the hematite system, yield close to two orders-of-magnitude higher U(VI) concentrations than under initial carbonate concentrations of 3 mM. Modeling of U(VI) bioreduction with concomitant reoxidation of UO_2 in the presence of ferrihydrite was also extended to a two-dimensional field-scale groundwater flow and biogeochemically reactive transport model for the South Oyster site in eastern Virginia. This model was developed to simulate the field-scale immobilization and subsequent reoxidation of U by a biologically mediated reaction network.

Comprehensive Proteome Characterization and Cytochrome c Expression in *Anaeromyxobacter dehalogenans* 2-PC as a Function of Electron Acceptor Growth Conditions

University-Led Research

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The overarching goals of this collaborative research effort are to identify new biomarkers for monitoring microbial activities at U-contaminated sites, to develop environmental metaproteomics tools, and to correlate metaproteomics profiles with geochemical parameters and U(VI) reduction activity (or lack thereof). The integrated analysis of geochemical, genomic, transcriptomic and proteomic data sets promises to provide a comprehensive view of the microbial community and how its activity affects (i.e., controls) radionuclide mobility in the contaminated subsurface.

Bioremediation efforts at the Oak Ridge Area 3 Integrated Field Research Challenge (IFRC) site revealed that *Anaeromyxobacter* spp. respond to ethanol biostimulation and are contributing to radionuclide immobilization. Using *Anaeromyxobacter dehalogenans* strain 2CP-C and available genome information, controlled laboratory experiments explored cytochrome c expression in cells grown with different electron acceptors, including fumarate, iron oxide, manganese oxide, nitrate and oxygen. Proteins from cell biomass were extracted using a heat-assisted-detergent-based lysis method and subjected to mass spectrometric interrogation. Computational analysis of the strain 2CP-C genome predicts 4,485 protein-coding genes, and ~2,000 proteins were identified in cells grown with fumarate, tryptic soy broth, nitrate and different oxygen concentrations provided as electron acceptors. Fewer proteins (~1100) were identified in biomass collected from cultures grown with metal oxides as electron acceptors. Overall, 2,872 proteins were identified across all growth conditions, representing 64% of the annotated genome. A total of 793 proteins were common to all growth conditions and likely represent the core proteome of *A. dehalogenans*. Based on genomic analysis, strain 2CP-C possesses 68 putative genes encoding c-type cytochromes, some of which have been implicated in metal reduction and may serve as activity biomarkers. Out of the possible 68 predicted c-type cytochromes in the genome, the lowest number of c-type cytochromes (i.e., 12) was detected in biomass grown with iron oxide as the electron acceptor, as compared with 34 c-type cytochromes identified in fumarate grown cells. Five putative cytochrome c proteins (*Adeh 2902*, *Adeh 2216*, *Adeh 1764*, *Adeh 1719*, *Adeh 1172*) were expressed under all growth conditions tested. Interestingly, the cytochrome c nitrite reductase *NrfA* was expressed under all growth conditions, suggesting this enzyme system plays a role even in the absence of nitrate/nitrite as electron acceptors. Localization maps identified soluble cytoplasmic and membrane-associated proteins demonstrating the effectiveness of the protein extraction method. Future research will evaluate the efficiencies of protein and nucleic acid extraction methods from mixed cultures, laboratory-operated soil columns, and Oak Ridge IFRC Area 3 sediments. The integrated analysis of geochemical information and metagenomic, metatranscriptomic and metaproteomic datasets promises to establish correlations between gene activity and protein expression (i.e., c-type cytochromes) and U(VI) reduction activity.

Development of a Self-Consistent Model of Plutonium Sorption: Quantification of Sorption Enthalpy and Ligand-Promoted Dissolution

University-Led Research

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The goal of this project is to improve our ability to predict the environmental behavior of plutonium through the development of a mechanistic model of plutonium speciation in subsurface environments. The speciation model will be a thermodynamic surface complexation model of plutonium sorption to mineral surfaces that is self-consistent with macroscopic batch sorption data, X-ray absorption spectroscopy (XAS) measurements, electron microscopy analyses, and quantum-mechanical calculations. Current efforts have focused on examining actinide interactions with hematite and sediments from the DOE Savannah River Site and Hanford site. Batch sorption experiments using plutonium and plutonium oxidation state analog surrogates (Eu(III), Th(IV), Np(V), and U(VI)) to pure minerals and sediments at 25oC have demonstrated the expected trend of An(IV) > An(VI) > An(III) > An(V) in terms of increasing sorption affinity as a function of decreasing effective ion charge. The data have been used to design ongoing experiments examining sorption to hematite as a function of temperature. Potentiometric titrations of hematite as a function of temperature have indicated the point of zero charge shifts to lower pH values with increasing temperature. For comparison with the batch sorption experiments, actinide adsorption onto the (001) surface of hematite was compared using quantum-mechanical calculations with periodic boundary conditions. For 100% surface coverage, adsorption energy decreases with increasing atomic number and oxidation state with the exception of neptunium. The distance between the adsorbing cation and the surface increases with increasing oxidation state and decreasing atomic number with the exception of uranium. The conflicting exceptions to the adsorption energy and adsorption geometry trends still have to be explained. Comparisons between actinide sorption at 100% and 50% surface coverage of U(V) on hematite indicates that the 100% surface coverage setup has, to a certain degree, the character of a co-precipitation model, as opposed to an adsorption model. Based on these results, further calculations will be performed using 50% surface coverage to more accurately model inner-sphere adsorption.

As a first attempt to quantify the laboratory batch sorption data, the potentiometric titration data and sorption data as a function of pH and temperature were combined into a surface complexation model describing actinide sorption to hematite based on bond valence calculations. This model will be further refined based on x-ray absorption spectroscopy (XAS) measurements, electron microscopy analyses, and future quantum-mechanical calculations.

Initial sorption experiments have also begun using sediments from the Savannah River Site and the Hanford Site. Sediments have been obtained from areas with known plutonium contamination and sorption of Th(IV), Np(V), Pu(IV), and Pu(V) has been examined. Based on sorption data, both sediments appear to facilitate surface-mediated reduction of Pu(V) to Pu(IV). Mineralogical characterization of the Hanford sediments has identified a significant fraction of calcite that strongly buffers the pH of sediment suspensions. This buffering has a significant impact on the extent of actinide sorption. As the thermochemical model described above is developed, sorption of the actinides to these sediments will be predicted using a component additivity approach.

Quantifying Microbe-Mineral Transformation Using Dielectric Spectroscopy

University-Led Research

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This project involves laboratory experiments to investigate three hypotheses:

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

Here, we present a series of measurements conducted on *D. Vulgaris* bacteria, which were grown in LS4D medium and re-suspended in 5 mM HEPES for measurements. Cells were also grown in presence of additional concentration of iron in LS4D medium to form different thicknesses of iron sulfide on them. The dielectric dispersion curves show a distinct change in the dielectric permittivity values for cells with different concentrations of iron. The dielectric permittivity at low frequency decreases with the increase in thickness of cell membrane. X-Ray Absorption Near Edge Structure (XANES) measurements using synchrotron radiations were also carried out on these cells at Brookhaven National Laboratories to determine the exact chemical composition of the metal sulfide. We also present a series of dielectric spectroscopy measurements carried out on iron sulfide (pyrrhotite) particles of dimension less than 38 µm. For these experiments, different concentrations of pyrrhotite samples were suspended in 1% by weight of Agar gel--the gel was allowed to set in the apparatus and measurements were carried out. These iron particles are assumed to be polarizable inclusions in an otherwise nonpolarizable medium. The lowest volume fraction of iron sulfate that our technique allows was also determined.

Hypothesis 3 (H3) will be tested with forward synthetic sensitivity trials using the physics-based modeling approach to explore the electrical signatures resulting from ongoing biostimulation treatments at the Rifle site based on our findings exploring H1 and H2. A critical aspect of this work will be elucidating the likely magnitude of electrical signals that could be detected with current field instrumentation, which records the electrical response over a limited frequency range and with less accuracy. We also anticipate the possibility of applying the modeling approach to time-lapse field datasets collected during various biostimulation experiments at the Rifle site that may be made available to us over the project duration.

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

University-Led Research

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

High-resolution three-dimensional Discrete-Fracture-Network-Matrix (DFNM) models based on well-defined local-scale transport equations can help to address some of these questions. However, such models have not been used extensively in the context of fracture-matrix interactions, because they are computationally demanding. Howver, due to tremendous advances in computational technology over the last 10 years, DFNM modeling in relatively large domains is now feasible. The overarching objective of our research is to use DFNM modeling to improve fundamental understanding of how effective parameters in conceptual models are related to fracture network structure and matrix properties. An advanced three-dimensional DFNM model is being developed, which combines upscaled particle-tracking algorithms for fracture-matrix interaction and a recently developed parallel fracture-network flow simulator. The particle-tracking algorithms will allow complexity in flow fields at different scales and track transport across fracture-matrix interfaces, based on rigorous local approximations to the transport equations. This modeling approach can incorporate aperture variability, multiscale preferential flow, and matrix heterogeneity. We anticipate that the code can handle computational domains with about 1 billion nodes for flow and 1 billion particles for transport. The overarching goal is to obtain insights into (i) the relationship between effective fracture-matrix interaction parameters, network structure, and matrix properties and (ii) their scale dependence in different types of fractured rock environments.

We will show results obtained using new and efficient schemes for tracking particles through fracture intersections, and upscaled particle-tracking rules for fracture-matrix systems, which are based on transition probabilities rigorously derived from the fundamental transport equations. Comparisons between simulations employing these efficient algorithms and "very-high-resolution" particle tracking approaches are ongoing as part of a benchmarking exercise. The upscaled particle-tracking rules offer substantial increases in computational efficiency with insignificant loss of accuracy, thus enabling the implementation of particle-tracking simulators with billions of particles. The next step is to incorporate both the intersection-tracking and upscaled algorithms into the parallel fracture-network flow simulator.

Our research will specifically target applications at the Oak Ridge Field Research Center, former nuclear test sites in Nevada (e.g., the Shoal and Bullion tests), and other field sites (e.g., Mirror Lake) where tracer tests were conducted to obtain fracture-matrix interaction parameters for site-scale transport models. The nature of the underlying fracture network, rock matrix properties, and the observed behavior of tracers (e.g., distinction between tracers with different molecular diffusivities) were vastly different at these sites. Our goal is to reproduce these differences from first principles using our high-resolution models, without involving any fitted parameters. Subsequently, we will simulate site-scale transport using parallelized flow and transport models.

From Nanowires to Biofilms: An Exploration of Novel Mechanisms of Uranium Transformation Mediated by *Geobacter* Bacteria

University-Led Research

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Surface-attached communities or biofilms, ranging from monolayers to highly structured, mature biofilms, play a key role in redox transformations of metals and could potentially be used as permeable biobarriers for the bioremediation of toxic soluble metals such as uranium. Although information about the role of biofilms in uranium transformations is scarce, recent field-scale studies indicate that particle-associated bacteria in the family *Geobacteraceae* are stimulated during the active phase of bioremediation. However, their specific physiological state and potential contribution to contaminant transformations remains largely unknown. Thus, we investigated biofilm formation in the model representative *Geobacter sulfurreducens* and as a developmental process, which is initiated as cell monolayers that grow into microcolonies and, then, as structured, mature biofilms. Although the removal activity of the biofilms increased linearly during biofilm development, more uranium was substantially removed per cell in the monolayered biofilms compared to microcolonies and mature biofilms. Despite the increased ability to immobilize uranium, the monolayered biofilms reduced significantly less uranium than the microcolonies and mature biofilms. These results are consistent with our previous finding that the uranium reductase activity of the biofilms is directly dependent on the expression of *G. sulfurreducens* conductive pili, which serve as electronic conduits and structural support to build multilayered biofilms. Thus, the genes encoding nanowire components could serve as molecular markers for mature biofilms and uranium reduction in the sediments.

To identify additional markers specific for each stage in biofilm development, we performed a genetic screen and identified mutants interrupted at the monolayer and microcolony stages. Out of 4,000 mutants screened, we identified 153 that had reproducible biofilm phentoypes and no planktonic growth defect. We identified the mutated gene and grouped the mutants in functional categories, corresponding to biofilm components whose expression is required for biofilm developmental transitions. Among the functional groups, we identified metabolic and regulatory genes whose expression is uniquely associated with the formation of microcolonies and biofilm maturation and that are not required for planktonic growth, suggesting they could serve as biofilm biomarkers. Also having potential as biomarkers were genes involved in the synthesis of biofilm-specific lipids. Moreover, we identified biofilm electron transport components, including proteins required for the synthesis and functioning of the pilus nanowires and yet uncharacterized outer membrane *c*-cytochromes.

These results demonstrate that specific genes are required for the transition of biofilm developmental stages, which could be used as markers to monitor biofilm growth and activity during *in situ* bioremediation. Furthermore, these studies also indicate that strategies based on the expression and/or manipulation of biofilm components such as pilus nanowires could serve as efficient tools for the bioremediation of uranium-contaminated sites.

The Use of Geophysical and Numerical Methods for Investigating Infiltration and Contaminant Transport at Oak Ridge National Laboratory

University-Led Research

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Waste handling at former S-3 storage ponds at Oak Ridge National Laboratory has resulted in leakage of strongly acidic, uranium-bearing water into the subsurface. The area has been capped with asphalt, but a portion of the subsurface remains contaminated and threatens the nearby receptor. Geophysical field investigations, laboratory experiments, and numerical modeling are used to image contaminant transport in the subsurface and to investigate effects of infiltration of meteoric water. Field surveying of two areas using self-potential (SP), electrical resistivity (ER), and induced polarization (IP) suggested significant spatial heterogeneity and detected preferential flow paths in the subsurface. Measured SP anomalies correspond to zones of low resistivity and are interpreted as a zone of high hydraulic conductivity, with large flux from the source area. The combination of several types of geophysical data allow for a more reliable hydrogeochemical data interpretation when combined with conventional geochemical and geological data.

We performed laboratory and numerical experiments to explore the spectral IP (SIP) signatures of contaminant transport. Changes in SIP signals of contaminated sediments as a result of infiltration of uncontaminated water were investigated using laboratory experiments and geochemical modeling. The computer programs PHREEQC and TOUGHREACT are used to predict changes in surface sorption, aqueous and mineralogical composition. Surface complexation on quartz and goethite surfaces based on the double layer model was incorporated into the geochemical model, such that the electrochemical properties of these surfaces could be predicted from geochemical reactions. The resulting surface potential was used to predict the SIP signal. This method may be used to model geophysical responses of contaminant transport at the S-3 Site.

New inversion algorithms have been developed for the time-lapse tomography of self-potential, electrical resistivity, seismic (based on first-break picking forward modeling), and induced polarization. The new approach is based on the use of an active time constraint (ATC). We demonstrate that regularization in both space and time domains effectively reduces inversion artifacts and improves the stability of the inversion problem. We are presently working on the joint inversion of these geophysical data and their connection to the reactive transport modeling code TOUGH2.

Microbial Redox Metabolism and U(VI) Reduction in ORFRC Area 2 Sediment Amended with Acetate or Ethanol

University-Led Research

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Understanding geochemical and microbial community responses to the addition of electron donors for stimulation of microbial metabolism is critical to predicting the efficacy of *in situ* bioremediation of uranium and other metal/radionuclide contaminants. This project is examining bulk terminal electron accepting processes (TEAPs) and U(VI) reduction in ORFRC Area 2 sediments undergoing biostimulation through ethanol or acetate amendment. The central hypothesis is that patterns of U(VI) reduction will be linked to shifts in the predominant TEAP and to changes in abundance and activity of different types of respiratory microorganisms.

A preliminary fed batch (with ethanol as the electron donor) experiment was conducted with a slurry of uncontaminated saprolite from 7-9 m depth at Area 2, to which ca. 150 umol/L of exogenous U(VI) (uranyl-acetate) was added from a stock solution. Repeated addition of ethanol led to concomitant reduction of Fe(III), sulfate, and both aqueous and solid-phase U(VI). At the end of this phase of the experiment, microbially reducible Fe(III) was depleted and sulfate reduction was the predominant TEAP. Subsequent addition of 1.5 mM ^{13}C-ethanol or ^{13}C-acetate stimulated sulfate consumption and U(VI) reduction in the sulfate-reducing sediment slurries; no changes in HCl-extractable Fe(II) content took place, suggesting that stimulated U(VI) reduction was linked to SRB activity. Q-PCR analysis verified an abundance of dsr genes of SRB in the sediment slurry, and bulk PLFA analysis indicated membrane lipids known to be associated with SRB. Analyses of ^{13}C-labeled PLFAs extracted from these slurries showed that of nine fatty acids or summed features identified, seven were labeled while two were not. The results indicate that acetate and ethanol differed in the components of microbial community that were stimulated, and that ethanol showed a greater stimulation across a wider range of community members. The fatty acids showing the highest ^{13}C incorporation were consistent known signature fatty acids for sulfate-reducing bacteria (br17:0a and 10Me16:0).

A large-scale experiment was initiated in August 2010 to examine bulk TEAPs, U(VI) reduction, and microbial community composition in semicontinuous culture reactors (SCRs) (10-day residence time) amended with different amounts acetate or ethanol (either 0, 0.1 or 0.2 mM/d, in duplicate, for a total of 10 reactors). Terminal electron acceptor metabolism and other geochemical parameters were monitored over a ca. 5-month period. Overall patterns of terminal electron metabolism were similar in the acetate and ethanol-amended reactors. Complete consumption of incoming nitrate was observed in all amended reactors. Complete reduction of solid-phase Fe(III) compounds (oxides and clays) took place in sediments receiving the higher levels of electron donor addition, in which sulfate reduction became active after 2-3 months. Roll tube enumerations confirmed major stimulation of Fe(III)- and sulfate-reducing populations. Patterns of U(VI) reduction indicated that the extent of reduction was similar in acetate vs. ethanol-fed reactors, and proportional to the amount of electron donor added. A major increase in the accumulation of residual HNO_3-extractable U (presumably uraninite) took place during the transition between Fe(III) and sulfate reduction. Analysis of the microbial community response to electron donor amendment is under way using 16S rRNA gene pyrosequencing, Q-PCR of taxa-specific 16S rRNA genes and selected functional genes, and ^{13}C stable isotope probing of bacterial PLFAs. After ca. 4 months of operation, the SCRs were allowed to sit with no aqueous phase throughput for a period of ca. 1.5 months. Medium throughput was then re-initiated, with one of the duplicate reactors for each treatment receiving ethanol or acetate at the previous level of loading, and its twin receiving no electron donor input. This change is designed to examine the ability of ethanol-based vs. acetate-based sulfate-reducing microbial communities (now dominant in the both the high ethanol and high acetate reactors) to carry-out sustained U(VI) reduction.

The Effects of Colloids, Organic Matter, and Ionic Strength on the Mobilization of Cesium and Strontium in Laboratory and Field Experiments

University-Led Research

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Colloid-facilitated transport of contaminants through the vadose zone has important implications for groundwater quality, and has received considerable attention over the last decade. Dissolved organic matter (DOM) is ubiquitous in the vadose zone, and its influence on mineral colloids and solute transport has been well documented. DOM sorption to colloids and sediment grains increases the mobility of colloids, but DOM could decrease dissolved-contaminant sorption to colloids, potentially leading to a reduction in contaminant mobility. The overall effect of DOM on colloid-facilitated contaminant transport is unclear. We conducted laboratory and field experiments to elucidate the effects of dissolved organic matter and colloids on the facilitated transport of cesium and strontium.

The laboratory experiments examined the effects of DOM on colloid-facilitated transport of a radioactive cation (Cs-137) in partially saturated repacked sand columns. The experiments revealed that the mobility of Cs-137 was limited when mineral colloids were absent from the porewater in the presence or absence of DOM. The addition of mineral colloids to the column influent increased Cs-137 mobility, and effluent Cs-137 was dominated by the colloid-associated form, indicative of colloid-facilitated transport. When DOM was added to an influent that contained both mineral colloids and Cs-137, the mobility of both the mineral colloids and Cs-137 significantly increased. The influence of DOM on colloid-facilitated transport is affected by the composition of the porous media and colloid mineralogy; however, the influence of changes in volumetric moisture content on colloid and Cs-137 transport is small.

The field experiments examined the effects of ionic strength and DOM on the mobilization and transport of cesium, strontium, and mineral colloids in a soil composed of fractured shale in the Melton Branch watershed at the Oak Ridge National Laboratory. Rainfall simulation experiments were conducted at a rainfall rate of 2.5 cm h^{-1} in an instrumented soil pedon. The rainfall included solutions of low and high ionic strength (0.01 to 5 mM sodium nitrate) and DOM (0, 2, and 8 mg C L^{-1}). The field experiments revealed a greater importance of the cation exchange mechanism over colloid-facilitated and DOM-facilitated mobilization of Cs and Sr. The infiltrating water passed predominantly through macropores. The mobilization of colloids increased at low ionic strength and higher organic carbon concentrations. Transport of DOM increased with decreasing ionic strengths. Increasing concentrations of colloids and DOM at low ionic strength did not mobilize significant fractions of absorbed Cs and Sr. Mobilization of Cs and Sr increased with an increase in ionic strength of the infiltrating water. Batch desorption experiments also showed that desorption of Cs and Sr increased with increasing ionic strength. The results indicate that ion exchange was the dominant mechanism for mobilization of Cs and Sr in the Oak Ridge soil under the conditions of these experiments.

Biogeochemical and Microbial Controls of I-129 Mobility in Groundwater Relevant to Long-Term Stewardship of DOE Sites

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University-Led Research

I-129 is one of the three major risk drivers (along with ^{99}Tc and ^{137}Cs) at existing and proposed DOE nuclear disposal sites. In aquatic environments, iodine, a biophilic element, mainly exists as I^-, IO_3^- and organic I. The high mobility of I in aquatic systems has led to ^{129}I contamination problems at sites where nuclear fuel has been reprocessed, such as the F-area of Savannah River Site. In order to assess the distribution of ^{129}I and stable ^{127}I in environmental systems, we developed a sensitive and rapid method that has enabled us to determine isotopic ratios ($^{129}I/^{127}I$) of speciated I via GC-MS [*1*].

Iodine occurs in multiple oxidation states in aquatic systems in the form of organic and inorganic species. That I occurs in multiple oxidation states leads to complex biogeochemical cycling of I and its long-lived isotope, ^{129}I, a major by-product of nuclear fission. In our studies, we investigated the sorption, transport, and interconversion of I species by comparing their mobility in groundwater samples at ambient concentrations of I species (10^{-8} to 10^{-7} M) to those at artificially elevated concentrations (~ 0.1 mM), which often are used in laboratory analyses. Results demonstrate that mobility of I species, as well as the type of species, greatly depends on the I concentration used [*2*], mostly due to covalent binding of I to a limited number of organic carbon moieties of the particle surface. At ambient concentrations, I^- and IO_3^- were significantly retarded; at concentrations of 0.1 mM, I^- traveled along with the water [*3*].

We determined if bacteria from a ^{129}I-contaminated aerobic aquifer at the F-area of SRS could accumulate I^- at environmentally relevant concentrations (0.1 µM I^-). Two previously described I^- accumulating marine strains, *Flexibacter aggregans* and *Arenibacter troitsensis* accumulated 2–50% total I^- (0.1 µM), whereas three SRS strains accumulated just 0.2–2.0%. I^- accumulation by FA-30 strain was stimulated by adding H_2O_2, was not inhibited by chloride ions (27 mM), and did not exhibit substrate saturation kinetics with regard to I^- concentration (up to 10 µM I^-). Moreover, strain FA-30 was able to accumulate I through intercellular and intracellular reactions. The results, however, indicate that bacterial I^- accumulation likely does not account for the high fraction (up to 25% of total I) of measured organo-I. However, enzymatic oxidation of I^- likely plays a greater role in iodination of NOM, and is currently being investigated further [*4*].

Our field and laboratory studies currently seek to find the cause for steady increases in ^{129}I concentrations (presently between 400 and 1000 pCi L^{-1}; drinking water limits is 1 pCi L^{-1} ^{129}I) emanating from radiological basins on the SRS. First-order calculations based on a basin sediment desorption study indicate that the modest increase of 0.7 pH units detected in study-site groundwater over the last 17 years since basin closure may be sufficient to produce the observed increased groundwater ^{129}I concentrations. This study underscores the need to identify the appropriate *in situ* stabilization technologies for all source contaminants, especially if their geochemical behaviors differ [*5*]. Spatial distributions of concentrations and speciation of radio-I (^{129}I) and stable I (^{127}I) in groundwater in the vicinity of the F-area seepage basin were investigated in order to test the hypothesis that I^- mobility can be controlled through engineered barrier systems. Near the source term, the majority (~55-86%) of I existed as I^- for both ^{127}I and ^{129}I. As the plume moved downgradient, the pH increased, and the Eh decreased iodide transformations into iodate and organo-I were measured. Considering that IO_3^- has a higher K_d value than I^-, we hypothesized that the production of IO_3^- and lower concentrations of ^{127}I and ^{129}I in downstream areas were due to removal of I from the groundwater. We inferred that removal of I from the groundwater through the formation of high molecular weight organo-I complexes is complicated by the release of other more mobile organo-I species [6]. Finally, through characterization studies of the nature of the interaction between iodine and NOM in the F-Area aquifer, it was shown that NOM clearly behaves as a sink for iodine. However, our work demonstrates that a small fraction of the SOM can also behave as a source, namely that a small fraction that may be readily dispersible under some environmental conditions and presumably release iodine in the organic-colloidal form.

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Uranium Attenuation and Release Investigated at the Molecular and Pore Scales: Responses to Geochemical Gradients in Geologic Media

University-Led Research

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We seek to establish the molecular-scale response of dissolved and solid-phase uranium to pH and selected ligand concentration gradients in soil, sediment, and fractured rock matrices that are representative of subsurface environments at contaminated DOE sites, with emphasis on the Oak Ridge Integrated Field Research Challenge (ORIFC) site. The overall approach of the project is to simulate geochemical gradients that prevail in groundwater uranium plumes at ORIFC, in an experimental setting that permits *in situ* analysis at pore- and molecular scales.

The first phase of research involved performing spatially resolved characterization of U-contaminated heterogeneous media collected from Area 2 and Area 3 downgradient of the S-3 Waste Disposal Pond. Microscale characterization was performed on seven samples at the Stanford Synchrotron Radiation Lightsource (SSRL). X-ray fluorescence microprobe maps collected at the U L(III) absorption edge show distinctly different element correlations in different samples, including *(a)* association with iron and/or manganese (both Area 2 and Area 3); *(b)* association with phosphorus (Area 3); and *(c)* no clear association (Area 3).

Map locations with relatively higher U concentrations were selected for microbeam X-ray Absorption Near Edge Spectroscopy (XANES). In all cases, uranium was present as U(VI). Sorption appears to be the main mechanism of association for uranium present with Fe and/or Mn, while uranium occurring with phosphorus appears in discrete particles consistent with a uranium phosphate mineral phase. Uranium with no clear elemental associations are likely uranium oxide phases.

The second phase of research, in progress, is a series of column experiments in which uranium uptake and/or release is measured over time as advective gradients in ligand concentration (phosphate, carbonate, and acetate, as well as pH) are imposed. Columns comprised of quartz, calcite, goethite, and natural soil layers are equipped with ports for porewater extraction from each layer. The influent solution is a synthetic groundwater similar to reported groundwater composition from Area 2, introduced at a rate of 0.1 mL/minute. Influent solutions are amended over time with increasing concentrations of the target ligands (one per column), with uranium concentration held constant at 100 µM. Porewaters, extracted weekly over a 3-month period, will be analyzed for U concentration by ICP-MS. Subsamples from the columns will be characterized using the X-ray analytical techniques noted above.

The third phase of research, in development, is an investigation of uranium distribution under spatially stable diffusive gradients in pH and carbonate, phosphate, and acetate concentrations developed through sediment mixtures. The sediments will be spatially characterized using the x-ray analytical techniques noted above.

We expect results to provide an improved understanding of hydrogeochemical factors that control uranium mobility, leading to improved predictive capability for subsurface U transport processes. We aim to extend the scientific framework used to develop decision-making strategies regarding remediation of contaminated sites, and in particular, those at the ORIFC site, by considering the pore- and molecular-scale impact of geochemical gradients characteristic of groundwater plumes.

Improved Understanding of Groundwater/Surface-Water Interaction at the Hanford 300 Area Using Spatially and Temporally Rich Datasets

University-Led Research

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The primary objective of this research is to advance the prediction of solute transport between the contaminated aquifer and the Columbia River at the Hanford 300 Area by improving understanding of how fluctuations in river stage, combined with subsurface heterogeneity, impart spatiotemporal complexity to solute exchange along the Columbia River corridor. Our work explores the use of continuous waterborne electrical imaging (CWEI), in conjunction with fiber-optic distributed temperature sensor (FO-DTS) and time-lapse resistivity monitoring, to improve the conceptual model for how groundwater/surface water exchange regulates uranium transport. We also investigate how resistivity and induced polarization can be used to generate estimates of the variation in depth to the Hanford-Ringold (H-R) contact between the river and the 300 Area Integrated Field Research Challenge (IFRC) site.

Strong natural contrasts in temperature and specific conductance of river water compared to groundwater at this site, along with periodic river stage fluctuations driven by dam operations, were exploited to yield new insights into the dynamics of groundwater-surface water interaction. Whereas DTS datasets have provided meter-scale measurements of focused groundwater discharge at the riverbed along the corridor, continuous resistivity monitoring has noninvasively imaged spatiotemporal variation in the resistivity inland driven by river stage fluctuations. Time-frequency (S-Transform) analysis of the DTS datasets, and cross correlation with time series of river stage and groundwater levels, has provided insights into the role of forcing variables, primarily daily dam operations, in regulating the occurrence of focused exchange at the riverbed. For example, high amplitudes in the DTS signal for long periods that dominate the stage time series appear to identify regions along the corridor where stage-driven exchange is preferentially focused. Time-lapse inversion of continuous resistivity imaging datasets has also identified spatial variability in the covariance between bulk resistivity in the Hanford unit and river stage, with higher covariance generally coincidental with the locations where DTS predicts focused exchange. Resistivity and induced polarization imaging between the river and the 300 Area IFRC has imaged spatial variability in the depth to the Hanford-Ringold contact in a region where borehole information is absent. However, synthetic studies based on this imaging effort have shown that successful induced polarization imaging is critically dependent on accurate quantification of measurement errors, and that paleochannels suspected to be locally incised below the Hanford-Ringold contact represent a challenging geoelectrical target. Our work has demonstrated how time-series analysis of both time-lapse resistivity and DTS datasets, in conjunction with resistivity/IP imaging of lithology, can improve understanding of groundwater-surface water exchange along river corridors, offering unique opportunities to connect stage-driven groundwater discharge observed with DTS on the riverbed to stage-driven groundwater and solute fluctuations captured with resistivity inland.

Remaining work on this project involves, (1) analysis of datasets from vertical temperature arrays and pressure transducers installed at locations along the FO-DTS to provide estimates of vertical groundwater fluxes, (2) completion of processing of an extensive new time-lapse resistivity dataset that has been collected on four shore-parallel lines extending inland towards the 300 Area IFRC over a four-month period, and (3) completion of a time-series analysis of the extensive FO-DTS dataset that has been collected during this study. Publications describing the findings of this work are in preparation, being led by collaborator Tim Johnson and Rutgers University Ph.D. student Kisa Mwakanyamale.

Molecular Mechanisms of the mer Operon: Computational Analysis of a Model Hg Trafficking System

University-Led Research

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Mercury is a key contaminant at DOE sites, and understanding biotic and abiotic processes of mercury mobility and transformation is critical for devising long-term solutions at sites. Ionic Hg(II), the most toxic form of mercury, is the substrate for synthesizing the neurotoxin of great public concern, methylmercury. Aerobic and facultative bacteria, which demethylate methylmercury and reduce Hg(II) to volatile, relatively inert Hg(0), abound at all mercury-contaminated DOE sites. These bacteria carry a genetic locus, *mer* operon, whose functions are classical models for interactions of mercury with large and small biotic molecules. We apply computational and experimental approaches to two hallmark *mer* proteins: (1) the metalloregulator, MerR, responsive even to femtomolar Hg(II), and (2) the mercuric reductase, MerA, which receives Hg(II) from a membrane Hg(II) transport system and reduces it to poorly reactive volatile Hg(0), which diffuses out of the cell. Structural insights that we derive from these well-defined proteins will apply to newly discovered proteins in Hg(II) methylating bacteria, and catalytic insights can inform models of Hg(II) trafficking with acellular components of NOM.

Experimental macromolecular structures and dynamics provide both a starting point and points of reference for atomic-level computational investigations. Much is known of the biochemical functions of MerR, but structure and dynamics analyses of MerR using X-ray and neutron scattering were hampered by its low solubility. We have recently increased MerR solubility up to 20-fold by optimizing pH and concentrations of various salts and small solutes. With these optimized buffers, we have grown crystals of MerR and its mutants suitable for X-ray diffraction. Similar conditions will be used for X-rays or neutron solution scattering of MerR to elucidate its unusual but widely found allosteric mechanism.

The rich background of structure, biochemistry, and kinetics of MerA makes it an apt subject for computational dissection of the unresolved atomistic details and energetics of Hg(II) trafficking and reduction by MerA. Structural (X-ray and NMR) and spectroscopic studies suggested possible transfer pathways involving cysteine residues on the N-terminal domain of MerA (NmerA) and within the catalytic core domain. In this study, we used classical molecular dynamics simulations to explore the dynamics of the core domain, with Hg(II) in various states along the transfer pathway. Analyses of these simulations are carried out and will be used to inform future studies that will use mixed quantum mechanics and molecular mechanics (QM/MM) methods. The computational cost of performing highly accurate *ab initio* and density functional theory calculations precludes the configurational sampling required to obtain converged free-energy profiles for enzyme-catalyzed reactions. To overcome these limitations, we report progress in the development and optimization of accurate and efficient semi-empirical QM methods appropriate to MerA catalysis.

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

University-Led Research

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

Our September 2010 experimental campaign at the Integrated Field Research Challenge (IFRC) site located at Rifle, CO, was conducted within an established experimental plot and was based on a continuous recirculation design; water extracted from well M-07 was amended with urea and molasses (a carbon and electron donor) and re-injected into up-gradient well M-02. A total of 168,000 L of re-circulated water amended with 42.5 kg of urea and 2.2 kg of molasses was injected (nominal pumping rate 10.5 L min^{-1}) over a 12-day period. Based on a KBr tracer test conducted as part of this experiment, we estimated a pore volume in our recirculation cell of ~19.3 m^3 and tracer recovery of ~60%. During the course of the experiment, pumping was periodically halted to allow collection of tomographic geophysical data (seismic, radar, electrical). The mass of urea injected upon complete hydrolysis would be sufficient to precipitate over 70 kg of calcite (~26 L).

Slug tests conducted after the completion of the recirculation experiment showed a 96% decrease in hydraulic conductivity in the injection well (M-02) compared to previous tests; the conductivity of the extraction well was essentially unchanged (-3%). The observed decrease in conductivity likely results from increased biomass or mineral precipitation, or both, in the injection well. Preliminary examination of time-lapse radar data indicates that this method was sensitive to induced subsurface property changes.

The urea and molasses treatment resulted in an enhanced population of sediment associated urea hydrolyzing organisms, as evidenced by increases in the number of *ureC* gene copies, increases in ^{14}C urea hydrolysis rates, and long-term observations of ammonium (a urea hydrolysis product) in the injection, extraction, and down-gradient monitoring wells. Modeling activities are under way to define field-scale urea hydrolysis rates, as well as quantify the spatial distribution of injected reactants and reaction products (ammonium and calcite). Long-term sampling/monitoring continues with assistance from the onsite Rifle IFRC staff.

Uranium Biomineralization by Natural Microbial Phosphatase Activities in the Subsurface

University-Led Research

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The project goal is to examine the role of phosphohydrolases in naturally occurring subsurface bacteria for the purpose of promoting the immobilization of uranium through the formation of insoluble uranium phosphate minerals.

Our prior studies with subsurface strains isolated from radionuclide and metal contaminated soils at the DOE Oak Ridge Field Research Center (ORFRC) demonstrated phosphatase phenotypes promoting metal- and uranium-phosphate precipitates during growth on [glycerol-3-phosphate (G3P)] as a sole carbon and phosphorus source. Liberated inorganic phosphate (PO_4^{3-}) precipitated >95% of U(VI) as low solubility uranium-phosphate minerals under oxic and anoxic conditions and different pH (5.5 to 7). Current objectives are to: (1) examine the diversity of the microbial communities present in Area 2 soil slurry incubations and Area 3 flow-through reactor experiments to characterize the subsurface microbial responses to organophosphate additions that promote slow (glycerol-2-phosphate [G2P]) and fast [G3P] release of PO_4^{3-}, (2) conduct flow-through incubations at low pH and in anaerobic conditions to study the competition between uranium adsorption, reduction, and biomineralization, and (3) conduct genome-enabled studies using sequence data of the ORFRC *Rahnella* strain Y9602 and reference strain *Rahnella aquatilis* 33071.

In collaboration with DOE-LBNL investigators (G. Anderson, C. Wu, and T. Hazen), we have characterized the ORFRC Area 2 subsurface microbial community structure and microbial population responses to G2P and G3P amendments under oxic and anoxic growth conditions. Microbial hydrolysis of G3P (20-day incubation) compared to G2P (36-day incubation) under acidic oxic conditions yielded 9.1 mM and 4.7 mM PO_4^{3-}, respectively. By using high-density 16S oligonucleotide microarrays (PhyloChip), significant changes in the richness of taxa belonging to the phyla A*ctinobacteria*, *Clostridia*, *Alpha*- and *Deltaproteobacteria* were identified. Preliminary analysis of 16S clone libraries of flow-through reactor Area 3 soils also demonstrated shifts in microbial community composition in soils treated with G2P compared to controls without organophosphate addition. *Betaproteobacteria* were dominant (>60%) in untreated soils with clones closely related to *Burkholderia* sp. previously identified in other ORFRC studies. *Betaproteobacteria* frequency decreased in all G2P-treated incubations. The frequency of *Actinobacteria* and *Alphaproteobacteria* clones increased in pH 7 columns, while *Gammaproteobacteria* and *Bacilli* clone frequency increased in pH 5.5 columns. Dominant clones in treated soils were most closely related to *Sphingomonas* sp. and *Paenibaccillus* sp. reported in other Hanford and ORFRC studies, respectively.

Additionally, the two *Rahnella* genome sequencing projects, (i.e., *Rahnella* sp. Y9602 and the *Rahnella aquatilis* ATCC 33071), led by JGI, are near completion. Comparative genomic studies between the two strains are under way to examine the potential genomic differences between the two strains. Currently, the Y9602 genome is complete and has allowed for the subcloning of five candidate low-molecular weight acid phosphatases. The sequences of these phosphatases will also be used for PCR screening of ongoing flow-through reactor studies. The combined multiphasic approach of soil slurry and column studies with differing organophosphate substrates, coupled to ongoing genome-enabled studies, will provide a greater understanding of microbial community dynamics involved in phosphate-mediated U(VI) sequestration and biomineralization.

Identifying Biomarkers and Mechanisms of Toxic Metal Stress with Global Proteomics

University-Led Research

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Hg can directly inactivate proteins by binding to their cysteine or selenocysteine residues. In addition, many such proteins are involved in membrane-bound respiration processes, and their inactivation can result in a burst of reactive oxygen species (ROS) amplifying the damage of each Hg atom. One of our goals has been to identify, quantify, and compare ROS- and Hg-damaged proteins under various Hg concentrations to test this model for two mechanisms of Hg toxicity. In doing this, we have defined the "Hg exposome" for proteins and benchmarked the bulk biochemical changes attending Hg damage. We have made great progress in this first aim. Our second goal has been to learn how the mer operon proteins prevent or repair Hg damage to the cell; for nontechnical reasons we must defer completion of this goal.

*The Hg Exposome.*Detection of post-translational protein modifications is one of the biggest challenges in global proteomics. However, looking for Hg-adducts on proteins is facilitated by mercury's 7 abundant stable isotopes. We have used this property to devise a computational filter that can be applied directly to mass spectrometry proteomics spectra to find peptides with inorganic Hg(II) or organic mercury, RHg, adducts (Polacco, et al, in press). We treated growing cells with monovalent organomercurials, phenylmercury acetate (PMA), or merthiolate (MT) (to avoid peptide cross-linking), and have devised variations on standard global proteomics protocols to minimize reassociation of RHg adducts among protein cysteines during preparation for proteomic analysis. These modifications also afforded excellent yields of peptides generally and improved recovery of cysteine-containing peptides, although the latter diminished on RHg exposure.

Using these methods with the model gamma-proteobacterium E.coli MG1655, we routinely recover ~1000-1200 proteins per experiment (three full-scale experiments tabulated). E.coli has 3,654 cysteine-containing proteins, of which 307 have been seen repeatedly to have at least one cysteine that is consistently 80-100% modified by either PMA or MT. They represent all metabolic functional groups; among these, the most frequently modified are proteins involved in energy generation (e.g., ATP synthase, GA3PDH, NADH-UbiQ oxidoreductase, succinic dehydrogenase), translation (e.g., five proteins each from the 30S and 50S ribosomal subunits, seven tRNA synthetases, IF3, EFTs, EFTu, and RF3) and amino acid biosynthesis (e.g., tryptophan synthetase, ornithine transcarbamylase, glutamate synthetase, and dihydropicolinic synthetase). All of these highly modified proteins are conserved among bacteria and archaea, and 30 of them are conserved in higher organisms including humans. Surprisingly, in PMA or MT exposed cells SEQUEST identified no more than background ROS damage in the proteome itself as measured by cysteic acids and methionine sulfoxide.

Bulk Biochemical Changes in the Cell. Total cellular thiols are blocked on exposure to organic or inorganic Hg; on a molar basis, the latter is much more effective at blocking thiols than the former. EPR revealed release of ~ 70% of cellular Fe, probably from Fe-S proteins. Fe was not leaked from the cell, nor were any of seven other essential metals. However, Hg exposure causes 3-fold depletion of potassium, likely from activation of the glutathione-regulated potassium efflux protein, and a corresponding increase in cellular sodium. We have observed Hg-sulfur adducts in many specific proteins consistent with these bulk physiological deficits.

Surprisingly, Hg continues to accumulate in the cells even in excess of the total cellular thiol pools. EXAFS revealed that at acute exposures, Hg increasingly takes N, O, or C ligands in addition to sulfur. In a lipid extraction procedure, we found 79% of the cell-associated Hg is in the macromolecular fraction and ~ 20% is soluble low-molecular-weight thiol compounds; both fractions offer many possible nitrogen and oxygen ligands. Although C, N, and O ligands are difficult to distinguish by EXAFS, unsaturated fatty acids could provide a substrate for oxymercuration. However, finding that <1% is in the organic phase suggests that does not occur under the conditions of our experiments.

Defining the Molecular-Cellular-Field Continuum of Mercury Detoxification

University-Led Research

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Sustainable stewardship requires eliminating both $MeHg^+$ and Hg^{2+}, the substrate for methylation. Enhancing removal $Hg^{2+}/MeHg^+$ from wetlands and waterways by natural Hg-resistant (HgR) microbes requires knowing how HgR works, including its metabolic demands on the cell. We work with proteobacteria that are abundant in high Hg areas of the Oak Ridge Reservation (ORR), and now extend our studies to HgR actinobacteria, also in high Hg regions of ORR. We examined mer protein interactions with each other and with specific host proteins to rapidly and completely convert Hg^{2+} or RHg^+ to Hg^0.

PROTEOBACTERIAL MerA/MerB: In the mer pathway, organomercurial lyase, MerB converts RHg^+ to Hg^{2+}, which is then reduced to Hg^0 by MerA, the mercuric ion reductase. In gamma-proteobacterial mer operons, MerA has an N-terminal metallochaperone-like domain, NmerA, tethered to each monomer of its homodimeric catalytic core. To simplify NmerA analysis, we have used separately expressed NmerA and catalytic core to show that NmerA can acquire Hg^{2+} from cellular proteins and deliver it to the catalytic core. (1) Recently, we showed that NmerA alone removes Hg^{2+} from MerB 100-fold more rapidly than the small cellular thiol, glutathione, indicating that specific interaction between NmerA and MerB expedites transfer. Here we show that a conserved MerB residue near the bound Hg(II) prevents premature release of Hg(II) until it is “triggered” by the NmerA domain of MerA. (2) We also recently developed a method to produce uncleaved, full-length MerA and used it to estimate the mobility of tethered NmerA domains and identify where NmerA interacts with catalytic core during Hg(II) transfer (with the ORNL SFA). We also determined the kinetic advantage of tethered NmerA by comparing Hg^{2+} transfer from a Hg-MerB complex to the catalytic core directly or via tethered NmerA.

ACTINOBACTERIAL MerA/MerB: Relatives of *Streptomyces lividans* are also found in high Hg regions of the East Fork Poplar Creek of the ORR. We have initiated to study properties of these co-evolved MerA/MerB proteins. *S. lividans* MerA lacks the tethered NmerA domain, and its MerB lacks a cysteine of proteobacterial MerB essential for Hg(II) transfer to NmerA, but has a distinct C-terminal cysteine pair. Studies of the role of this cysteine pair in RHg binding and Hg(II) release will be presented.

PROTEOBACTERIAL REGULATION OF mer EXPRESSION: In proteobacteria, repressor-activator MerR captures RNA polymerase (RNAP) in an inactive pre-initiation complex at the structural gene operator-promoter, MerOP. Hg(II)-binding to MerR causes underwinding of MerOP DNA, allowing RNAP to transcribe the mer mRNA. To dissect Hg(II) interactions in this complex, we first located Hg(II) binding sites in the AT-rich MerOP. Calorimetry reported three Hg(II) binding sites in a 38-bp MerOP, and EXAFS showed that nitrogen ligands are involved as expected from known binding of Hg(II) to thymidines. Since MerR tightly binds MerOP and Hg(II), an anti-activator protein, MerD, is used by proteobacteria to turn expression off when MerA has reduced all Hg(II). We have purified MerD, mutated it, and are producing antibodies for interaction studies with MerR, MerOP, and RNAP. We are also measuring mRNA lifetime by Northern and RT-qPCR to discern how MerD turns off mer transcription when Hg(II) is low, although Hg(II)-MerR stays bound to MerOP.

ACTINOBACTERIAL REGULATION OF mer EXPRESSION: *Actinobacteria* control their mer operons with a simple repressor of the structurally distinct ArsR family, which includes regulators for Cd, Zn, and Cu efflux. Other ArsR-family proteins are poorly soluble, so we work with the UT-ORNL group to optimize solubility of *S. lividans* MerR (SLMerR) for biophysical and biochemical studies to dissect the basis for its specific response to Hg(II). Also, since there are 3D structures of ArsR-family metalloregulators, we can use homology models to begin computational studies with the ORNL group on its metal-specific responses.

Manganese Redox Mediation of UO_2 Stability and U Fate in the Subsurface: Molecular- and Meter-Scale Dynamics

University-Led Research

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Long-term stability of U(IV) is imperative to the success of U bioremediation in the environment. Injection of electron donors such as acetate and establishment of reducing conditions is being tested to reductively precipitate U(VI) as U(IV) species. During this process, reductive dissolution of Fe and Mn oxides will occur, followed in time, by the establishment of sulfidogenic conditions. After the injection of electron donor ceases, groundwater conditions will slowly return to more oxidizing conditions. As this occurs, strong oxidants such as MnO_2 can form through microbial activity under suboxic conditions, jeopardizing the stability of U(IV). The focus of our project since it started in June 2010 has been (1) to investigate whether MnO_2 formation can occur under low O_2 conditions in both laboratory and *in situ* field experiments, (2) to examine the fate of U once it is oxidized by MnO_2 and the effects of pH and carbonate concentration, and (3) to determine whether direct contact between MnO_2 and U(IV) is necessary for U re-oxidation and where the reaction is localized.

Laboratory experiments with a model Mn-oxidizing bacterium demonstrated that Mn oxidation may proceed at relatively high rates, even when the O_2 concentration is very low (0.1%). Kinetic analysis revealed that the bacteria had a high affinity for O_2. To test whether Mn oxidation can proceed under field-relevant conditions, we developed a method to encapsulate active Mn(II)-oxidizing bacteria in a diffusible gel (gel puck) that can be incubated *in situ* in the wells at the Rifle IFRC site. We have isolated a variety of strains from Rifle sediments and are testing their efficacy in the gel pucks. We are planning to deploy these gels at the Rifle site later this spring. During this past field season at Rifle, we deployed sediments in mesh bags and suspended at various depths to probe the field DO concentrations that support Mn oxidation. We also deployed *in situ* column experiments, which received an inflow of 25 µM Mn^{2+} to probe the accumulation of Mn oxides. Finally, we have performed *in situ* experiments to verify that U(IV) can be reoxidized by MnO_2 at the Rifle aquifer through the use of diffusive gels. Mesh bags, columns and gel pucks with U(IV)/MnO_2 were retrieved in November 2010 and are being characterized using ICP-OES, XAS, and SEM/TEM. Beamtime at SSRL was allocated to this project, and XAS measurements will be made in March and April 2011.

To understand the reaction between MnO_2 and U(IV), biogenic uraninite or monomeric U(IV) were embedded in gels along with a Mn oxidizer and incubated in a basic medium with 5% oxygen and 100µM Mn(II). Although Mn oxidation was evident in the gel pieces, no U(VI) was released from the gel into the medium, although U(VI) was present in the gel. These gel pieces will be analyzed by electron microscopy, XAS, and microXAS to explore the spatial relationships among U(VI), Mn oxides, and the Mn(II)-oxidizing bacteria.

Finally, experiments are being conducted to further explore the reactions occurring at the MnO_2 mineral interface, specifically the adsorption of U(VI) to MnO_2 and the oxidation of U(IV) on MnO_2 surfaces. Results to date show that U(VI) adsorbs strongly to MnO_2 above pH 4, and that carbonate has a strong negative effect above pH 8 due to the formation of U(VI)-carbonate complexes. Samples from these experiments will be analyzed by XAS to determine the molecular-scale structure of the adsorbed U(VI) and the effects of pH and carbonate.

Phylogenetic Structure and Functional Profiling of Uranium-Contaminated Sediments Enriched *In Situ* and *In Vitro*

University-Led Research

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Our primary objectives were to better understand the demographic shifts and functional capabilities of bacterial communities in response to uranium as a contaminant and electron acceptor, and to identify previously unknown populations capable of reducing uranium. We used both *in situ* and *in vitro* approaches to address these goals.

In situ bioremediation of uranium with a slow-release electron donor, emulsified vegetable oil (EVO), was tested in Area 2 of the U.S. DOE IFRC site, Oak Ridge, TN. The EVO containing (w/w) 60% vegetable oil, 6% biodegradable surfactant, 0.3% yeast extract, and 0.05% $(NH_4)_3PO_4$, was injected one time into the subsurface. Groundwater samples were collected from one up-gradient (W8) and seven down-gradient wells (W1–7) to monitor temporal dynamics of geochemistry and functional genes of microbial communities over nine-months using GeoChip. Detrended correspondence analysis (DCA) of all detected genes suggested that significant changes in microbial communities occurred down-gradient but not in the up-gradient control. Corresponding to the geochemical changes, genes involved in biodegradation of the EVO (acetogenesis, CH_4 production and oxidation) and reduction of the electron acceptors (denitrification, cytochrome, sulfate reduction, dissimilatory and assimilatory nitrate reduction) were significantly enriched. The detected genes revealed enrichment of denitrification genes narG, nosZ, and dsr from *Desulfotomaculum* and *Desulfovibrio,* and cytochromes of *Geobacter*, *Anaeromyxobacter*, and *Desulfovibrio* after EVO injection, followed by a decline as EVO was consumed. These changes closely correlated with the concentration changes of acetate and the electron acceptors. This study demonstrated that a one-time injection of EVO provided e-donors for the effective bioreduction of U(VI) and *Geobacter*, A*naeromyxobacter*, *Desulfovibrio*, and *Desulfotomaculum* were detected in abundance.

In vitro enrichments tested a broader range of electron donor and acceptor combinations. Three uranium contaminated sediment samples from the DOE IFRC site (FW107, FW102-2 and FW102-3) were collected from Area S3 and used as microcosm inocula in enrichments with either nitrate, Fe(III), or U(VI) as electron donors. Enrichments were sequentially transferred every 35 days for three cycles. Permutational MANOVA revealed that the bacterial communities were driven by both the inocula community (P=0.01) and electron acceptors (P=0.01). GeoChip analysis was performed on the second sequential enrichment and revealed that a diverse group of genes encoding for aromatic degradation, cytochromes and metal resistance (e.g., copA, chrA, and czcAD) was found in all microcosms. It was also observed that nitrogen fixation (nifH) and hydrogenase genes were more abundant in uranium-grown microcosms than nitrate-grown ones. In light of the GeoChip results, we tested two sets of previously designed Fe-Fe hydrogenase primers to investigate the influence of uranium on the diversity of hydrogenase genes. Clone libraries of hydrogenase genes were generated from a separate set of uranium-supplemented microcosms inoculated with identical amounts of FW107 or FW102-2 derived cell mass. The results indicated that FW107 inoculum contained hydrogenase genes most closely affiliated with *Clostridium botulinum* as well as *Alkaliphilus*, *Paenibacillus*, *Pelotomaculum*, and *Ruminococcus*. When the community was enriched in the presence of U(VI), a less diverse collection of Fe-Fe hydrogenase genes were detected that included additional species of *Clostridium* as well as *Desulfotomaculum*. Fe-Fe hydrogenase coding genes affiliated with *Clostridium*, *Symbiobacterium*, and *Shewanella* were amplified from the initial inoculum of FW202-2 whereas under U(VI) selection, sequences affiliated with *Clostridium*, *Shewanella* and *Desulfotomaculum* were detected.

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

University-Led Research

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Natural redox conditions regulate technetium (^{99}Tc) species and mobility. Under reducing environments, highly mobile pertechnetate ($Tc^{VII}O_4^-$) is readily reduced to sparingly soluble Tc^{IV} oxides, (e.g. $TcO_2 \cdot nH_2O$). Numerous studies have investigated the reduction of TcO_4^- by naturally occurring abiotic and biotic processes, on the premise that these could be used for long-term immobilization of ^{99}Tc. The majority of those processes involve biogenic generation of Fe(II) and iron minerals, which subsequently reduce Tc. While effective for short-term immobilization of ^{99}Tc, one major difficulty with this strategy is that the (re)oxidation of Tc^{IV} oxides is relatively facile, and remobilization is likely to occur under oxidative environmental conditions.

Our research objective is to provide the fundamental understanding necessary to evaluate the feasibility of reductive immobilization of TcO_4^- by controlled application of nanoscale zero-valent iron (nZVI) to stimulate sulfidogenic conditions and, consequently, microbial sulfate reduction. Under sulfidic conditions, ^{99}Tc forms either discrete Tc-sulfide solid phases or co-precipitates with FeS phases, both expected to be less susceptible to oxidation. ZVI deployed in subsurface permeable reactive barriers can scavenge natural oxidants, including O_2 and NO_3^-, creating a reducing environment ideal for sulfate-reducing bacteria (SRB). In addition, H_2 generated from ZVI corrosion can serve as an electron donor to boost the growth of SRBs.

Biotic experiments utilized *Desulfotomaculum reducens* in a standard SRB medium (WP, 37^{o}C) with or without 0.5 g/L nZVI. A large-scale experiment (500 mL cultures) indicated that nZVI stimulated sulfidogenesis, resulting in an earlier start to biotic sulfate reduction and about a 3-fold increase in cell numbers, trends confirmed in a small-scale (60 mL) trial. Mössbauer and micro-XRD analysis revealed that mackinawite was the primary FeS phase formed during sulfate reduction in the presence of nZVI. For subsequent biotic experiments, *D. reducens* culture conditions were optimized to more closely mimic the Hanford subsurface: a synthetic groundwater medium (HS-300) was used, and cultures were grown at 27^{o}C. Following a preliminary small-scale experiment under these conditions, which indicated that *D. reducens* or nZVI alone or in combination effectively removed TcO_4^- from solution, a large-scale trial was set up to investigate any ^{99}Tc-containing sulfides generated.

To clarify the role of individual chemical species in sequestering ^{99}Tc in a biotic system, we performed series of abiotic reduction experiments. Sulfide was added in doses varying from 0 to 10 mM to simulate different stages of the biotic development of sulfidogenesis. At low sulfide doses (0, 0.1, 0.5, and 1 mM), mimicking the initial period of biotic sulfate reduction, all aqueous sulfide was scavenged during pre-equilibration by nZVI. Following $^{99}TcO_4^-$ addition, ^{99}Tc removal rates fit first-order kinetics and correlated strongly with the sulfide dose. However, the two higher sulfide doses (5 and 10 mM), representing the intermediate and end stages of biotic sulfate reduction, resulted in a significant level of aqueous sulfide present before $^{99}TcO_4^-$ addition. This excess aqueous sulfide appeared to substantially drive the reaction away from first-order kinetics, resulting in low but persistent levels of aqueous ^{99}Tc, even after 100 hours of reaction. Reoxidation experiments indicated that sulfidic conditions resulted in slower ^{99}Tc reappearance rates and significantly lower aqueous ^{99}Tc concentrations after more than 100 hours, compared to nonsulfidic conditions. The amount of ^{99}Tc released to the aqueous phase decreased as the sulfide dose increased.

Ongoing research involves characterization of solids recovered at different stages of the experiments, including Electron Microprobe and Microscopy, XRD, Mössbauer, and XPS. The data obtained, combined with that from wet chemical analysis, should provide a fundamental basis to understand the complex (bio)geochemistry of this alternative strategy that utilizes sulfidogenic conditions to sequester ^{99}Tc in the subsurface environment.

Influence of Natural and Synthetic Organic Ligands on the Stability and Mobility of Reduced Tc(IV)

University-Led Research

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Our work aims at better understanding the fate and transport of reduced technetium [Tc(IV)] in the presence of natural and synthetic organic ligands that commonly co-exist with the contaminant, in order to design better strategies for remediating Tc-contaminated DOE sites. We have successfully determined the stability constants of the complex formed between $TcO(OH)^+$ and acetate at varying ionic strengths (NaCl) and, using the Specific Ion Interaction Theory (SIT) equations, we determined the stability constant at zero ionic strength, $\log\beta_{1,-1,1}^0$, to be 2.8 ± 0.3 [1]. We have also quantified the binding constants of complexes formed between Tc(IV) species and natural humic (HA) and fulvic (FA) acids, including those isolated from the Oak Ridge Integrated Field Research Challenge (IFRC) site, as a function of the solution ionic strength [2,3,4]. Results showed that neither the ionic strength, the origin of the humic acids (IFRC HA, purified Sigma Aldrich HA, or International Humic Substances Society Elliot Soil HA), nor the type of humics (i.e., humic versus fulvic acid) significantly affect the humate complex binding constants. Interestingly, the dominant neutral species $TcO(OH)_2^0$ form a humate complex, with a binding constant $\log\beta_{1,-2,1}$ of ca. 4. The value of $\log\beta_{1,-1,1}$ for the humate complexes with $TcO(OH)^+$ is 6.5. Based on these data, PHREEQC calculations showed a predominance of the TcO(OH)-HA complex at $pH < 6$, while the $TcO(OH)_2$-HA complex and $TcO(OH)_2^0$ are the major species at $pH > 6$. The complexation constants of Tc(IV) species with EDTA and citrate have also been determined as a function of the solution ionic strength (0.5-3 M) [5,6]; $\log\beta_{1,-1,1}$ varies between 7 and 9 for EDTA, and between 5 and 6 for citrate. Results also showed the presence of an EDTA complex with the neutral $TcO(OH)_2^0$ species at neutral pH, with $\log\beta_{1,-2,1}=5$, over the range of ionic strengths studied, while citrate does not form such a complex. In agreement with the binding constant presented above, all natural and synthetic organic ligands were found to enhance the dissolution of reduced Tc(IV) by 5–10 fold [7,8], although EDTA and Elliot soil HA were among the most effective in causing the dissolution of Tc(IV) under strict anaerobic conditions. Interestingly, however, under oxidizing conditions, EDTA was found to decrease the oxidative dissolution of Tc(IV) due to their strong complexation, whereas Elliot soil HA enhanced the dissolution, which was attributed to its redox reactive functional groups by accepting electrons from reduced Tc(IV), thereby causing its enhanced oxidative dissolution. This study indicates that both natural and synthetic organic ligands may significantly impact the stability and mobility of Tc(IV), and should be considered in the long-term stewardship and design of remediation strategies for soils and groundwater contaminated with Tc.

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Viral infection of Subsurface Microorganisms and Metal/radionuclide Transport

University-Led Research

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Microbially mediated metabolisms have been identified as a significant factor either directly or indirectly impacting the fate and transport of heavy metal/radionuclide contaminants. To date, several microorganisms have been isolated from these contaminated environments, including metal-reducing bacteria. Examination of annotated finished genome sequences of bacterial isolates obtained from environments containing uranium and nitrate, *Geobacter uraniumreducens* Rf4, *G. daltonii* FRC-32, *Anaeromyxobacter* sp. Fw109-5 and *Acidovorax ebreus* TPSY, revealed phage genes integrated into the chromosome. Presence of these gene sequences indicates that these indigenous subsurface bacteria are susceptible to viral infection. To date the role that viruses play influencing microbial mortality and the resulting community structure under varying redox conditions in subsurface sedimentary environments remains poorly understood.

The objective of this project is to investigate viral infection of subsurface bacteria and the formation of contaminant-bearing viral particles. This objective will be approached by examining the following working hypotheses: (i) subsurface microorganisms are susceptible to viral infections by the indigenous subsurface viral community, and (ii) viral surfaces will adsorb heavy metals and radionuclides. In an effort to initially assess the significance of viral infection in subsurface microbial communities, the production of viral like particles in response to biostimulation of the microbial community was investigated by inoculating low-nutrient culture medium containing acetate and nitrate with uranium and nitrate containing shallow subsurface sediment (25% mass/vol). Acetate was oxidized coupled to the reduction of nitrate, resulting in an increase in bacterial abundance. Biostimulation led to a concurrent increase in viral like particles (VLP’s) resulting in a virus-to-bacteria ratio that ranged from ca. 480 to 2,400 over the course of the study. No significant production of VLP's was observed when acetate or nitrate was omitted from culture medium. Efforts are ongoing to identify and link the infective viral community to the metabolically active microbial community, as well as to assess the role of viral surfaces in adsorping heavy metals and radionuclides. Similar to bacterial surfaces, viral surfaces could adsorb heavy metals and radionuclides subsequently influencing contaminant transport. It is therefore necessary to establish the potential relationship(s) between viruses, subsurface microbial communities, and contaminant metals/radionuclides, to provide sufficient scientific understanding such that DOE sites would be able to incorporate coupled physical, chemical, and biological processes into decision making for environmental remediation and long-term stewardship--by establishing viral-microbial relationships and the subsequent fate and transport of heavy metals and radionuclides.

Imaging Biofilms in Opaque Porous Media

University-Led Research

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This exploratory project focuses on development of quantitative imaging tools for characterizing biofilm architecture in porous media. The overarching objective is to generate new knowledge about the spatial arrangement of biofilms and to develop data sets that can be used for verification of existing and new numerical models that can predict biofilm growth and resulting architecture.

Quantitative imaging of biofilms has historically been approached using various types of microscopy (light, CLSM), magnetic resonance imaging of limited resolution, index-matched porous media and fluids, or other visualization techniques that are limited to biofilms grown on a flat substrate or in two-dimensional micromodels. Most of these techniques are inherently limited in visualizing much of the third dimension or provide insufficient resolution for delineation of biofilms.

Our approach has been to use x-ray microtomographic imaging in combination with novel use of x-ray contrast agents. The main obstacle to using x-rays for visualization is the fact that biofilms and their aqueous environment have very similar x-ray absorption capacities and are therefore difficult to separate in a reliable and quantitative manner. At this point, we have tested two different techniques: (1) Physical straining or trapping of an x-ray contrast agent on the outer surface of the biofilm, and (2) physical separation of biofilm and aqueous solution based on size-exclusion of a suspension. Both approaches have been tested in two-dimensional micromodels and in three-dimensional flow columns using the GSECARS computed tomography facility at the Advanced Photon Source.

Overall, both techniques show promise; however, both also have some limitations in terms of use. A verification study has been successfully completed for the physical straining approach (using Ag colloidal particles), but the technique is being further explored to outline optimal conditions of use. Current research on the use of a barium sulfate suspension is focused on optimizing the concentration such that sufficient contrast is obtained in the images, without the suspension potentially compressing the biofilm.

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

University-Led Research

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Most reactive surfaces in clay-dominated sediments are present within nanopores (pores of nanometer dimension). The behavior of geological fluids and minerals in nanopores is significantly different from those in normal non-nanoporous environments. The effect of nanopore surfaces on U(VI) sorption/desorption and reduction is likely to be significant in clay-rich subsurface environments. Our research objective is to test the hypothesis that U(VI) sorption on nanopore surfaces can be greatly enhanced by nanopore confinement environments. In order to test this hypothesis, we are proceeding with the following tasks: (1) Synthesize and characterize nanoporous alumina and goethite as analog materials; (2) Investigate U(VI) sorption and desorption on nanopore surfaces in controlled carbonate-bearing model systems; (3) Investigate U (VI) sorption and desorption of natural sediments from FRC site (4) Investigate effects of nanopore sizes on the susceptibility of the sorbed U(VI) to reduction by a range of commercially available quinones with well-defined redox potentials, as well as well-characterized dissimilatory metal-reducing bacteria (e.g., *Geobacter sulfurreducens*); and (5) Carry out EXAFS studies of U(VI) sorbed on normal surfaces, on nanopore surfaces, and in post-reduction samples in order to understand the chemical environments of the sorbed U(VI) at the molecular level.

U(VI) sorption affinity was evaluated with non-nanoporous alpha-alumina (SSA of 8 m^2/g) and crushed natural goethite (SSA of 5 m^2/g), as well as nanoporous natural goethite (SSA of 60 m^2/g; nanoporosity of 30 μL/g for < 5 nm pore) and alumina materials with large nanopores (SSA of 147 m^2/g; nanoporosity of 90 μL/g for < 5 nm pore) and small nanopores (SSA of 230 m^2/g; nanoporosity of 145 μL/g for < 5 nm pore). Sorption of 100 μM U(VI) onto 0.2 g solid material in 10 mL of 5 mM HCO_3^- solution under near-neutral pH was rapidly completed for nanoporous goethite and Al oxides, whereas only ~30-60% sorption occurred on non-nanoporous oxides. Bicarbonate and pH exerted a minor effect on U(VI) sorption on nanoporous goethite and Al oxides, but played a significant role in hindering U(VI) sorption on non-nanoporous goethite and Al oxides. The majority of U(VI) remained on the surface of nanoporous goethite and Al oxides after extraction with 50 mM $NaHCO_3$, whereas nearly all U(VI) was released from non-nanoporous materials. Sorption and desorption results collectively suggest that nanoporous materials have significantly higher U(VI) sorption affinity than non-nanoporous materials.

Microbial reduction of soluble U(VI) to sparingly soluble uraninite (UO_2) is considered an efficient remediation technique for subsurface uranium contamination. An important unanswered question for *in situ* bioremediation is whether U(VI) sorbed on a reactive mineral surface is subject to effective and rapid reduction, as is the case for aqueous U(VI). To investigate the chemical linkage between U(VI) sorption affinity and the redox reactivity on a mineral surface, U(VI) bound to the surfaces of non-nanoporous alpha-alumina and nanoporous alumina (2~5 μmole U/g) was reduced by 1 mM hydroquinone (AH_2DS) over 1 day to 3 weeks in an anaerobic chamber. The reduction of U(VI) sorbed on the alumina surfaces was estimated by comparing the U(VI) desorption in anoxic bicarbonate solution (10~1000 mM) between 1 mM AH_2DS- reduced and unreduced samples. Duplicate samples were analyzed by U L_{III}-edge x-ray absorption spectroscopy for uranium speciation. The results indicate complete reduction to nanoparticulate uraninite of U(VI) sorbed on non-nanoporous alumina in 2 days, whereas little to no reduction was observed with U(VI) sorbed on nanoporous alumina with large pores (15% U(IV)) and small nanopores (5% U(IV)) after 1 weeks. This result is attributed to the higher sorption affinity of U(VI) to nanoporous surfaces than to non-nanoporous surfaces.

Federal Agency-Led Research

Upscaling of U(VI) Desorption and Transport from Decimeter-Scale Heterogeneity to Plume-Scale Modeling

Federal Agency-Led Research

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Previous studies of a uranium-contaminated aquifer at the Naturita CO UMTRA site showed that a calibrated reactive transport model that used an equilibrium controlled surface complexation model to simulate adsorption matched the uranium plume observed in 2001 reasonably well. However, predictions from this calibrated model over the last ten years show that observed uranium concentrations decreased significantly faster than predicted with the uniform model, particularly in the upgradient region of the aquifer. The focus of this project is the development of approaches for upscaling reactive transport models by investigating U(VI) desorption across spatial scales that are larger than typical bench-scale studies, with the aim of providing more robust predictions at the plume scale. The project is using extensive datasets previously collected at the uranium mill tailings site at Naturita, CO, at bench-, decimeter-, and tracer-test scales to evaluate the extent to which subsurface physical and geochemical heterogeneities need to be understood to improve model upscaling to the plume scale.

We previously collected large U(VI) desorption datasets in batch experiments, column experiments, experiments with known decimeter-scale physical and chemical heterogeneity (laboratory tanks with controlled grain size sediment packing structures), and finally in meter-scale, natural- and forced-gradient tracer tests. Key issues in upscaling to the decimeter-scale are the chemical gradients introduced as a result of water mixing caused by physical heterogeneity and the heterogeneous chemical properties of sediment grain size fractions. Intensive modeling studies of batch desorption data for a <2mm composite material and two size fractions (<0.25 mm and 0.25 to 2 mm) are being conducted to evaluate both one- and two-site equilibrium surface complexation models. These equilibrium surface complexation models are being incorporated into multicontinuum mass-transfer models of U(VI) elution in the column experiments to determine mass-transfer models and parameters. These models will provide a basis for simulating observed behavior of the tank experiments. High-resolution simulations of the tank experiments will provide a direct test of the bench-scale models in decimeter-scale experiments.

Tracer tests planned for summer 2011 will observe *in situ* desorption processes over longer time scales compared with previous experiments. These tests will be monitored by groundwater sampling and electrical tomography measured with both surface electrodes and two rows of downhole electrodes. The goal is to evaluate upscaling approaches at two distances from the injection gallery. Knowledge of the spatial distribution of sedimentary facies defined by previous geophysics studies, coupled with new sediment acquisition and characterization, will provide a better understanding of heterogeneity at the plume scale. Ultimately, plume-scale predictions of U(VI) transport for 13 years (1999-2012) will be compared with measured values in the aquifer, with upscaling of reactive transport parameters in accordance with the meter-scale studies.

Multiscale Assessment of Prediction Uncertainty in Coupled Reactive Transport Models

Federal Agency-Led Research

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Reactive transport simulations provide a systematic framework for integrating hydrologic and biogeochemical conceptual process models into a quantitative description of subsurface behaviors, which can then be used to project future concentrations. Assessing the uncertainty associated with these projections is difficult, because subsurface environments are open and complex, and subject to multiple interpretations and conceptualizations. In this project, we embrace alternative conceptualizations in a Bayesian Model Averaging (BMA) approach, which considers uncertainty resulting from both model parameters and alternative conceptualizations.

The methodology is being applied the Naturita UMTRA site to investigate how uncertainty of adsorption processes impacts uranium transport at scales ranging from column tests to the plume scale. Analysis of column studies packed with purified quartz powder showed that the response surfaces were often highly complex, indicating the need for global optimization approaches. Seven alternative surface complexation models were simultaneously calibrated to three datasets for the quartz columns and predictions were made for a fourth dataset. Of the seven postulated models, only three had a significant probability. Model uncertainty significantly exceeded parametric uncertainty even in these well-controlled laboratory experiments, and model averaging gave significantly superior predictions relative to any single model. This model-averaging approach is being applied to a pair of field tracer tests where the alkalinity of the groundwater was either increased or decreased, which caused uranium desorption or adsorption respectively. An exhaustive calibration study of alternative adsorption stoichiometry demonstrated that the most probable adsorption models all contained dicarbonato surface complexes. Models calibrated to the increased alkalinity experiment gave good predictions of the experiments with decreased alkalinity. In contrast, calibration to the decreased alkalinity dataset gave predictions of the increased alkalinity experiments with slightly larger mean errors. Two-dimensional synthetic studies with alternative geostatistical models of hydraulic conductivity have shown that the Kashyap Information Criterion (KIC) provides a suitable measure of model probability when compared with the computationally demanding Markov Chain Monte Carlo technique. In addition, it was found that linear and nonlinear confidence intervals reflect only parametric uncertainty, and therefore model uncertainty cannot be implicitly considered and compensated for by measures of parametric uncertainty. Instead, model uncertainty should be explicitly considered using model-averaging methods.

Simulations are also being applied at the Rifle IFRC site to evaluate the parametric and conceptual model uncertainty for the stimulated bioreduction of U(VI) by microbial processes. A synthetic model of *in situ* uranium bioremediation based on the Rifle site has been developed to evaluate the interplay of transport rates, biogeochemical reaction rates, and spatially variable material properties that determine the location and magnitude of key reaction products. Synthetic observations were generated by vertically averaging 3-D reactive transport simulations that considered 3 facies present at the site having varying physical and reactive properties. One-dimensional simulations successfully reproduced vertically averaged synthetic observations for the first 100 days of the simulation. However, simulations for next 50 days predicted a rebound in the uranium concentration that was not indicated by the data--this difference could be attributed to microbial dynamics.

Scientific Discovery through Advanced Computing (SciDAC) Research

PFLOTRAN: The Next-Generation Peta-Scale Subsurface Reactive Flow and Transport Code

SciDAC Research

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The next-generation, peta-scale, reactive flow and transport code PFLOTRAN developed under a SciDAC-2 project for modeling subsurface processes, is being augmented to incorporate adaptive mesh refinement (AMR), based on the SAMRAI package developed at LLNL and mimetic finite difference (MFD) methods, to model highly anisotropic porous media using a mass conserving, second-order accurate scheme with scalar quantities obtained at the grid cell faces. These new techniques have been implemented into PFLOTRAN for the Richards equation and are currently being extended to multiphase flow and reactive transport. When completed, they will enable ground-breaking advances in modeling DOE legacy contaminated sites such as Oak Ridge, Savannah River, and Hanford, as well as application to geothermal energy and CO_2 sequestration. Finally, in anticipation of next-generation heterogeneous computing architectures, the geochemistry routines within PFLOTRAN are currently being restructured for vectorization through OpenMPI and CUDA directives to enable vector operations on hybrid multicore/GPU architectures.

Hybrid Numerical Methods for Multiscale Simulations of Subsurface Biogeochemical Processes

SciDAC Research

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The traditional approach to reactive transport modeling represents porous media as an effective continuum, with continuum models and properties representing upscaled averages of processes defined at the pore scale. However, for some problems in subsurface biogeochemistry, conditions necessary for the validity of upscaling closure approximations are violated, requiring an alternative approach. One such approach is the multiscale hybrid method, in which pore-scale subdomains are embedded in a continuum domain in those areas where continuum approximations are invalid. Past work under this project has focused on the quantification of conditions under which pore-scale simulation is needed, and on development of high-performance codes for computationally efficient 3D simulation at pore- and continuum-scales. Now, these tools are being combined into a comprehensive multiscale hybrid modeling framework and tested using a mixing-controlled precipitation reaction problem based on experiments conducted at INL.

We have developed two approaches for hybrid multiscale coupling of pore- and continuum-scale models. In the first approach, developed at UCSD, an iterative approach is used to match boundary conditions between continuum- and pore-scale domains. This approach was tested on a model system of transport in a fracture with reactive walls (Battiato et al., *Advances in Water Resources*, 2011). Future work will expand this method to a complex pore geometry (with solution of Stokes flow equations) and patchy heterogeneous reaction properties. The second approach is based on a dimension-reduction method in which short bursts of pore-scale simulation are performed and numerically upscaled to provide parameters for selected subdomains of a continuum-scale model. The continuum-scale model is then advanced on a longer time step, providing initial and boundary conditions for another short period of pore-scale simulation. This approach avoids the need to impose general closure approximations by performing the upscaling closure numerically and updating it periodically, while at the same time minimizing the computational demands associated with pore-scale simulation. This method was applied to a mixing-controlled precipitation reaction problem (Tartakovsky and Scheibe, submitted).

We are now conducting a full-scale application of the dimension-reduction method to intermediate-scale experiments on calcite precipitation.Two Science Application Partnership projects support this effort. In the first, continued development of the pore-scale Smoothed Particle Hydrodynamics (SPH) and continuum-scale STOMP parallel codes has proceeded. The focus has been on incorporation of multi-component reactions and testing of a load-balancing approach for reactions based on task sharing using Global Arrays counters. In the second, the SALSSA workflow and data management system has been expanded to provide mechanisms for multitasking job control. This is needed for the full-scale application of the dimension reduction approach, which requires simultaneous solution of multiple pore-scale simulation subdomains, each of which can be run on multiple processors (i.e., there is a dual parallelism inherent to the problem). Over the course of a single multiscale hybrid simulation, many such jobs will be adaptively started and stopped, requiring a sophisticated multiprocessor task management tool.

Environmental Molecular Sciences Laboratory (EMSL)

Increasing Impact through Integration—The Environmental Molecular Sciences Laboratory (EMSL), a DOE User Facility

EMSL DOE User Facility (Director - Allison Campbell; Interim CSO and Lead Scientist for Interfacial Chemistry—Don Baer; Lead Scientist for Geochemistry, Biogeochemistry, and Subsurface Science—Nancy Hess)

N.J. Hess, D.R. Baer (PI)—*PNNL*

Credible, predictive models of contaminant fate and transport models in the subsurface require understanding and identification of key geochemical and biogeochemical reactions that control contaminant mobility. The ability to identify and adequately probe dynamic processes at the molecular scale can provide information needed to accurately simulate these processes with computational models, and incorporate them into reactive flow and transport models. This is an important goal of many SBR researchers addressing the nation's environmental challenges, including carbon sequestration, contamination from past nuclear weapons production, and a scientific basis for the long-term stewardship of nuclear waste. Linking of experimental and theoretical information from molecular to field scale requires the integration of diverse experimental and computational techniques and collaboration with experts from multiple disciplines.

The Environmental Molecular Sciences Laboratory (EMSL), a DOE national user facility in Richland, Washington, provides integrated experimental and computational resources and expertise for scientific research and discovery in subsurface biogeochemical research to users free of charge. Three capability sets are particularly relevant for biogeochemical research.

(1) Next-generation imaging and surface-characterization experimental capabilities that can be used to provide the spatially resolved elemental analysis, oxidation state determination, chemical speciation, mineral identification, and microbe-mineral associations necessary for understanding the chemical fate and mobility of contaminants in the biogeochemical environment. Within the next 12 months EMSL users will have access to nano-SIMS capability.

(2) The comprehensive quantitative proteomics/ metabolomics platforms; whole transcriptome analysis platforms, platforms for gene expression profiling, small RNA analysis, novel transcript identification and many genome- and epigenome-directed applications that provide EMSL users extensive capabilities for unraveling the interplay between microbial communities and geochemistry.

(3) An integrated suite of capabilities to support research in subsurface flow and transport from the micron to the intermediate scale. Users have access to experts who can assist with every step of the research process, from pre-experiment modeling to hydraulic characterization, analytical chemistry, numerical modeling, and post-process analysis on custom-built flowcells. On site lithographic fabrication capabilities are available for the creation of custom pore-scale micromodels in a variety of materials.

In 2012, a fourth capability set, a new Radiochemistry Facility, will be available to users that will greatly expand the range experimental capabilities for analysis of environmental samples contaminated with radionuclides. The new facility consists of ~6000 sq ft of lab space. The surface analysis-imaging suite contains FIB-SEM, TEM-EELS, SPM, XPS and EMP. The Magnetic Resonance Facility houses wide-bore 100 and 750 MHz NMR spectrometers with a wide range of specialized sample environments and nuclide probes, and an EPR spectrometer. The radiochemistry facility will also house sample preparation, solution chemistry, and analytical tools including micro-XRD and spectroscopic capabilities. These capability sets, together with NWChem, EMSL's premier computational modeling code, enable users to address subsurface biogeochemical research challenges from both experimental and modeling vantage points.

Integrated Field-Scale Subsurface Research Challenges (IFRC)

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

Hanford IFRC (Principal Investigator: John Zachara)

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The Integrated Field-Scale Subsurface Research Challenge (IFRC) at the Hanford 300 Area addresses multiscale mass-transfer processes controlling U(VI) plume dynamics in a complex hydrogeologic setting. A series of forefront science questions on reactive mass transfer are posed for research which relate to the effect of spatial heterogeneities; the importance of scale; coupled interactions between biogeochemical, hydrologic, and mass-transfer processes; and measurement approaches needed to characterize and model a mass-transfer dominated system. The site has 35 instrumented wells and an extensive monitoring system. It includes a deep borehole for microbiologic and biogeochemical research that provides access to the entire thickness of the unconfined 300 A aquifer and that is now being used for down-hole biogeochemical studies.

Significant, impactful progress was made in CY 2010, including the quantification of wellbore flows in the fully screened wells and the testing of means to mitigate them; the development of site geostatistical models of hydrologic and geochemical properties, including the distribution of U; developing and parameterizing a reactive transport model of the smear zone that supplies contaminant U to the groundwater plume; performance of a second passive experiment of the spring water table rise and fall event with a associated multipoint tracer test; performance of down-hole biogeochemical experiments where colonization substrates and discrete water and gas samplers were deployed to the lower aquifer zone; and modeling of past injection experiments for model parameterization, deconvolution of well-bore flow effects, system understanding, and publication. We continued efforts to assimilate geophysical logging and 3D ERT characterization data into our site-wide geophysical model, and have now implemented a new and improved strategy for this activity.

An important focus of CY 2010 activities has been infrastructure modification to the IFRC site to eliminate vertical well bore flows in the fully screened wells. The mitigation procedure was carefully evaluated and has now been completed, yielding a monitoring system isolated to the upper aquifer and smear zone. A new experimental campaign is planned for early spring 2011 that will utilize the modified well-field for a U reactive transport experiment in the upper aquifer zone. Geophysical monitoring experiments of rainwater recharge and vadose zone transport have been initiated, with promising results The increasingly comprehensive field experimental results, along with the field and laboratory characterization, are leading to a new conceptual model of U(VI) flow and transport in the IFRC footprint and the 300 Area in general, and insights into the microbiological community and associated biogeochemical processes.

Uranium and Strontium Isotopes as Tracers of “Smear Zone” (Lower Vadose Zone) Contributions to 300 Area Groundwater

Hanford IFRC (Principal Investigator: John Zachara)

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The 300 Area at the Hanford Site in Washington is situated along an ~2 km stretch of the Columbia River. Past operations in the 300 Area included the disposal of chemical and radioactive waste derived from fuel rod fabrication and other experimental processes into a series of settling ponds and disposal trenches. This resulted in a persistent groundwater U plume in the 300 Area, with U concentrations reaching 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 as predicted. The persistence of the groundwater U plume is likely due to replenishment from contaminated vadose zone sediments influenced by the dynamic groundwater chemistry resulting from incursions of Columbia River water due to highly variable river stage (see review in Zachara et al. 2007, and references therein). The 300 Area Integrated Field Research Challenge (IFRC) site was established to conduct field-scale experiments and modeling regarding U mobility in a hydrologically dynamic environment.

One type of experiment utilizing the IFRC well field (consisting of a triangular array of 38 wells, ~70 m to a side) is to sequentially sample the well array during springtime when the water table typically undergoes a series of rises and falls through the lower vadose zone (termed the “smear” zone). From observations of U concentration in groundwater, it is evident that U is transferred from the smear zone to groundwater during these water table excursions. Our previous work has demonstrated significant U isotopic variation in contaminated sediments both vertically and horizontally in the 300 Area down to the scale of the IFRC well array. Tracking the U isotopic composition of groundwater at three shallow IFRC wells, we are able to relate changes in U isotopic composition to the local vadose sediment U isotope stratigraphy, allowing us to evaluate the fraction of U coming from the “smear” zone during rise and fall of the water table. During the relatively low stands, the groundwater returns to a consistent and recurring “background” U isotopic signature and concentration. We have also found that ^{87}Sr/^{86}Sr provides a second signature of input from the “smear” zone. Shifts in U isotopic composition accompanying water table rise/fall are correlated with ^{87}Sr/^{86}Sr, but unlike U, the Sr isotopic signature from the vadose zone appears uniform across the IFRC site.

Anaerobic Biogeochemical Processes in Hanford 300 Area IFRC Subsurface Sediments

Hanford IFRC (Principal Investigator: John Zachara)

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Microbial terminal electron accepting (TEA) processes involving nitrate, native Fe(III), and sulfate, and the biogeochemical behavior of U(VI) and Tc(VII) were investigated in Hanford 300A IFRC subsurface sediments. The objective of this research was to characterize biogeochemical redox processes influencing radionuclide behavior across a subsurface redox transition zone spanning the coarse-grained Hanford formation and the underlying fine-grained Ringold Formation subunit. In Hanford- and Ringold-sediment microcosms incubated anaerobically in synthetic groundwater and amended with acetate, lactate, and glucose, nitrate was reduced relatively rapidly (<7 d), with the transient appearance of nitrite. Fe(III) and sulfate were reduced more slowly (2-3 weeks) in organic-amended Hanford, but were not reduced in Ringold microcosms, probably due to low biomass concentrations and metabolic activities in the Ringold sediments. ^{57}Mössbauer analyses revealed that the Fe(III) content in oxide and phyllosilicate phases in the Hanford sediments decreased upon bioreduction, with a concurrent increase in phyllosilicate Fe(II). In Hanford sediment incubated with 60 µM U(VI) amended with organic carbon, 86-92% aqueous U(VI) was removed from solution within 2 months. X-ray absorption spectroscopy (XAS) indicated that 67-77% of Hanford sediment-associated uranium was U(VI), probably as an adsorbed species on new or modified reactive mineral phases. ^{99}Tc(VII)O_4^- was reduced in live Hanford sediment microcosms as well as those that were bioreduced and then heat-killed prior to incubation with Tc(VII), indicating the potential for both biotic and abiotic reduction pathways. The initial aqueous Tc(VII) concentration (20 µM) was reduced to below the detection limit (3.98×10^{-9} M) within days to months, depending on reduced sediment concentration. Tc(VII) reduction by the pre-bioreduced sediments was found to be proportional to the weak acid-extractable Fe(II) and sediment-associated sulfide concentrations. X-ray microprobe analyses, including fluorescence mapping, elemental multichannel analysis, and micro-diffraction, revealed that reduced and precipitated Tc was likely associated with a mica phase. XAS analyses showed that the reduced Tc species in bioreduced sediment without sulfate was predominantly a Tc(IV)O_2-like phase, while some Tc(VII) was also reduced by sediment-associated sulfide in bioreduced sulfate-amended sediment, as indicated by Tc-S bonding in the reaction product as indicated by EXAFS. This work demonstrates the potential for anaerobic microbial reduction of nitrate, native Fe(III), and sulfate in coarse-grained Hanford formation subsurface sediments of the Hanford 300A IFRC--generally considered as being a globally oxic environment. Moreover, U(VI) and Tc(VII) are subject to biogeochemical transformation that can influence the aqueous concentrations of these important Hanford subsurface contaminants.

U(VI) Transport during Water Table Fluctuations

Hanford IFRC (Principal Investigator: John Zachara)

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U(VI) desorption from the capillary fringe at the 300 Area Hanford IFRC site, WA, is strongly related to frequent groundwater table fluctuations induced by rapid stage changes in the Columbia River. In the "smear zone" (zone through which the groundwater table sweeps) at the IFRC site, the sediment can experience more than one saturation cycle every day. These groundwater fluctuations complicate the current understanding of U(VI) transport because the smear zone is likely a persistent source of long-term U(VI) contamination. To assess these complex U(VI) dynamics, we conducted laboratory column experiments to inform field-scale modeling. We used two comparison columns (4.28 cm × 44.4 cm) to examine the U(VI) desorption affected by varying saturation/unsaturated conditions. Less than 8 mm site-wide composite sediments collected from the Hanford IFRC site were packed into both columns. Water contents and matric potentials at different depths were measured via TDRs and tensiometers placed in the columns. U-free synthetic groundwater was injected into the columns at a constant rate to desorb U(VI). Two stop flow events were embedded into the desorption process in each column. Before each stop flow event, one of the columns was freely drained; the sediment represents the smear zone with the groundwater table dropping to the bottom of the column. The other column remained saturated. After each stop flow event, the unsaturated column was re-saturated, and regular desorption continued in both columns. The pH, U(VI), major ions, and alkalinity were measured in all the effluent and drained samples. The results showed that the U(VI) breakthrough curves in both columns were almost identical, indicating that the unsaturated conditions during the stop flow events did not have a significant impact on the U(VI) desorption. U(VI) concentrations in the drained samples were lower than the U(VI) concentrations in the effluent samples. This lowering of U(VI) can be explained by introducing a conceptual dual-domain system: a domain dominated by macropores and a domain dominated by micropores. In our experiment, the adsorbed U(VI) was largely associated with fine sediment in the micropore domain, and the fluctuated water table only affected the macropore domain. To quantify the mass-transfer parameters within macropores and micropores, as well as between two domains, a reactive transport model (STOMP) was coupled with a multirate surface complexion model to simulate the results. The calibrated model results will be extended to a 1-D field scale. The conceptual dual-domain pore model is expected to partially explain the existence of the long-term U(VI) plume in the Hanford 300 Area.

Uncertainty Quantification Using High Performance Computing for the October 2009 Uranium Desorption Experiment at the Hanford 300 Area IFRC Site

Hanford IFRC (Principal Investigator: John Zachara)

G.E. Hammond (PI), X. Chen—*PNNL*; P.C. Lichtner, *LANL*

Reactive flow and transport modeling is being employed to simulate the October 2009 uranium desorption experiment within the Integrated Field Research Challenge (IFRC) site at the Hanford 300 Area. This modeling effort considers uncertainty within the underlying conceptual model including hydrologic and geochemical heterogeneity in the aquifer, geochemical process models (equilibrium vs. multirate kinetic surface complexation), and flow boundary conditions (choice of monitoring wells and strategy for mapping head to model boundary). Uncertainty arising from imprecise conceptual models and parameters is quantified within a Monte-Carlo framework for tracer and uranium concentrations observed at monitoring wells within the IFRC site. Due to the large computational demand (e.g., hundreds to thousands of realizations, each composed of millions of unknowns and requiring a ~4.5 hour simulation time), this work would not be possible without high-performance computing. To this end, the massively parallel PFLOTRAN simulator provides the efficient scalability and multi-realization simulation capability necessary to complete this analysis with reasonable turnaround. Each ensemble of multi-realization simulations was carried out on 64,000 processor cores with 128 cores per realization. Results from this analysis suggest that observed breakthrough is most sensitive to the flow boundary conditions imposed on the model, with aquifer heterogeneity and the geochemical process model still playing important secondary roles. The research clearly demonstrates the need for additional uncertainty quantification at the Hanford 300 Area IFRC site.

Electrical Geophysical Characterization and Monitoring at the Hanford 300 Area IFRC

Hanford IFRC (Principal Investigator: John Zachara)

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The Hanford 300 Area IFRC wellfield was installed with a high performance electrical geophysical monitoring system, with the objective of supporting characterization and monitoring of properties and processes influencing uranium transport. 3D characterization efforts will fill a critical data need for the IFRC modeling team if the distribution of electrical properties can be related to or otherwise inform the distribution of hydrogeologic properties governing flow and influencing uranium transport at the Hanford 300 area. The electrical geophysical array is also being used to monitor time-lapse changes in subsurface electrical properties, which serve as proxy measures of properties or processes governing uranium transport. These include time-lapse monitoring of changes in subsurface electrical conductivity caused by surface infiltration and subsequent downward fluid migration, time-lapse monitoring of changes in subsurface conductivity caused by the intrusion of Columbia River water into the 300 Area aquifer during high river stage, and monitoring of tracer migration during a uranium desorption experiment.

A dense 3D borehole electrical resistivity tomography (ERT) survey was conducted at the IFRC wellfield, and a parallel inversion code was developed and used to produce a geostatistically accurate estimate of the electrical conductivity distribution of the IFRC at submeter resolution, including uncertainty estimates. The results of this characterization are being used by the IFRC modeling team in conjunction with borehole logging and tracer test data to inform estimates of hydraulic properties within the IFRC wellfield. The electrode array was augmented with a 2D surface array in late 2010 to monitor meteoric water infiltration and downward migration. Time-lapse imaging results show a wetting front moving from the surface to approximately 2 meters depth in 5 days after a rain event of 0.5 cm in 0.5 days. This suggests that focused periodic infiltration events may play an important role in vadose zone transport at the 300 area, and has prompted a detailed induced infiltration test scheduled for fall 2011.

Geophysical efforts at the 300 area IFRC are transitioning from characterization to process monitoring. Geophysical monitoring efforts are planned for FY2011 in support of three experiments. (1) Transient changes in fluid conductivity associated with a tracer/uranium desorption experiment will be imaged to monitor tracer migration within the IFRC wellfield. (2) In order to investigate the hydrogeologic link between the IFRC wellfield and the Columbia River, a series of 2D ERT/IP surface arrays will be installed between the river and wellfield, and changes in bulk conductivity associated with river water intrusion at high stage during spring runoff will be imaged. (3) An experiment in the fall of 2011 will be initiated to simulate a focused, episodic natural infiltration event in order to investigate vadose flow and uranium transport to the water table.

The Effects of Columbia River Stage on Contaminant U Concentrations and Groundwater Compositions at the 300 Area IFRC

Hanford IFRC (Principal Investigator: John Zachara)

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Seasonal variations in contaminant uranium concentrations have long been observed at the Hanford site's 300 Area. A significant pool of uranium persists in the vadose zone; of particular interest is the uranium within the smear zone, the area traversed by the water table. The springtime snowmelt and the resultant spasmodic rise in river stage are accompanied by rises in the water table at the IFRC. Our objective was to determine whether groundwater captured uranium from the smear zone during water table rises, and whether the groundwater composition was affected by infiltration of river water during high-stage events. Groundwater was sampled by pumping from the screened interval of the aquifer, and by bailing from the top of the aquifer, across the site, from May 15 to July 29, 2010. Samples were analyzed for major dissolved components and uranium.

Uranium concentrations prior to the seasonal groundwater rise were 30–50 µg L^{-1} across the site. On June 8, the water table rose abruptly, and had risen 1.4 m by June 26. The uranium concentration increased differentially across the site, with the largest contributions occurring proximal to the southernmost well cluster (Well 3-30). At that well, the uranium concentration increased from 50 µg L^{-1} to 330 µg L^{-1} over the course of four days. Uranium concentrations decreased as the water table crested. Over the test period, the uranium concentration across the site—the "background" concentration—increased broadly to 60–70 µg L^{-1}. Anion concentrations were used to assess the impact of river water infiltration. For example, the Cl^- concentration, decreased at the eastern site boundary (Well 2-24) from pre-test values of 21 mg L^{-1} to 4 mg L^{-1}. The initial change in concentration lagged the rise in the water table by 2–3 days, then coincided with water table rise and fall, and represented a maximum river water component of 80%. The groundwater impact was less toward the west, with the river water invading across the site to the northwest, and comprising less than 25% of the total at the western site boundary (Well 2-9). The results demonstrated that the smear zone represented a significant source of uranium to groundwater, with river water imposing significant compositional variation to the aquifer over the course of the test.

A more limited passive test is planned for 2011, and we are deploying aquifer-skimming pumping systems to sample the uppermost centimeters of the aquifer and test whether there are significant contributions of uranium to the aquifer during periods of low water.

A Geochemical Heterogeneity Model for a Contaminated Vadose Zone-Aquifer System

Hanford IFRC (Principal Investigator: John Zachara)

C. Murray (PI), J. Zachara, J. McKinley, Y.-J. Bott—*PNNL*

The Hanford IFRC is investigating multiscale mass-transfer processes that control seasonally variable concentrations in the 300 A uranium plume. The plume has displayed remarkable persistence over the past 20 years, and questions remain as to whether the causes are hydrologic or geochemical. Field injection experiments that promote U(VI) desorption and adsorption are under way at the IFRC site, as well as passive experiments that monitor the release of contaminant U(VI) from the lower vadose zone to the aquifer during periods of spring high water table. The vertical zone through which the water table fluctuates due to changing river stage is known as the "smear zone." Key to the understanding and simulation of experiments at the IFRC is information on the spatial distribution of U(VI) contaminant concentrations, and reaction properties that determine solid-liquid distribution. Approximately 750 grab samples were collected during the installation of 35 wells within the 1600 m^2 IFRC site. Approximately 125 of these samples from the middle and lower vadose zone and saturated zone were air dried and screened to < and > 2 mm. Particle size distribution was measured on each size fraction, and the < 2 mm fraction was characterized for the total U, 1000 h bicarbonate extractable U (BEU), and surface area. Twenty-four hour adsorption distribution ratios (Kd's) were measured from synthetic groundwater on bicarbonate extracted sediments that had been washed repeatedly to remove residual bicarbonate. We used a subsample of 90 measurements from the smear zone to generate a preliminary geostatistical model of BEU and Kd, which included the generation of 100 realizations of the 3D spatial distribution of each variable. After generation and evaluation of this model, an additional set of 31 smear zone samples was analyzed from six locations, to provide better spatial coverage of the IFRC site. This provided the opportunity to test predictions of the BEU and Kd from the existing geostatistical model at the new measurement locations. All but one of the new measured values of BEU, and all measurements of Kd, fell within the 95% probability intervals from the existing model, and most values were close to the mean estimate from the geostatistical model, suggesting that the model does a reasonable job of representing the geochemical heterogeneity of the system. We updated the geostatistical model of both variables to include all available data. The updated geostatistical model has been used to provide an estimate of the mass of BEU present in the smear zone at the IFRC, based on integration of the mass of BEU in each of the 100 realizations. This also included an assessment of the uncertainty in that estimate, based on the variability in the integrated mass from the suite of realizations. The mean mass of BEU in the smear zone of the IFRC was estimated to be ~58 kg, with the 95% probability interval ranging from 45.8 kg to 73.4 kg. The suite of geostatistical realizations of BEU and Kd will be used as input for reactive flow and transport modeling to assess the potential for the smear zone to provide a continuing source for the groundwater uranium plume in the IFRC and surrounding 300 Area.

Facies Delineation Using Core, Borehole Geophysical Log, Electrical Resistivity Tomography, and Electromagnetic Borehole Flow Meter Data

Hanford IFRC (Principal Investigator: John Zachara)

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Y. Rubin, *U. of California-Berkeley*; J. Zachara, *PNNL*

Subsurface characterization at the Hanford 300 Area IFRC Site has included grain size analyses, borehole geophysical logging, electrical resistivity tomography (ERT), short-duration, constant-rate injection tests, electromagnetic borehole flow meter profiling (EBF), and multiple tracer tests. These various data types represent different volumes of interrogation and provide different types of information about the physical, hydraulic, and geochemical properties of the subsurface and their connectivity. Multivariate and geostatistical analyses of the physical property and geophysical log data were performed to evaluate the use of different combinations of data for delineating facies. Objectives of this work include evaluating data worth and developing methods for more efficient and effective multiscale parameterization of subsurface flow and transport properties.

Borehole geophysical logs represent the highest density data sets available for the 300A IFRC site. The spectral gamma log data have been shown to correlate relatively well with mud fraction, and thus provide a means for easily differentiating between the mud-rich and low-permeability Ringold fm and the overlying gravel- and cobble-dominated Hanford fm. However, within the Hanford fm, the spectral gamma log data appear to have a large random component, with variogram analyses showing nugget values that are 30-50% of the total variance. Therefore, different clustering algorithms are used to aggregate the spectral gamma log data for better delineation of larger-scale structural features. ERT data show that the central portion of the Hanford fm is more resistive than the top and bottom portions. Transforming electrical conductivity results to porosity using Archie's Law yields lower porosity values for the central portion of the Hanford fm. EBF profiling shows the central portion of the Hanford fm to also have lower permeability, which is consistent with the lower porosity values inferred from ERT data.

Facies models developed using the various data sets described above will be used for flow and reactive transport modeling with high-performance computing. Criteria for delineating facies boundaries, as well as flow and transport parameters assigned to different facies, will be estimated through forward and inverse modeling of field tracer tests.

Multiscale and Multitype Data Assimilation for Hydrogeological Characterization at the Hanford 300 Area

Hanford IFRC (Principal Investigator: John Zachara)

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Data assimilation at the Hanford 300 Area focuses on enhancing the characterization of the hydraulic conductivity field using multiple types of data. Our efforts thus far were based on assimilating data from constant injection rate pumping tests, EBF profiling, and tracer tests. More recently, we expanded our focus to include additional data, including hydraulic heads and geophysical data. The data assimilation is performed using the Method of Anchored Distribution (MAD) that was developed to meet the challenges of multi-type, multi-scale data assimilation.

The introduction of additional data types and additional insight from field investigation raised new issues that we are currently addressing. This includes assessment of the effects of ambient flow on EBF profiling and the development/auditing of a comprehensive and accurate database for the borehole logs and geophysical surveys. The expansion of the data types available for assimilation required us to enhance the computational efficiency of MAD. Issues of particular significance in this context include subset reduction, reduction in the number of forward simulations required for accurate estimation of the likelihood function, and reduction in the number of target parameters.

Results are presented that describe the contributions of the various types of data and data assimilation methods toward improvement in the hydrogeological characterization. We also present progress made with the geophysical database analysis.

Characterizing and Modeling Grain-Scale Uranium Mass Transfer at the Hanford IFRC Site

Hanford IFRC (Principal Investigator: John Zachara)

D.L. Stolker (PI), D.B. Kent—*USGS*; C. Liu, J. M. Zachara—*PNNL*

The Hanford IFRC Team's objective in this research is to develop a grain-scale model for uranium(VI) mass transfer that can be incorporated into models to simulate U(VI) reactive transport at larger spatial scales. A surface complexation model has been developed to describe equilibrium uranium adsorption onto <2 mm IFRC smear-zone sediments over the relevant range of chemical compositions, both for samples from specific locations and a composite sample prepared from sediments collected across the IFRC site (site-wide composite, SWC). Optimal fits were obtained with the equation:

$$> \quad SOH + UO_2^{2+} + 2H_2CO_3 = {>}SOUO_2(CO_3HCO_3)^{2-} + 4H^+ \qquad (1)$$

Fitting equilibrium uranium adsorption-desorption data for the SWC using equation 1 and a site density of 3.84 umoles/m^2 yields a conditional logK value of -11.73±0.11. The conditional equilibrium constant and site density were found to be inversely proportional over a wide range of site-density values such that the following equation:

$$\log K = \log Sa - 11.141 \qquad (2)$$

where Sa is the site density in umoles/m^2, can be used to calculate the logK value for any site density down to 0.003 umoles/m^2. The SWC was dry-sieved to six size fractions ranging from <0.063 mm to 2 mm, in order to characterize the adsorption properties of individual grain sizes. Nitrogen gas adsorption and desorption was performed on each size fraction. Static batch reactor experiments were conducted on individual size fractions to evaluate labile uranium and adsorption properties. Rates of uranium desorption, under constant chemical conditions, were studied in flow-through batch reactor experiments with stop-flow events. Also, rates of release from the bulk <2 mm SWC were measured in waters similar to Hanford groundwater and Columbia River water to probe the impact of variable chemistry on grain-scale desorption rates. Nitrogen adsorption-desorption isotherms indicate that all IFRC composite samples and all size fractions of the SWC exhibit evidence of complex pore networks and connectivity in the intragrain domains, with access to larger pores restricted by micropores (pores with widths < 2 nm). Micropore volume increased by a factor of two between the largest and smallest size fractions. While the volume of these micropores is small (1-2 mm^3/g), they account for 20-30% of total surface area for each size fraction. Labile uranium increased by a factor of two from the smallest to largest size fraction. In flow-through batch reactors, apparent initial rates of uranium desorption increased with decreasing particle size by nearly an order of magnitude between the smallest and largest size fractions. However, after subsequent stop-flow events, the variation of rates between individual size fractions was less than ten percent. Increasing alkalinity by a factor of four resulted in an increase in the rate of initial uranium release from the bulk <2 mm composite sample by a factor of two.

Hydrologic Characterization of the Reconfigured Hanford IFRC Wellfield

Hanford IFRC (Principal Investigator: John Zachara)

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Evidence for river-induced wellbore flow in long-screen wells, and their impact on aqueous sampling results, have been observed in Hanford IFRC monitoring wells during both active and passive field-scale experiments. These impacts greatly complicate interpretation of both conservative and reactive tracer transport processes, and thus identification of an effective mitigation strategy was a primary project objective. Another objective was to use the reconfigured wellfield to better characterize the upper portion of the Hanford unconfined aquifer where the resupply of soluble U(VI) has been shown to occur.

Installation of a bentonite plug within the lower portion of the well screen was identified as an effective strategy for eliminating well-induced intercommunication between the upper and lower high hydraulic conductivity zones, and this approach was implemented throughout the wellfield. Prior to well mitigation, additional electromagnetic borehole flowmeter (EBF) profiling was performed to better characterize the vertical distribution of horizontal hydraulic conductivity across the full saturated thickness and full lateral extent of the wellfield. Following well mitigation activities, an independent EBF profiling campaign was performed over the upper zone of all site monitoring wells. These measurements were collected to provide a more targeted interrogation of the upper zone after eliminating the strong ambient flows present during profiling of the full saturated thickness. A large-scale tracer injection/U(VI) desorption experiment, which involved a continuous two-week-long injection of low U concentration waters spiked with conservative tracers, was also conducted in the reconfigured wellfield. This test was the first large-sale injection test at the IFRC site focused on interrogating U(VI) mass transfer and transport processes within the upper portion of the aquifer. Previous large-scale injection experiments were dominated by high groundwater velocities in the lower portion of the aquifer.

In addition to the upper zone tracer injection test, a suite of constant-rate injection tests will be conducted in the reconfigured wellfield to better characterize hydraulic properties of the upper zone. These results, along with constant-rate injection tests and EBF profiles conducted over the full saturated thickness of the aquifer, will be used to develop revised 3D hydraulic conductivity fields for the Hanford IFRC site and will inform future modeling efforts.

Solute, Heat, and Geochemical Modeling Studies at the Hanford IFRC Site

Hanford IFRC (Principal Investigator: John Zachara)

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Numerical simulation studies of Br and heat tracer tests at the Hanford IFRC site clearly show that groundwater head data alone were insufficient to calibrate the hydraulic conductivity distribution. The Br tracer data were critical for constraining and improving the estimation of hydraulic conductivity and determination of boundary conditions. However, depth-discreet Br concentration measurement was not available at most of the existing fully screened observation wells at the Hanford IFRC site, as iis often the case in other field sites where multi-level solute tracer sampling might be prohibitively expensive. On the other hand, depth-specific temperature data were easy and inexpensive to obtain. Nevertheless, temperature data were more difficult to interpret because of additional processes involved in heat transport and significant noise in the temperature data.

Here, we show that the simulated temperature breakthrough curves can match reasonably well with the observed, even though the hydraulic conductivity distribution calibrated for the Br tracer was directly used for modeling heat transport without any adjustment. This suggests that temperate data can be used as a cost-effective proxy for conservative solute tracers to help constrain and improve the calibration of the hydraulic conductivity distribution, especially in the vertical direction. The advantage of such a proxy is obvious, considering the ease and low cost in obtaining depth-specific temperature measurements.

In the hydraulically connected system between river and aquifer at the Hanford IFRC site, calcite dissolution was caused by Columbia River water intrusion during high river stages, and correspondingly calcite was depleted within parts of the hyporheic zone. The influence of calcite dissolution and its distribution on U(VI) mobility was evaluated by employing a 2D reactive transport model which runs across the IFRC site. The simulation results indicated that the mobility of U(VI) and the contaminant flux discharged into the river were noticeably affected by calcite dissolution and the calcite distribution within the hyporheic zone. The calcite dissolution mobilized the U(VI) by producing more calcium and carbonate and enhancing their subsequent complexation with aqueous U(VI), which promoted the discharge of more U(VI) mass into the river. Less depletion of calcite in the hyporheic zone also led to more U(VI) mass discharged into the river.

Coupled intragrain diffusional mass-transfer and nonlinear surface complexation processes play an important role for the transport behavior of U(VI) in contaminated aquifers. Two alternative model approaches for simulating these coupled processes were analyzed and compared: (1) the physical nonequilibrium approach that explicitly accounts for aqueous speciation and instantaneous surface complexation reactions in the intragrain regions and approximates the diffusive mass exchange between the immobile intragrain pore water and the advective pore water as multirate 1st-order mass transfer; and (2) the chemical nonequilibrium approach that approximates the diffusion-limited intragrain surface complexation reactions by a set of multiple 1st-order surface complexation reaction kinetics, thereby eliminating the explicit treatment of aqueous speciation in the intragrain pore water. Model comparison was conducted for column and field scale scenarios, representing the highly transient hydrological and geochemical conditions in the U(VI)-contaminated aquifer at the Hanford 300A site, Washington, USA. It was found that the response of U(VI) adsorption/desorption kinetic behavior to hydrogeochemically induced changes in U(VI) adsorption strength was more pronounced in the physical than in the chemical nonequilibrium model. The magnitude of the differences in model behavior depended particularly on the degree of disequilibrium between the advective and immobile phase U(VI) concentrations. While a clear difference in U(VI) transport behavior between the two models was noticeable for the column-scale scenarios, only minor differences were found for the Hanford 300A field-scale scenarios, where the model-generated disequilibrium conditions were less pronounced as a result of frequent groundwater flow reversals.

Data Management Efforts for the Hanford 300 Area and Rifle IFRCs

Hanford IFRC (Principal Investigator: John Zachara)
Rifle IFRC (Principal Investigator: Philip Long)

R. Versteeg (PI), *Sky Research, Inc.*

Data curation is a required task for all IFRCs. As both the scope of the IFRCs (in terms of duration, number of participants, and diversity of disciplines involved in each IFRC) as well as the amount of data collected is substantially larger than previous geoscience research projects sponsored by DOE, the specific implementation of this curation (colloquially referred to as the IFRC data management task) is notrivial and required a novel approach. Over the past several years, an approach for data management was developed and implemented—initially only for the Hanford 300 Area IFRC, but recently also for the Rifle IFRC. The 300 Area (www.300areaifrc.org) and Rifle IFRC (www.ifrcrifle.org) thus share a common approach, software components and (where appropriate) data models, which have allowed for synergistic development and resource utilization. This general approach has two main elements: (1) implementing data management as a web application (resolving issues of distributed applications and data inconsistencies) and (2) using the maximum amount of existing software components (both commercial and open source). This extends both to the standard components of a web application (such as the database and the server), and to the software components for mapping, display, and data presentation. The web application has been implemented in Zend Framework, an open source, object-oriented web application framework implemented in PHP 5, and uses ESRI ArcGIS, Google Maps, and several other Google components, as well as several open-source packages for wikis, graphing, and data analysis.

The data management effort has several objectives. The first is to capture all data collected as part of the IFRC in such a way that this data is easily available and accessible, both for the core IFRC group and for future researchers. The second is to provide tools for basic data exploration and visualization. The approach for the data collection includes (1) iterative development of a comprehensive data inventory; (2) implementation of a structure allowing individual scientists to upload their data in a relatively unstructured format in so-called data packages (a loose free form assembly of data with some basic amount of documentation—typically identical to the scientist specific organization); given that the IFRC project PIs (John Zachara and Phil Long) strongly encourage data package assembly and submittal, compliance with this effort is high; and (3) full integration of selected parts of the data contained in the data package into the database (note that the metadata describing what is in the data package is entered into the database and is searchable as soon as a data package is uploaded).

Data management requires an ongoing effort over the life of the IFRC, both in the continued integration of novel data, as well as in the expansion of features in the web interface and in the refactoring of the database and underlying implementation. Thus, the system status is evolving continuously. The system has the following elements (1) a Mysql database that holds all the data required for system operation (each IFRC has a dedicated database); (2) a PHP back end that uses the Zend Framework architecture and components, and is fully object oriented; and (3) a rich front end that provides for user management, authentication, and authorization, and allows for different levels of data access depending on user privileges. Current capabilities provide for on-demand graphing and contouring of different datasets, generation of time series animations, cross analysis of different datasets, and download of user selectable subsets of the data. In addition, by aggregating components such as Google calendar, weather, twitter feeds, and webcams, the website provides a central point of access for field activities. At the SBR meeting, an overview and live demonstration of the system will be given.

In addition to the continuous enhancements of the IFRC databases and associated functionalities, one of the primary open challenges remaining is the effective capture of data from laboratory experiments (as such experiments are typically highly variable) and the integration of proteomics and genomics data. Finally, one open issue is how the efforts performed under the 300 Area IFRC and Rifle IFRC can be integrated with other efforts in geoscience data management.

ORNL IFRC: Multiscale Investigations on the Rates and Mechanisms of Targeted Immobilization and Natural Attenuation of Radionuclides and Co-Contaminants in the Subsurface

ORNL IFRC (Principal Investigator: Scott Brooks)

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The Oak Ridge Integrated Field Research Challenge (ORIFRC) project comprises an integrated multidisciplinary, multi-institutional research program whose goal is to provide an improved scientific understanding and predictive capability of subsurface contaminant fate and transport through experiments and observations at scales ranging from the molecular to the watershed. The influence of coupled processes on U, Tc, and co-contaminant NO_3^- fate and transport are being quantified along numerous contaminant pathways, using subsurface manipulations and the assessment of natural attenuation processes throughout the watershed. These investigations are being integrated with multiscale numerical simulations to address several coupled processes and assess their impact on contaminant transport. The research is identifying and quantifying key reactions, previously not addressed, that control contaminant fate and transport. The research approach is designed to enable the understanding gained and models developed to be broadly applicable to individual DOE sites.

The overall goal of the project is to advance the understanding and predictive capability of coupled hydrological, geochemical, and microbiological processes that control the *in situ* transport, remediation, and natural attenuation of metals, radionuclides, and co-contaminants at multiple scales, ranging from the molecular to the watershed. The specific objectives of this research are to: (1) quantify recharge pathways and other hydraulic drivers for groundwater flow and dilution of contaminants along flow pathways, and determine how they change temporally and spatially during episodic events, seasonally, and long term; (2) determine the rates and mechanisms of coupled hydrological, geochemical, and microbiological processes that control the natural attenuation of contaminants in highly diverse subsurface environments; (3) explore novel strategies for enhancing the subsurface stability of immobilized metals and radionuclides; (4) understand the long-term impacts of geochemical and hydrologic heterogeneity on the remobilization of immobilized radionuclides; and (5) improve our ability to predict the long-term effectiveness of remedial activities and natural attenuation processes that control subsurface contaminant behavior.

To meet the research objectives, integrated multidisciplinary activities from the watershed to the microscopic scale investigate (i) the use of geophysical techniques to define subsurface heterogeneity within pathways and to relate those observations to processes of interest (recharge, distribution of subsurface amendments) (ORIFRC poster by Hubbard et al.), (ii) influence of spatially and temporally variable groundwater recharge on hydrobiogeochemical processes affecting contaminant fate and transport (ORIFRC posters by Hubbard et al., Kostka et al.), (iii) targeted manipulations for enhanced immobilization of U/Tc using slow-release carbon sources and controlled pH adjustment to co-precipitate U and Tc with aluminum hydroxides (ORIFRC posters by Wu et al., Gu et al.), and (iv) the use of microbiological techniques to elucidate the biogeochemical mechanisms controlling natural attenuation and targeted immobilization through interrogation of *in situ* microbial communities (ORIFRC presentation by Schadt et al., posters by Kostka et al., Wu et al.). The various observation scales are integrated with numerical modeling (ORIFRC poster by Parker et al.) and advanced data analysis techniques to investigate the complex nonlinear relationships in and between the microbial community and hydrogeochemistry data.

Pilot-Scale Field pH Manipulation for Immobilizing Uranium in a Highly Contaminated Acidic Environment

ORNL IFRC (Principal Investigator: Scott Brooks)

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Few effective remedial options are available to remove or immobilize uranium *in situ* in a highly contaminated acidic soil environment like the one at the Integrated Field Research Challenge (IFRC) site in Oak Ridge, Tennessee. The objective of this research was to evaluate and demonstrate the feasibility of a controlled base addition technique (or pH manipulation) for immobilizing uranium (as uranyl, UO_2^{2+}) and other contaminant metals or radionuclides, such as technetium (Tc), *in situ* through neutralization, which leads to the precipitation or co-precipitation of contaminant metals or radionuclides with aluminum oxyhydroxides in the subsurface. A field plot (~2×3 m) was established following preliminary laboratory studies, which showed that the addition of strong base can provide a rapid yet effective means of sequestering U(VI), Tc(VII), and other toxic metals such as Ni(II) and Co(II) in the soil and groundwater. Greater than 94% of soluble U(VI) and greater than 83% of Tc(VII) can be immobilized at pH above 4.5 by the co-precipitation and/or adsorption with Al-oxyhydroxides. Previous studies also indicate that the presence of sediment minerals facilitates the immobilization of contaminants, and the immobilized U(VI) and Tc(VII) were found relatively stable against leaching by high levels of $Ca(NO_3)_2$ (up to 50 mM). In addition, our research has shown that pH is a master variable controlling the structure and function of subsurface microbial communities.

In situ pH-manipulation through controlled base addition began at the site following hydrological characterization with bromide tracer test in late October 2010. Preliminary field results (pH adjusted up to 4.2) indicate that the system is performing as expected: the decrease in Al and U concentrations has been observed in the monitoring wells, which are connected with an injection well as groundwater pH increased from about 3.5 to above 4.0. On the other hand, the monitoring wells with poor hydrological connection with the injection well have shown little responses in geochemical change. Since the initiation of the pH adjustments, six 2-D first-arrival seismic tomography (FST) profiles have been collected on a transect 1 m from the injection well and orthogonal to the predicted dominant flow direction. The FST profiles indicate little change in seismic *P*-wave velocity in proximity to the injection well within the first 30 days of the experiment; however, statistically significant localized *P*-wave velocity anomalies exist >30 days within the injection region. The microbial response to pH manipulation is being determined by quantifying the abundance, community composition, and activity of groundwater microbial communities. To date, microbial abundance and activity remain at baseline levels, and bacterial communities are predominated by facultative denitrifying microorganisms. A generic geochemical model, based on aqueous complexation, precipitation, and surface complexation reactions, has been developed to describe aqueous and solid-phase concentrations of metals and anions based on groundwater and soil titration data from laboratory studies. The model is being further developed and integrated with field groundwater flow and transport models on the basis of *in situ* pumping tests, tracer tests, geophysical data, and other geochemical characteristics. Ultimately, this model will be used to simulate hydrogeochmical dynamics in the field experiment and to demonstrate that subsurface pH control may offer a potential means for *in situ* immobilization of U(VI) in acidic soils and groundwater contaminated with high levels of Al.

Development and Application of a dsrA Microarray for Examining Sulfate-Reducing Prokaryotes

ORNL IFRC (Principal Investigator: Scott Brooks)

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Microorganisms are the most diverse group of life known and can be found in almost any environment; however, the extent of that diversity is still largely unknown. The uncultivated status of the majority of microorganisms in nature makes understanding their ecological functions even more difficult. New technologies are urgently needed to meet the challenge of characterizing uncultivated microorganisms and better understanding their role in ecosystem functioning. The objectives of this project were to evaluate a new primer set for sequencing of dsrA genes from sulfate-reducing prokaryotes (SRP) in environmental samples using a high-throughput pyrosequencing approach, to evaluate the effectiveness of a new probe design strategy, and to develop a prototype functional gene array for monitoring SRP in subsurface environments. We first developed a degenerate primer set to amplify the dsrA gene (dsrA-F: ACSCACTGGAAGCACG; dsrA-R: GATGTCRTCYYKCCAG) and tagged both the forward and reverse primers with different 8 bp barcodes so that we can use both forward and reverse sequences separately, in order to provide longer sequences for biological analysis, increase sequence accuracy and reliability, and double the sequencing output. We then tested the dsrA primers with samples from the subsurface biogeochemical research (SBR) field research center, Oak Ridge Integrated Field-Scale Subsurface Research Challenge (OR-IFC) site. At OR-IFC, emulsified vegetable oil (EVO) was injected into one subplot of Area 2 with high U(VI) contamination, where it serves as a slow-release carbon source and electron donor. Groundwater samples were collected from one upgradient control and seven downgradient wells over a 9-month period. The dsrA gene was amplified from all samples with appropriate clear band size under low stringent conditions (54°C for annealing), suggesting that the designed primers appeared to work well with these subsurface samples. Next, amplified sequences were sequenced using 454 titanium technology. Approximately 200M in sequence information with 500K reads were obtained. After preprocessing, 435,725 sequence reads remained. A total of 2064 OTUs were obtained for each tag based on the combined samples. Rarefaction analysis indicated that the diversity of the abundant populations in these communities was recovered in this study. From these sequences, we designed 1,227 dsrA perfect match (PM) probes and corresponding mismatch (MM) probes based on the MPDNN model. We incorporated the MM probes as a part of the NimbleGen-based GeoChip fabrication. GeoChip was then hybridized with 12 environmental DNA samples from pond sediments. On average, 151 dsrA genes were detected in these samples. Most of these PM probes (95%) had at least 1.2 fold higher signal than MM probes, and 89% of PM probes were four times brighter than the MM probes, indicating the MPDNN-based MM probe design performed well on real environmental samples. With this new probe design strategy, we have constructed the SRPoChip, a prototype GeoChip-like array for monitoring SRP.

Quantification of Recharge Processes at the ORNL IFRC Using Geophysical and Numerical Approaches

ORNL IFRC (Principal Investigator: Scott Brooks)

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The impact of large episodic, seasonal, and annual recharge on remediation and natural attenuation is not well understood. At humid DOE sites, such as the ORNL IFRC, where recharge is the main driver for groundwater flow, it could represent a critical control on long-term immobilization and dilution of contaminants such as uranium and nitrate, respectively. Average annual precipitation at the site (1220 mm) induces interactions between low ionic strength and poorly buffered, acidic (pH 5.6) rainwater and high ionic strength, highly buffered, acidic (pH ~3.5,) groundwater, as well as with aqueous and solid-phase contaminants, including nitrate and U(IV). Due to the high concentration of nitrate in the source region (up to 50,000 mg/L), the longitudinal extent of the nitrate plume (~1 km), and the role of nitrate as an oxidant for uranium, to date we have primarily focused on investigating the impact of recharge on nitrate concentrations.

Because induced geochemical processes occur within a multiscale hydrological framework (that includes perched water bodies, matrix diffusion, and fast-path preferential pathways), understanding recharge-induced geochemical transformations is challenging using wellbore data alone. To improve understanding of these processes and the implications for natural attenuation, we are advancing and implementing a variety of geophysical and numerical approaches at the ORNL IFRC. Our multifold experimental and numerical efforts will be described in this presentation, including:

- The development and implementation of a coupled hydrogeochemical-geophysical numerical modeling approach to explore the sensitivity of time-lapse electrical tomographic (ERT) methods for elucidating recharge-related processes, such as changes in infiltration, moisture content and nitrate concentration due to rainfall. The coupled model allows us to jointly honor time-lapse hydrological, geochemical, and crosshole geophysical datasets as we explore recharge-induced hydrogeochemical responses at the local scale and as a function of distance from the source;
- The use of time-lapse surface ERT datasets for exploring how groundwater nitrate concentrations change as a function of precipitation events and distance from the source;
- The use of time-lapse surface seismic refraction datasets for monitoring rainfall-induced vadose zone parameters and processes, including the estimation of effective unsaturated hydraulic conductivity and the spatiotemporal evolution and distribution of perched water bodies;
- Co-analysis of 50 years of precipitation and nitrate groundwater/stream data to explore clustering and intermittency behavior;
- Laboratory column experiments to explore the impact of recharge-induced nitrate concentration variability on U geochemistry and geophysical signatures (SIP, ERT) as part of a collaborative ERSP project with PI Andre Revil; and
- Numerical flow simulations to explore the influence of episodic precipitation events on nitrate dynamics at the plume scale and the influence of these variations on U behavior.

Our geophysical and numerical research is leading to advances in geophysical methodologies for quantifying subsurface hydrogeochemical processes over field-relevant scales, and to an improved understanding of recharge-induced phenomena at the ORNL IFRC.

Microbial Community Structure and Microbially Catalyzed Nitrate Attenuation across the Oak Ridge IFRC Watershed

ORNL IFRC (Principal Investigator: Scott Brooks)

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The fate and transport of uranium and nitrate are intimately linked through a variety of biogeochemical reactions. The primary mechanisms for nitrate removal are dilution and microbial denitrification. The structure-function relationships of microbial communities have been poorly characterized in the terrestrial subsurface. At the ORIFRC site, however, we have made significant progress in both characterizing the microbial community (bacteria and fungi) and in demonstrating the activity of denitrifying organisms in the source zone. Previously, very few bacteria have been isolated and physiologically characterized, and rarely have environmentally relevant isolates been acquired. Our isolation and characterization of *Rhodanobacter* spp. is a significant advance for coupling *in situ* analyses with genomic and physiological assays.

The mechanisms and controls of microbial denitrification are investigated through: (1) unprecedented genetic characterization of subsurface microbial diversity using deep sequencing methods and quantitative analysis of various microbial taxa, (2) generation and data mining of genomic and metagenomic sequence data from site materials, (3) isolation and molecular and physiological characterization of novel denitrifying organisms from the highly U(VI) contaminated ORIFRC site subsurface, (4) design of new molecular tools to detect the abundant denitrifiers present in the contaminated ORIFRC site subsurface, and (5) the use of stable isotope tracers to track denitrification activity in situ and in rate assays. A total of 22 well locations and depths were sampled from 2008 to the present. During recent sampling, we focused on subsurface microbial function by applying quantitative molecular methods targeting RNA, rate measurements, and cultivation work in parallel on representative groundwater samples. Massively parallel pyrotag sequencing at the watershed scale confirmed that pH is a master variable driving bacterial community composition and activity in the groundwater at the ORIFRC site. The bacterial community in the acidic, contaminated source zone, is predominantly composed of denitrifying microorganisms from the bacterial genus *Rhodanobacter*. These organisms are shown to be metabolically active in source zone groundwater, and in some cases, are essentially the only active organisms detected (comprising constituting 50% to 100% of rRNA detected). Genomic and metagenomic analyses of near-source-zone groundwater and bacterial isolates from site groundwater and sediment demonstrate the presence of genes for complete denitrification in *Rhodanobacter* spp. (nitrate to nitrite to nitric oxide to nitrous oxide to nitrogen). Six denitrifying bacteria, including a member of the genus *Rhodanobacter*, have been isolated from the site and subject to genome sequencing by ORNL and JGI. Draft genomes are available for 5 of the 6 organisms, and the organisms are publicly available to SBR PIs in the DSMZ, the German Collection of Microorganisms and Cell Cultures. Genome analyses reveal the presence of great sequence novelty in denitrification genes within bacterial isolates from the site, and this sequence novelty challenges our ability to accurately quantify denitrification activity at the genus level using molecular techniques. In addition, multiple nitrite reductase genes were observed in several of the genomes, suggesting that it will be necessary to incorporate lateral gene transfer into modeling of nitrogen transformation in the subsurface.

Assessment of Coupled Plume-Scale Processes at the Oak Ridge IFRC Using High Performance Computing

ORNL IFRC (Principal Investigator: Scott Brooks)

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An effort was undertaken to implement a high-resolution numerical model to simulate complex coupled geochemical and hydrologic processes at the ORNL IFRC site using PFLOTRAN, a massively parallel, variably saturated, subsurface reactive flow and transport model. The first phase of the work involved creating a 2D vertical slice model through the main axis of the contaminant plume emanating from the former S-3 Ponds, in which liquid waste from the Y-12 plant was disposed. Baseline simulations of nitrate and uranium plumes were conducted to study the response to steady-state flow fields associated with average recharge rates during S-3 Pond operation and after their decommissioning and capping. Simulations were also performed subject to transient recharge rates averaged over various periods (e.g., monthly, daily) to evaluate coupling between transient flow and reactive transport. Realistic representations of transient recharge were obtained from inverse hydrologic modeling of rainfall, water level, and geophysical observations near the S-3 Ponds. Using a Python-based toolchain, we generated PFLOTRAN structured grids of various resolutions using a Digital Elevation Map (DEM) of the Oak Ridge Reservation and hydrogeologic datasets.

In addition, a 3-D model was initiated making use of the full anisotropic permeability tensor capability in PFLOTRAN. The geology at the BCV (Bear Creek Valley) site is complicated by dipping beds of limestone, shale, and sandstone with strike N 55º E and dip 45º SE, over which is superimposed a highly porous, horizontally oriented, saprolite weathering profile. A narrow, highly permeable, transition zone lies between the saprolite zone and bedrock. Fluid flow occurs primarily through secondary porosity produced by diagenesis, fracturing, and weathering of the bedrock. Groundwater flow paths are predominately parallel to geologic strike and dip. To model this system in 3D, we constructed a grid with *x*-axis aligned with the strike of the geologic formation and *z*-axis vertical. This formulation requires a full-permeability tensor with off-diagonal components obtained by counterclockwise rotation of the principal axes tensor through the formation dip angle. A full tensor capability is implemented in PFLOTRAN using the mimetic finite difference (MFD) method, a mass conserving, second-order accurate scheme with auxiliary pressure degrees of freedom at grid cell faces. Although this approach requires over three times the number of unknowns compared to a conventional cell-centered finite volume approach, it is applicable to highly anisotropic porous media requiring full tensor coefficients on general polyhedral meshes. In these preliminary calculations, an equivalent continuum formulation is used to represent the fractured rock mass.

Modeling Coupled Biogeochemical and Hydrologic Processes in Field Experiments at the Oak Ridge IFRC Involving pH Manipulation and Electron Donor Injection

ORNL IFRC (Principal Investigator: Scott Brooks)

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A field plot in saprolite downgradient of the former S-3 Ponds site was used to study the biogeochemical dynamics of highly contaminated acidic groundwater, when base is added to reduce aqueous radionuclides via geochemical precipitation and bioreduction. Pump tests and a bromide tracer study were performed to characterize the site (ORIFRC poster by Gu et al.). Injection and extraction wells were utilized to create a recirculation field. Base was added to the recirculated effluent to slowly increase the aquifer pH. Pump tests and tracer tests were performed involving bromide injection in the planned injection well. Inverse modeling of pump and tracer tests was performed to estimate hydraulic and dispersion characteristics of the aquifer. A 3-D numerical flow and reactive transport model was formulated to simulate the experiment. A comprehensive geochemical reaction model was coupled with the transport model to simulate surface complexation and ion exchange reactions using PHT3D. The geochemical model was verified against laboratory experimental data using literature equilibrium constants for surface complexation reactions with iron and aluminum hydroxides, and was successfully applied to the transient field experiment.

A second field experiment was performed involving injection of emulsified vegetable oil (EVO) in a carbonate gravel zone located between the shale/saprolite region adjacent to the capped S-3 Ponds and Bear Creek over a >300 day period (ORIFRC poster by Wu et al.). A numerical model was implemented to interpret results of the field experiment. Simulations considered aqueous and surface complexation reactions and various redox reactions involving EVO, EVO degradation products, sulfate, nitrate, Fe, Mn, U, and Tc mediated by specific microbial populations. Two approaches to modeling the biologically mediated reactions were investigated: (1) a Monod kinetic model, and (2) a thermodynamic model proposed by Istok et al. (2010) in which microbial growth is assumed to be transport-limited rather than kinetic-limited. The numerical simulations enable analysis of the complex interactions among specific physical, chemical and microbial processes in a more comprehensive way than before, and provide a platform to conduct numerical experiments of these interactions.

Biological Reduction of Uranium in the Contaminated Subsurface by Slow-Release Electron Donor

ORNL IFRC (Principal Investigator: Scott Brooks)

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In situ bioremediation of a uranium-contaminated aquifer with the slow-release electron donor emulsified vegetable oil (EVO) was tested in Area 2 of the Oak Ridge IFRC site, where U contaminated groundwater flows to Bear Creek through a highly permeable gravel fill zone (hydraulic conductivity of ~0.038 cm/sec). Groundwater at the site contains 5-6 µM U, 1.0-1.2 mM sulfate, 0.2-1.5 mM nitrate, 2.5-4.0 mM Ca^{2+}, and a pH of ~6.8.

The EVO selected contained (w/w) vegetable oil (60%), biodegradable surfactant (6%) and yeast extract (0.3%). Prior to the field test, laboratory tests were conducted to verify U(VI) reduction by EVO using microcosms. A bromide tracer test characterized the subsurface transport pathways prior to the EVO injection. Diluted EVO solution (20%, 900 gal total volume) was injected simultaneously into three injection wells within 1.5 hours. EVO distribution and consequent biogeochemical processes have been monitored through analysis of groundwater samples, and surged sediment samples over a two-year period. A large fraction of EVO injected was trapped or adsorbed in the subsurface. Sequential bioreduction of nitrate, Mn(IV), Fe(III) and sulfate and U(VI) occurred after EVO injection. Transient accumulation of acetate was observed as an intermediate in the EVO degradation. An initial increase in U in groundwater was observed, but within 2-4 weeks of EVO injection, reduction and removal of U was observed in all monitoring wells connected to the injection wells. U concentrations in groundwater were reduced to below 0.126 µM (U.S. EPA drinking water standard) at several well locations. The U concentration in groundwater seeps flowing into Bear Creek decreased by >80% within a four-month period and remained at less than 50% of original level after >365 days. Nitrate concentrations discharging to the creek were reduced to below detection limit and remained at low levels (<0.005 mM). Dissolved methane concentration in monitoring wells increased after EVO injection, indicating the enhancement of methanogenic activity. After the EVO was consumed, rebound of U in groundwater was observed, together with the rebound of sulfate concentrations as acetate production decreased. The rebound generally occurred first in the monitoring wells closest to the injection area, where upgradient groundwater containing oxidants like nitrate and sulfate, and U flow into the field plot. Rebound in downgradient wells were observed as EVO was sequentially consumed in upgradient locations. Uranium (VI) reduction to U (IV) in the microcosm and *in situ* field tests was confirmed by XANES spectroscopy. Reduced U was present in the solid phase predominantly as a nonuraninite phase, such as U(IV) atoms bound to Fe oxides or complexed to phosphoryl ligands.

Bacterial populations were analyzed using 16S rRNA gene libraries and Geochip 3.0 microarrays. The activities of major Fe(III)- and sulfate-reducing bacteria with U(VI)-reducing capability as well as methanogens were stimulated after injection of EVO. *Desulforegula*, known for LCFA oxidation to acetate, also dominated shortly after EVO injection and are thought to catalyze this process. Growth of known U(VI) reducing microorganisms, including *Desulfulvibrio*, *Anaeromyxobacter*, and *Geobacter* spp., were stimulated after the EVO injection. The EVO injection resulted in significant changes in both microbial functional communities and microbial diversities. Pyrosequencing and quantitative PCR of 16S rRNA in groundwater from monitoring well samples revealed a rapid decline in groundwater bacterial community richness and evenness after EVO injection, concurrent with increased 16S rRNA copy levels. Conversely, the richness and evenness increased due to EVO injection based on the functional gene analysis. The microbial communities and diversities reverted back close to original conditions as the EVO was consumed.

Biogeochemical Dynamics Controlling Uranium Mobility and Bioremediation in Contaminated Aquifers: Overview of Results from the Integrated Field-Scale Subsurface Research Challenge Site at Rifle, CO

Rifle IFRC (Principal Investigator: Philip Long)

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The Rifle IFRC Science Team (Multiple Institutions)

The Integrated Field Research Challenge Project (IFRC) at Rifle, CO, USA conducts research on a shallow alluvial aquifer adjacent to the Colorado River that is contaminated with uranium from former uranium and vanadium milling operations. The site is ideal for electron-donor-amendment experiments at the field scale, in which soluble U(VI) is reduced to insoluble U(IV) by *Geobacter sp*. stimulated by the electron donor (dilute acetate). Reduction and removal of U from groundwater is achieved even in a flowing aquifer system that is continuously challenged by up-gradient U. Use of novel proteomics and metagenomics analyses of groundwater and natural sediments from in-well columns have enabled tracking of the microbial community changes from dominance of Fe reducers to dominance of sulfate reducers after about 30 days of acetate amendment. Based on a preliminary interpretation of sequencing data from an in-well column dominated by sulfate reduction, a novel sulfate-reducing, *Desulfobacter*-like bacterium is the most abundant member of the community. The two next most abundant organisms include a *Sulfurovum*-like species and a distinct species related to *Sulfurimonas denitrificans*. Both are related to sulfur-oxidizers capable of anaerobic, nitrate-dependent sulfur oxidation, an observation consistent with geochemical reaction networks that predict production of native sulfur during sulfate reduction. Several smaller partial genomes include a *Geobacter*-like bacterium that was likely continuing to reduce Fe(III) minerals during dominance of sulfate reduction, and a low-abundance organism apparently related to a phosphate-accumulating bacterium. Proteomic analysis of sediment samples from the in-well column confirmed activities of sulfur oxidation and nitrate reduction, along with sulfate reduction, by detection of proteins from the relevant biochemical pathways in these microorganisms. The presence of active *Geobacter sp*. during sulfate reduction suggests their importance to ongoing U(VI) reduction.

Uranium desorption tracer tests and non-biostimulated Fe(II) amendment experiments were initially conducted separately from biostimulation experiments to understand abiotic processes controlling U mobility; however, during 2010, desorption by bicarbonate amendment and bioreduction by acetate amendment were successfully combined to assess the impact of bicarbonate on U(VI) bioreduction rates and to estimate the total available sorbed pool of U(VI) at the field scale. Results indicate that aqueous species dominated by enhanced stability of Ca-UO_2-CO_3 ternary complexes do not decrease the rate of U(VI) reduction under Rifle geochemical conditions. A three-dimensional reactive transport model similar to that used for the Big Rusty Experiment (2008) is currently under development to assess the interaction of U(VI) sorption-desorption processes and bioreduction of U(VI) to U(IV). Constraints provided by the results of the Super 8 experiment are expected to enhance uncalibrated model accuracy across a range of biogeochemical conditions.

The Rifle IFRC science team has also begun to examine the unexpected persistence of the U plume at Rifle, with the goal of significant improvement in our ability to predict natural attenuation of uranium plumes. Monitoring of the Rifle site over the last decade shows differing patterns of U(VI) concentration, and analysis of sediments has revealed the presence of naturally occurring U(IV). In some wells, U concentration in groundwater varies on approximately an annual cycle (~ 0.4 to 0.8 uM), whereas more recent detailed monitoring in other wells shows stable U concentration, but significant differences in Fe, Mn, V, As, and Se associated with the rise or fall of the water table during spring runoff. An increase in dissolved oxygen near the water table and its entrainment into the aquifer and subsequent consumption during falling water levels indicate the importance of biogeochemical cycling associated with the annual runoff cycle in the Colorado River. Clearly there are multiple sources of U at the Rifle site contributing to persistence of the plume, and we anticipate that quantification of various sources will greatly improve prediction of long-term behavior of U plumes.

Proteomics Analysis of Planktonic and Sediment-Associated Communities During Biostimulation at the Rifle IFRC

Rifle IFRC (Principal Investigator: Philip Long)

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The application of proteogenomic techniques at the Rifle IFRC site has greatly advanced our knowledge of microbial community structure and function during biostimulation. During the 2007 and 2008 field experiments, planktonic biomass was recovered from the aquifer during acetate amendment, and analyzed using both 2D-LC-MS/MS and an Accurate Mass and Time (AMT) tag approach. The generated spectra were searched against "synthetic metagenomes", consisting of concatenated isolate genomic data resulting in peptide and protein identifications. During the 2009 and 2010 field biostimulation experiments at Rifle, a series of both planktonic and sediment-associated microbial communities were sampled. A high-resolution temporal series of planktonic biomass samples (14 in total) were recovered from a downgradient well over a 48-day period. Sediment associated biomass was obtained by using "in-well" flow-through sediment incubators that allowed the recovery of this material without expensive and disruptive drilling operations. Previous planktonic biomass samples have been obtained at discrete time points either at the beginning or end of acetate amendment; the aim during the Super8 experiment was to use proteogenomics to track community function and structure over the whole period of biostimulation in a previously unamended portion of the aquifer. Initial results suggest that planktonic biomass was greatly reduced both at the very beginning of acetate amendment (prior to acetate impacting the sampling well), and following the cessation of amendment. During the period of active injection, data searches using a "synthetic metagenome" consisting of isolate genomes from common metal- and sulfate-reducing subsurface bacteria indicate dominance of *Geobacter* populations with lower abundances of other species such as *Rhodoferax* and *Anaeromyxobacter* spp. However, the availability of new metagenomic data from planktonic biomass has indicated the presence of other key microbial populations during biostimulation; these observations have been confirmed using proteomic data. Sediment samples were recovered at Rifle to investigate the microbial community associated with sediment particles during sulfate reduction. We carried out 454 metagenomic sequencing on extracted DNA from the sediments, with the results yielding at least three near-complete genomes or essentially complete genomes. The sediment is highly dominated by a single sulfate-reducing bacterial population, with lower-abundance populations of sulfur-oxidizing and nitrate-reducing bacteria. Subsequent proteomic analysis on these samples has confirmed the presence of proteins associated with both sulfur oxidation and nitrate reduction. These data suggest a role for element cycling at Rifle that is currently not fully understood.

An Ion exchange, Surface Complexation and Grain-Scale Mass-Transfer Model to Describe U(VI) Reactive Transport at the Rifle IFRC Site: Application to Laboratory Columns and Field Experiments

Rifle IFRC (Principal Investigator: Philip Long)

J.A. Davis (PI), *LBNL*, M.B. Hay, *USGS*, P. M. Fox, K.H. Williams—*LBNL*

Predicting uranium mobility in the subsurface requires detailed knowledge of geochemical processes controlling the sorption dynamics of U(VI). This is a particular challenge in cases where aqueous solution conditions are highly variable on short time scales, which may be the case during remediation schemes. Desorption of U(VI) from mineral surfaces is strongly dependent on aqueous chemistry (e.g., pH, HCO_3, and Ca concentrations). Thus, in a remediation scenario, U(VI) transport is controlled by the chemistry of the injectate and by biogeochemical processes that buffer or alter aqueous chemistry. Further, kinetic processes such as intragranular/intra-aggregate diffusion affecting U(VI) desorption equilibrium may become increasingly important under conditions where changing solution chemistry causes relatively steep U(VI) concentration gradients.

The objectives of this work are to quantify the effects of aquifer sediment properties on the transport dynamics of U(VI) under variable chemical conditions, and to develop a reactive transport model that can be applied to Rifle field settings. An ion exchange model was developed from batch experiments conducted with the aquifer sediment <2 mm fraction, which was then applied in combination with a surface complexation model to describe U(VI) transport in laboratory columns. Chemical conditions within the columns were varied through the injection of influent solutions with differing pH, bicarbonate, and major ion concentrations. Initial conditions within the columns were impacted by slow calcite dissolution and dissolution of salts derived from groundwater that dried onto sediments after subsurface collection. Stop-flow events during column elution demonstrated U(VI) desorption was not at local equilibrium, and U(VI) elution data were used to calibrate a distributed-rate mass-transfer model for U(VI) desorption, at flow rates and alkalinity concentrations relevant to the shallow aquifer at the Rifle site. Oversaturation with respect to calcite persisted throughout this column experiment and was also observed in the aquifer at the Rifle site despite the presence of calcite. In a column experiment with high influent bicarbonate, calcite oversaturation exceeded one order of magnitude but eventually declined to equilibrium, suggesting new calcite nucleation and precipitation within the column. Consistent with this interpretation was the fact that U(VI) elution was observed at a level inconsistent with aqueous speciation and kinetic model predictions, indicating that other processes were affecting results, such as co-precipitation with calcite. Finally, the model was successfully applied to describe previous groundwater injection experiments after correction for local surface sediment properties (surface area, ion exchange capacity, and initial adsorbed U(VI)).

Geochemical Effects of Iron and Sulfate Reduction in the Rifle IFRC Aquifer: Mineral Transformations, Solid-Phase U Accumulation, and Abiotic Reduction

Rifle IFRC (Principal Investigator: Philip Long)

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Uranium mobility in contaminated aquifers is controlled by a complex assortment of biogeochemical parameters, including aqueous speciation, microbial activity, mineralogical and hydrologic properties, and redox status. There is particular interest in understanding the factors governing U mobility under reducing conditions, which exist both naturally and as a result of biostimulation at the Rifle IFRC site. The overarching goal of this work is to investigate the geochemical effects of iron and sulfate reduction on mineral transformations and U mobility under conditions relevant to the Rifle IFRC field site. Through a combination of laboratory batch and field column experiments, we have investigated the following: (1) the degree and mechanism of Fe(II) uptake onto Rifle sediments, (2) U(VI) uptake and abiotic reduction by Fe(II)-bearing sediments, (3) abiotic U(VI) reduction by dissolved sulfide, and (4) U(VI) uptake and reduction on sediments during biostimulation.

Batch experiments performed on Rifle sediment indicated that Fe(II) uptake increases with increasing pH. Comparison of Mössbauer spectra from pristine and ^{57}Fe(II)-spiked sediments demonstrated that a large fraction (40-100%) of added ^{57}Fe(II) is oxidized to Fe(III) on sediment surfaces, presumably by Fe- and Mn-bearing minerals, and that the degree of oxidation decreased as Fe(II) loading increased. It appears that spiking sediments with ^{57}Fe(II) resulted in ferrihydrite transformation to a nanoparticulate Fe(II)/Fe(III) mineral phase. This suggests that the reactivity of Fe(II)-exposed sediments towards U(VI) (i.e., abiotic reduction) may only be important at relatively high Fe(II) loadings when a significant amount of Fe(II) is present on mineral surfaces. Experiments also demonstrated that U(VI) uptake was enhanced in the presence of Fe(II), either through abiotic reduction of U(VI) by Fe(II) or through the creation of new sorption sites from the addition of Fe(II). Separate batch experiments in the absence of sediment demonstrated that dissolved sulfide was capable of reducing U(VI) to nanoparticulate uraninite, with the rate of reduction dependent on a number of solution variables, including concentrations of U(VI), sulfide, bicarbonate, and Ca(II). It appears that aqueous U(VI) speciation in particular may affect the rate of reduction due to the nonreactivity of calcium uranyl carbonate complexes [$Ca_2UO_2(CO_3)_{3(aq)}$ and $CaUO_2(CO_3)_3^{2-}$]. While geochemical conditions typical of Rifle groundwater were not favorable for abiotic U reduction by dissolved sulfide over the experimental time scale of 48 hours, abiotic reduction of U(VI) by sulfide may be an important pathway for U-sequestration under certain chemical conditions and over longer time scales.

U sequestration onto Rifle sediments was investigated during column experiments, known as *in situ* column experiments, performed in the field during the Super 8 field experiment, which was designed to investigate the degree of U(VI) bioreduction in the presence of elevated bicarbonate concentrations (40-50 mM) compared to that in ambient Rifle groundwater (~9 mM bicarbonate). While levels of U accumulation on sediments were very similar under the two bicarbonate concentrations during periods of iron reduction, accumulation of U on sediments was 1-2 orders of magnitude higher during periods of sulfate reduction, indicating that sulfate-reducing conditions may be much more effective at sequestering U in the solid phase.

Proteogenomics Analysis of Microbial Communities Stimulated during Bioremediation at the Rifle IFRC Site

Rifle IFRC (Principal Investigator: Philip Long)

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There is increasing interest in harnessing metabolic capabilities of subsurface microbial communities to transform and remediate environmental contaminants. Here we use a proteogenomic approach to characterize the bacterial sediment and groundwater communities from an acetate amended aquifer in Rifle, CO, during uranium bioremediation.

Phylogenetic and genomic analyses from an in-well sulfate-reducing column sediment revealed the dominance (~64% of the community) of bacteria closely related to *Desulfobacter postgatei* (99% 16S rRNA gene identity). Other highly abundant organisms, each comprising ~6% of the community, included bacteria most closely related (94-97% identity) to the sulfur-oxidizing *Epsilonproteobacteria*, *Sulfurovum* and *Sulfurimonas*, and to *Geobacter bemidjiensis*. Both the sulfate-reducing and sulfur-oxidizing bacteria were sampled at depths for near-complete genome reconstruction. Liquid chromatography-based mass spectrometry and peptide identification using paired genomics data indicated proteins used for sulfate reduction (such as Dsr and APS) and nitrogen-fixation proteins (Nif) were expressed by *Desulfobacter*, at the same time as proteins important for nitrate, nitrite, and nitrous oxide reduction (Nap, Nir, and Nos) and the sulfur oxidation pathway (Sox) were expressed by *Sulfurovum* and/or *Sulfurimonas*. These results indicate anaerobic sulfur oxidation, and the reduction of nitrogen species, occur concomitant with sulfate reduction in the Rifle aquifer during uranium remediation.

Similarly, metagenomic analysis of three temporal groundwater samples, collected during acetate-stimulated Fe(III) reduction in 2008, demonstrated high abundance of *Desulfobacter*. The experiment was conducted in a section of the aquifer that had also been stimulated in the previous year, resulting in differences in the *Geobacter* populations of each year (Wilkins et al., 2009; Callister et al., 2010). Members of the Fe(III)-reducing *Geobacteraceae*, including organisms closely related to *G. bemidjiensis* and strain KN400, as well as *Desulfocapsa* and *Rhodobacter* spp., were detected in all three 2008 samples. In addition, organisms related to phylogenetically distinct candidate divisions composed of uncultured bacteria - previously identifiable only by 16S rRNA gene analysis - were abundant, and displayed partially overlapping distributions across the time course samples. These novel lineages increased in abundance over time as sulfide concentrations increased. Proteomic data confirmed the activities of multiple novel lineages during Fe(III) reduction.

Together, our phylogenetic, metagenomic, and proteomic results show that *Desulfobacter* and *Geobacter* spp. are ubiquitous and respond to acetate amendment across an iron-sulfate redox gradient in both planktonic- and sediment-attached biofilm communities. Our findings also show that acetate amendment stimulates phylogenetically diverse communities, including organisms contributing to the biogeochemical cycling of nitrogen and reduced sulfur species.

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Callister SJ, Wilkins MJ, Nicora CD, Williams KH, Banfield JF, VerBerkmoes NC *et al*. (2010). Analysis of biostimulated microbial communities from two field experiments reveals temporal and spatial differences in proteome profiles **44**: 8897-8903.

Post-Biostimulation Field Experiment and C-13 Stable Isotope Probing of Column Experiments to Identify Acetate Utilizers

Rifle IFRC (Principal Investigator: Philip Long)

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In order to determine which microorganisms take up acetate during biostimulation and how the uptake of acetate by specific organisms, especially *Geobacter* species, changes over time, a 120-day column biostimulation experiment was performed. A total of eight columns were loaded with Rifle sediments and operated under continuous flow conditions using Rifle groundwater, amended with 3 mM C-12 acetate. At regular time intervals, C-12 acetate flow into a specific column was switched to C-13 acetate. That column was then operated under C-13 acetate amendment for 36 hours before it was sacrificed for detailed geochemical and microbiological analyses. Column operation started under iron reduction (based on the measured Fe(II) in the column effluent), while sulfate reduction (based on removal of sulfate between influent and outflow), was noted at about 25 days of operation. The microbial characterization consisted of phospholipid fatty acid analysis (PLFA) and stable isotope probing (SIP). All microbial characterization was done to differentiate between the C-12 and C-13 incorporation into the biomass. Results showed that there was a differentiation between the community that was taking up acetate actively throughout the 120 days of operation and the overall microbial community. Of interest was that the fraction of *Geobacter* population remained fairly constant throughout the duration of the experiment, as well as its acetate uptake. Results also showed that of the acetate incorporated into the overall biomass, about 40% was incorporated into *Geobacter* biomass. These results are key for the proper numerical simulations of biostimulation via acetate amendment and the biostimulation of *Geobacter*.

A field-post-biostimulation experiment was performed to assess the stability of bioreduced uranium under field conditions, and to track the post-biostimulation microbial community dynamics. Eleven columns were packed with Rifle sediment and biostimulated in the laboratory for 70 days with 3 mM acetate. The column influent to the columns contained 20 nM U(VI). After this biostimulation period, the columns were transported to the Rifle site and submerged below the water level in one of two different wells U01 and P104. Well U01 is located upstream and well P104 downstream of the injection gallery of a recently performed field biostimulation experiment. Columns were connected to a peristaltic pump, and groundwater was pumped from a depth of 18 ft. below groundwater level into the columns and then up to the surface, where the column effluent as well as groundwater was collected and analyzed at least on a monthly basis. Individual columns were withdrawn and sacrificed over a one-year period for detailed geochemical and microbiological characterization. Results showed that there were significant changes in groundwater chemistry (dissolved oxygen and dissolved organic carbon) over time, such that there were episodic periods of sulfate reduction and sulfite and uranium oxidation in the columns. Uranium remained relatively stable in the columns for a period of about 200 days, after which 70% of the previously bioprecipitated uranium was remobilized during the next 100 days. The onset of the uranium remobilization coincided with higher dissolved oxygen levels in the groundwater. Microbial characterization is still in progress.

Persistence of the Uranium Plume at the Rifle IFRC: Importance of Naturally Reduced Zones

Rifle IFRC (Principal Investigator: Philip Long)

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The uranium plume at the Rifle IFRC has not attenuated as predicted via a reactive transport model developed more than 10 years ago. We hypothesize four sources of U to account for this observation, (1) Downward flux of U from the vadose zone that is entrained into the aquifer during times of high water table associated with high runoff in the Colorado River, (2) Mobilization of reduced U from naturally reduced zones (NRZ's), (3) Slow oxidation of U(IV) dispersed through the saturated zone, and (4) Influx of natural background U(VI) from upgradient groundwater recharge. We are currently attempting to quantify the contribution of each of these sources of U, creating a site-wide U inventory in the process. In this work, we focus on the NRZ's. A survey of groundwater data from uranium mill tailings sites in the Western U.S. indicates that more than half of them (11 out of 18) have exhibited persistently high levels of Fe(II) at one or more well locations on a given site. These observations suggest that natural reduction is a common feature in these and similar alluvial aquifers. NRZ's have also been directly observed at four uranium-contaminated sites, further indicating their general importance to uranium mobility via either sorption to organic detritus or via enhanced microbial activity and associated reduction of U(VI) to U(IV) in these zones. At the Rifle IFRC, NRZ's have an important and unique implication to plume maintenance. While uranium speciation is likely to be an important control on the release of uranium from NRZ's to groundwater, other factors are likely to be equally important, including redox-poising constituents and permeability interfaces along boundaries of NRZs, which give rise to diffusion-limited reaction rates. In addition to U, NRZ's contain elevated concentrations of NOM, Fe(II) and reduced inorganic sulfides. All of these constituents can scavenge oxidants introduced following seasonal incursion of (sub)oxic groundwater or the sustained release or accumulation of endogenous oxidants (e.g., MnO_2), promoting retention of U(IV). Moreover, studies of biogenic uraninite corrosion in the Old Rifle aquifer implicate diffusion-limited solute transport as an important contributor to strongly retarded U(IV) loss rates, with observed rates >100-fold slower than those observed in stirred laboratory reactor studies. Diffusion-limited solute transport retards oxidation of both solid phase and nonprecipitated forms of U(IV). Thus, a combination of speciation, an abundance of redox-poising constituents, slow U(IV) oxidation rates, and permeability interfaces and/or low-permeability facies within which U(IV) resides are believed to impede flushing of U from the system, effectively enhancing the stability of U(IV) sequestered through natural (i.e., nonstimulated) processes. At the Rifle IFRC, an NRZ has been instrumented with a closely spaced well network. Passive Flux Meters (PFM) deployments in 2009 and 2010 show that the U flux in the vicinity of the NRZ has a mean of 0.39 ug/cm^2-day (std. dev. 0.43, n=99) and ranges from 0.01 to 2.17 ug/cm^2-day. The flux of U is generally greatest in the lower part of the wells, and average flux varies significantly between wells. Flux-averaged uranium concentrations for each well were consistent with observed aqueous concentrations, suggesting that this NRZ is not currently a sink for U. An *in situ* column test using ^{236}U as a reactive tracer is planned to assess the rate of U reduction under ambient conditions in the reduced zone. NRZ's at the Rifle IFRC exhibit differing geometries, intensity of reduction, and concentrations of U and organic carbon. For example, one NRZ at Rifle contains up to 50 times more U than the typical alluvial sediment at Rifle. Consideration of various factors controlling NRZ's suggests that defining "biogeofacies" in contaminated aquifers may be useful in predicting contaminant mobility and persistence of groundwater plumes of redox-sensitive metals.

Sedimentology and Physical/Geochemical Property Estimation to Support 3D Reactive Transport Modeling

Rifle IFRC (Principal Investigator: Philip Long)

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The Rifle Integrated Research Field Challenge (IFRC) is working to understand the microbiological, geochemical, and hydrologic processes controlling uranium mobility, and provide a clearer picture of the processes controlling natural attenuation of the uranium plume in groundwater at the Old Rifle Site. Reactive transport modeling is one of the most important tools being used to integrate the available data and test hypotheses on the relative importance of those processes. We are characterizing the physical and chemical heterogeneity of the subsurface sediments at Rifle so that we can develop the numerical models of the site required for reactive transport modeling.

Wells for a new experimental plot, Plot C, were drilled at Rifle in the summer of 2010. Characterization methods include laboratory measurements of physical and reactive properties of sediment samples; geophysical logging of the new boreholes; hydrologic testing of the wells before and after injection experiments at the new experimental plot; and collection of electrical resistivity tomography (ERT) and induced polarization (IP) data. Geological characterization of the sediments from the new plot included geologic descriptions of the sediments during drilling, particle size analysis, and measurements of surface area. Particle size and surface area measurements were focused on CD-08, the well with the most complete set of samples. Samples were analyzed on 15 cm spacing throughout that well. As we found for previous experimental plots at the Rifle IFRC, the sediments were dominated by gravel, ranging from 43% to 84%, with less than 7.5% being mud (< .063 mm). We have developed a model for predicting surface area for Rifle sediments from the particle size distribution (psd) based on the principle of superposition. Analysis of coarse-fraction coatings for one sample showed that 6% of the total sample consisted of grain coatings, which could impact the estimate of surface area. Borehole geophysical data collected at the new site included density, spectral gamma (KUT), and electromagnetic logs. Logging was conducted in 25 wells, and provides data that will be used to estimate a select suite of physical and reactive transport properties, including the porosity and one or more measures of the psd. Static and time-lapse ERT and IP data were collected for 16 boreholes that were modified to include 11 copper electrodes per well. That data has been used to generate preliminary estimates of the spatial distribution of porosity and Spor (ratio of specific surface area to porosity). Hydrologic characterization has included pump tests, slug tests, and electromagnetic borehole flowmeter (EBF) profiles. Interpretation of the pump tests suggested a specific storage of 0.19 to 0.23 with an average hydraulic conductivity of ~8.5 m/day. The suite of measurements collected in Plot C will be used to complete a property transfer model that will be used to estimate the required hydraulic and reactive transport properties from well data. We will then perform a geostatistical analysis of the heterogeneity at the site and generate realizations of the numerical grids of hydraulic and geochemical properties required for reactive transport modeling of experiments at the new experimental plot.

The Rifle IFRC "Super 8" Field Experiment: Biogeochemistry and Uranium Mobility Accompanying Concomitant Acetate and Bicarbonate Amendment

Rifle IFRC (Principal Investigator: Philip Long)

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Research activities at the IFRC site in Rifle, CO are designed to integrate geochemical, biological, and hydrological studies to enhance our understanding of subsurface uranium mobility. Initiated in August 2010, the "Super 8" field experiment was designed to achieve three primary objectives: (1) replication and extension of the "Little Rusty" (2008) nonbiostimulated uranium desorption experiment; (2) quantification of uranium mobility during acetate amendment under conditions of varying alkalinity and where microbial iron reduction is the dominant metabolic process; and (3) assessing the extent to which uranium speciation impacts the thermodynamic controls on enzymatic uranium reduction.

In advance of the experiment, a new flowcell consisting of 34 wells was installed in an undisturbed area of the site. The flowcell traverses a region of significant topographic relief along the aquifer-aquitard contact, with variations in aquifer thickness along the primary flow direction exceeding 1.5 m. Injection wells were configured such that downgradient locations could selectively be exposed to sodium acetate (ca. 5 mM), sodium bicarbonate (ca. 40 mM) or a combination of the two. Distinct conservative tracers (NaBr and D_2O) were used to discriminate the acetate and bicarbonate plumes within the flowcell. Pre- and post-injection hydrologic tests revealed minimal change in aquifer flow properties as a result of the amendments, with slight decreases in permeability observed in just three of the acetate injection wells.

Although Ca/Mg-UO_2-CO_3 ternary complexes comprised >95% of the aqueous uranyl species, acetate amendment resulted in rapid removal of U(VI) from groundwater. Statistical analysis of removal rates indicating negligible difference between locations impacted and unimpacted by elevated bicarbonate. Analysis of 16S rRNA microarray data indicated little variation in microbial community composition as a function of bicarbonate concentration. Increases in hybridization intensity of probes specific to metal and sulfate-reducing bacteria were observed following acetate injection, with increases in aqueous Fe and Mn accompanying stimulated microbial activity. Fe(II) concentrations within the region impacted by $NaHCO_3$ were considerably higher (400–500 µM) than previously documented at the Rifle site, likely the result of ion exchange with elevated Na^+. Limited release of arsenic during metal reduction was observed at multiple locations, with the effect greatest for locations proximal to the region of acetate injection; arsenic concentrations fell to levels at or below baseline values once injection ceased.

Results indicate that ca. 20 days of bicarbonate amendment was sufficient to exhaust a large fraction of the labile, sorbed pool of U(VI) within 1 m of the injection zone. The extended period of U desorption accompanying bicarbonate injection generated abundant sorption sites and dramatically impacted post-biostimulation changes in groundwater U(VI). The rate of uranium rebound in locations impacted by elevated bicarbonate was much slower than locations amended solely with acetate, where U(VI) began to rebound as soon as acetate concentrations fell to levels below detection. In contrast, U(VI) concentrations in bicarbonate-impacted locations remained significantly below pre-injection values well in excess of 100 days. This observation suggests that removal of sorbed U by microorganisms may be limited, consistent with previously reported batch experiments. Such results suggest that enhanced desorption via *in situ* alkalinity adjustment is a potentially viable means of accelerating attenuation of U, either in combination with biostimulation or as a stand-alone remedial approach.

Variably Saturated Flow and Multicomponent Biogeochemical Reactive Transport Modeling of a Uranium Bioremediation Field Experiment at the Rifle IFRC Site

Rifle IFRC (Principal Investigator: Philip Long)

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Three-dimensional, variably saturated flow and biogeochemical reactive transport modeling of the Rifle IFRC 2008 “Big Rusty” *in situ* uranium bioremediation field experiment was used to develop a systematic understanding of the impacts on uranium behavior of pulsed acetate amendment, seasonal water-table variation, and highly resolved spatially variable physical (hydraulic conductivity, porosity) and geochemical (reactive surface area) material properties. A principal challenge was the mechanistic representation of biologically mediated terminal electron acceptor process (TEAP) reactions whose products significantly altered geochemical controls on uranium mobility through increases in pH, alkalinity, exchangeable cations, and highly reactive reduction products. Based on new knowledge from proteomic studies, simultaneously active Fe(III) reducing bacteria (FeRB) and sulfate-reducing bacteria (SRB) were incorporated into the modeling. In this case, an initially small population of slow-growing SRB was active from the initiation of biostimulation. Biomass and proteomics data from the 2008 experiment provided an unprecedented opportunity to assess the capabilities of a genome-scale metabolic (“in silico”) model of *Geobacter sulfurreducens* (Mahadevan et al. 2006) that replaced the field calibrated Fe(III) TEAP reactions. The constraint-based *in silico* model accounts for over 500 intracellular and exchange reactions. Most importantly, 77 of the 168 *Geobacter sulfurreducens* proteins detected during the Big Rusty experiment were associated with *in silico* model reactions.

The simulated water table decline during the field experiment resulted in the isolation of residual reactants and products, as well as unmitigated uranium, in the newly unsaturated vadose zone. High permeability sandy gravel structures led to locally high flow rates in the vicinity of injection wells that increased acetate dilution. In downgradient locations, these structures created preferential flow paths for acetate delivery that enhanced local zones of TEAP reactivity and subsidiary reactions. The most abundant sampled proteins (acetyl-CoA transferase and citrate synthase) corresponded to the highest central metabolism reaction rates in the *Geobacter sulfurreducens in silico* model For example, pyruvate ferredoxin oxioreductase activity agrees with the prediction that this enzyme converts acetate to pyruvate. The proteomics data detect some activity through the “other” acetyl CoA transferase pathway (GSU174), whereas the *in silico* model has all the flux going through GSU490. The absence of phosphotransacetylase in the proteomics data may be due to sequence divergence in environmental copies of this protein, resulting in poor matches when proteomic measurements are searched against the *G. sulfurreducens* genome.

The large memory and computational performance of the DOE BER EMSL Chinook computing cluster were indispensible for the efficient exploration of alternative conceptual process models and hypotheses in the context of the observed experimental behaviors. In this case, the 160-day simulations performed with the eSTOMP massively parallel processing simulator required 12 hours of wall clock time. An equivalent simulation without parallel processing would have taken 60 days, assuming sufficient memory was available.

Scientific Focus Areas (SFA)

The Argonne Subsurface Scientific Focus Area

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

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The Argonne Subsurface Scientific Focus Area (SFA) integrates synchrotron-based biogeochemistry with microbiology, molecular biology, and protein biochemistry to pursue the long-term scientific goal of elucidating the interplay, at the molecular level, between specific microbial metabolic activities, solution chemistry, and mineralogy contributing to the transformations of contaminants at DOE field sites. Hypotheses directed toward achieving this goal are tested by experiments that capitalize on unique Argonne capabilities, together with key collaborative efforts at Oak Ridge National Laboratory (ORNL), Pacific Northwest National Laboratory (PNNL), Michigan State University, University of Iowa, University of Illinois Urbana-Champaign, Stanford University, Hamilton College, Virginia Commonwealth University, University of Tennessee, and the Illinois Institute of Technology. The objective of the ANL SFA is to characterize coupled biotic and abiotic, molecular-scale contaminant transformations to provide fundamental knowledge necessary for predicting contaminant reactivity and transport processes. This objective drives the development and optimization of synchrotron methods for measurements at the molecular level pertinent to understanding contaminant transformations in subsurface environments. Two scientific themes drive the development of hypotheses. These themes are: (1) *Microbial metabolic activity—together with changes in solution chemistry, mineralogy, and solid-phase surface reactivity, and the presence of electron donors, acceptors, and shuttles—affects the formation and distribution of (bio)mineral phases, as well as the rate, extent, and mechanisms of biotic and abiotic molecular-scale contaminant transformation; and (2) multiple coupled biological, chemical, and physical processes can affect the molecular-scale transformation of contaminants in the vicinity of mineral-microbe microenvironments.*

Research is focused on the transformation of uranium and mercury at different spatial scales and in the context of iron and sulfate reduction. Research strives to (1) determine which aspects of microbial metabolism, solution chemistry, and mineralogy are key to the distribution of mineral species and to contaminant transformations; (2) evaluate the reactivity of biogenic mineral phases with respect to the chemical transformations of U^{VI} and Hg^{II}; (3) provide fundamental knowledge concerning the molecular-scale transformations of contaminants at the mineral-microbe microenvironment; and (4) evaluate the effect of diffusion and advective water flow on contaminant transformations. Research emphasizes laboratory-based experimentation with single-crystalline-phase iron oxide powders, fabricated iron-rich mineral assemblies designed to mimic mineralogical conditions in the SBR Integrated Field Centers (IFCs), iron oxide thin films, and geomaterial collected from all three of the SBR IFRC sites. Inocula for promoting iron- and sulfate-reducing conditions include (1) monocultures of dissimilatory iron reducing bacteria (e.g., *Geobacter* spp., *Anaeromyxobacter* spp.) and dissimilatory sulfate-reducing bacteria (e.g., *Desulfovibrio* spp.) representative of organisms identified at contaminated sites and (2) microbial consortia collected from all three of the SBR IFC sites.

In addition to an overview of the ANL SFA, this poster will also provide results of hard x-ray micro(spectro)scopy investigations of interactions among lepidocrocite thin films, metal-reducing bacteria (e.g., *Anaeromyxobacter* spp. and *Shewanella oneidensis* MR-1) respiring on the films, and uranium. These results identify at least two different interactions with uranium within five microns of the mineral-microbe interface.

Microbial Community Development under Sulfate- and Iron-Reducing Conditions Based on Electron Donor and Electron Shuttle Amendment

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

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The biotic contribution to contaminant transformations has yet to be characterized in terms of which metabolic processes are essential, although numerous reports have documented the relationship between changes in microbial community population composition and contaminant transformations. New methods for high-throughput genome sequencing of environmental samples now offer the opportunity for analysis of microbial communities in greater depth, as well as prediction of the predominant metabolic processes involved in the biotic component of contaminant transformation. A critical research need is the highly integrated, in-depth monitoring of the microbial community dynamics, proteomics, and geochemistry of a subsurface system during biostimulation to identify the metabolic processes directly related to the resulting transformations.

Two experiments are described here that explore the impact of specific electron donors (acetate, lactate, and glucose) as well as the addition of an electron shuttling compound (2,6-anthraquinone disulfonate [AQDS]) on the evolution of microbial communities originating from the US DOE SBR Old Rifle IFRC site. For both experiments, mixed batch experiments containing iron-rich material inoculated with sediments from the Old Rifle IFRC site were characterized by microbial community analysis (using 454 XLR70-based tag sequencing of the V3-V4 region of the 16S rRNA-encoding gene) and geochemical measurements (wet chemical and synchrotron-based analyses) to elucidate natural biogeochemical processes that can be augmented to foster stabilization of subsurface contaminants. According to electron donor available, distinct communities developed over time as they reduced iron and/or sulfate over the course of the experiment. Geochemical characterization of an acetate-amended system supplemented with or without AQDS identified an increase in the concentration of reduced iron and a decrease in the concentration of sulfate with time. An increase in the relative abundance of the *Deltaproteobacteria* was observed early in the experiment (post Day 2) for both systems. For the AQDS-amended system, the majority of the community was dominated early by *Geobacter*, whereas a greater increase in the relative abundance of *Bacteroidetes* was observed for the batch not containing AQDS. In both systems a transition occurred (post Day 23) when the *Firmicutes* became dominant (specifically members of the genus *Desulfosporosinus*).

Presently, research is focused on determining how the combination of Fe(III) oxide mineralogy and specific electron donors affects microbial community development under iron(III) and sulfate-reducing conditions. Future investigations will focus on identification of metabolic pathways via metagenomic analysis, and microbial community and biogeochemical dynamics with populations from other DOE field sites under advective flow conditions.

Non-Uraninite U(IV) Phases in Biostimulated Sediments from the Oak Ridge IFRC

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

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The reduction of relatively soluble and mobile U^{VI} to less-soluble U^{IV} species by native bacteria is a promising immobilization strategy for subsurface uranium plumes. The ability to reduce U^{VI} has been established for a number of field-relevant bacteria, but the coupled interactions among uranium, bacteria, iron minerals, and soluble electron acceptors (e.g., sulfate) remain relatively unexplored. In addition to addressing these interactions in controlled laboratory systems, the Argonne Subsurface SFA project is involved in studying the U speciation in sediments from the Oak Ridge IFRC, both in microcosm systems and in materials collected during field-scale biostimulation campaigns.

Recently, it has been established that U^{VI} can be reduced to U^{IV} phases that are different from uraninite. One factor leading to nonuraninite U^{IV} phases was the presence of dissolved phosphate [1], but nonuraninite phases also formed during biotic and abiotic U^{VI} reduction in phosphate-free medium [2]. Since natural systems contain a variety of ligands and complexing surfaces, the sequestration of uranium in nonuraninite phases at field sites can be hypothesized.

In this study, synchrotron x-ray absorption spectroscopy was used to track the spatial distribution, valence state, and speciation of U and Fe in ethanol-stimulated sediments from the Oak Ridge IFRC, in parallel with [SO_4], [U], [ethanol], and pH characterizations of the solution phase. Diffusion limited reactors at two sulfate concentrations were set up in x-ray transparent bottles and interrogated *in situ* over a period of three years. Ethanol addition to the overlying solution caused the development of a dark layer at the solution-sediment interface, concurrent with the removal of U^{VI} from solution. Uranium L_{III}-edge XANES indicated reduction of U^{VI} to U^{IV} in the dark band, but no initial reduction in the bottom tan portion. The originally uniform distribution of U^{VI} in the bottom part of the sediment evolved to uniformly dispersed U^{VI} hotspots <200 microns in size. EXAFS revealed that U^{IV} in the top dark portion formed a non-uraninite phase in which the U^{IV} atoms were not complexed to phosphate groups. Similar non-uraninite U^{IV} phases were observed in fully reduced sediments from the injection wells of the 2009 biostimulation experiment with emulsified vegetable oil (EVO) at the Oak Ridge IFRC.

These observations suggest that reductive sequestration of uranium at field sites may be more complex than originally thought. More studies will be required to elucidate the factors controlling the speciation of reduced uranium phases and their susceptibility to remobilization. Future directions include studying the effects of complexing surfaces and ligands on the identity of U^{IV} phases, as well as studies of the remobilization properties of field-relevant U^{IV} phases.

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Role of Sorbents in Abiotic Redox Transformations of Hg(II)

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

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The reduction of Hg^{II} to Hg^0 can increase Hg mobility due to the volatility of Hg^0. Magnetite and green rust have been shown to reduce aqueous Hg^{II} to Hg^0. Since Hg is typically present in aquatic and terrestrial systems at low concentrations, binding of Hg^{II} to high affinity sites on bacteria could have important implications for the potential reduction of Hg^{II} to Hg^0 and the overall mobility of Hg in biostimulated subsurface environments. In this study, we have tested the ability of magnetite and green rust to reduce Hg^{II} adsorbed to *Bacillus subtilis* cells (2 g/L). The experimental conditions were high (50 µM) and low (5 µM) Hg concentrations, at pH 6.5 and 5.0. At high Hg:biomass loading, the majority of solid-phase Hg^{II} was bound to carboxyl groups. Addition of 2.5 mM magnetite ([magnetite]:[Hg]=50:1) resulted in Hg^{II} reduction to Hg^0, as evidenced by Hg L_{III}-edge x-ray absorption spectroscopy (XAS). Reduction was complete within 2 hours and 2 days at pH 6.5 and 5.0, respectively. At low Hg:biomass loading the majority of solid-phase Hg^{II} was bound to sulfhydryl functional groups on the cell. Sulfhydryl-bound Hg^{II} was not reduced by magnetite ([magnetite]:[Hg]=50:1) at pH 6.5 or 5.0 after 2 months of reaction. Green rust, which is generally a stronger reductant than magnetite, reduced about 20% of sulfhydryl-bound Hg^{II} to Hg^0 in two days. These results suggest that binding of aqueous Hg^{II} to sulfhydryl cell-wall functional groups severely inhibits the ability of mixed $Fe^{II/III}$ phases to reduce it to Hg^0, whereas binding to the carboxyl groups on the cell does not.

While the interaction between Hg^{II} and mixed $Fe^{II/III}$ phases is an important reduction pathway in the Hg cycle, Fe^{II} associated with clays and minerals is ubiquitously present in the environment and may also have a significant influence on Hg^{II} redox transformations. We have tested the ability of Fe^{II} amended goethite suspensions to reduce Hg^{II} in the presence of Cl, an environmentally relevant anion known to form aqueous complexes with Hg. Micron- and nanometer-sized goethite particles were equilibrated with 1 mM $FeCl_2$ at pH 7.2 and reacted with 0.25 mM Hg^{II}. Reduction of Hg^{II} was observed in both systems, but they differed in the products of reduction. Hg^I was formed in the micron-sized goethite system, whereas Hg^0 was formed in the nanoparticulate geothite system. Since natural and contaminated environmental systems contain particulate matter with a wide range of particle sizes, understanding the effects of particle size on electron-transfer processes may provide key insight into understanding the abiotic redox transformations of radionuclides and heavy metals.

Effects of Incorporated P on the Bioreduction of Fe(III) Oxides

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

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Natural Fe^{III} oxides typically contain a range of incorporated trace elements that can include P (phosphate). Although solution phase and adsorbed P have been shown to have a significant impact on the bioreduction of Fe^{III} oxides, little is known about the potential effects of incorporated P. We have examined the bioreduction of Fe^{III} oxides (lepidocrocite (g-FeOOH) and maghemite (g-Fe_2O_3)) containing 0 –3 mass% P. Kinetic dissolution studies showed congruent release of Fe and P, indicating that the P in these materials was incorporated within the particles. In the absence of P, lepidocrocite was rapidly and stoichiometrically reduced to magnetite by *Shewanella putrefaciens* CN32, and over time the magnetite was partially transformed to ferrous hydroxy carbonate (FHC). Doping with 0.2–0.7 mass% P significantly inhibited the initial reduction of lepidocrocite, but ultimately resulted in greater Fe^{II} production and the formation of carbonate green rust as the dominant secondary mineralization product; doping with 3.0% P resulted in the formation of green rust and vivianite. However, the bioreduction of both maghemite and P-doped maghemite resulted in solid-state conversion to magnetite, with subsequent formation of FHC.

Additional experiments examining the effects of different electron donors and the presence of electron shuttles on Fe^{III} oxide bioreduction were conducted with Bayferrox 943, a commercially available lepidocrocite that contains 0.2 mass% P. Acetate, alanine, citrate, ethanol, glucose, glutamate, glycerol, glycine, malate, propionate, and succinate were not effectively utilized as electron donors for the bioreduction of Bayferrox 943 by *S. putrefaciens* CN32; however, substantial Fe^{II} production was observed when formate, lactate, H_2, pyruvate, serine, or *N*-acetylglucosamine (NAG) was provided as an electron donor. Maximum rates of Fe^{II} production varied considerably, from 5.7 mM Fe^{II} d^{-1} for serine to 0.5 mM Fe^{II} d^{-1} for NAG, as did the overall extent of Fe^{II} production, which ranged from 69 mM Fe^{II} for pyruvate to 17 mM Fe^{II} for H_2. Differences were also observed in the types of Fe^{II}-bearing secondary mineralization products formed with different electron donors. Carbonate green rust was the dominant biomineralization product when formate, H_2, lactate, or NAG was provided, while siderite formed with pyruvate or serine; however, magnetite was never observed as a secondary mineralization product. The formation of carbonate green rust in cultures amended with NAG suggests that NAG was at least partially oxidized to carbonate; cultures containing H_2 had carbonate added to the medium prior to inoculation. The presence of various electron shuttles did affect the rate of Fe^{II} production during the bioreduction of Bayferrox 943; however, carbonate green rust was formed regardless of the presence or absence of soluble exogenous electron shuttles.

These results clearly indicate that the inclusion of impurities like phosphate can have significant effects on the bioreduction of Fe^{III} oxides and secondary mineral formation.

Reduction of U(VI) and Hg(II) by Magnetite

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

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The mobility of heavy metal and radionuclide contaminants in subsurface environments is strongly influenced by their chemical speciation, especially their oxidation state. For example, the reduction of U^{VI} to U^{IV}, which can decrease the mobility of U in the subsurface, is the basis for many *in situ* remediation technologies, including bioremediation-based approaches. Alternatively, the reduction of Hg^{II} to Hg^0 can result in increased volatilization. The successful application of bioremediation-based *in situ* treatments, however, requires a thorough understanding of the key microbiological and geochemical processes controlling contaminant transformation and mobility in the subsurface. The objective of our work is to better understand the abiotic controls on the reduction of U^{VI} and Hg^{II} by biogenically formed reactive Fe^{II} species, such as magnetite (Fe_3O_4). Of the reduced iron minerals, magnetite is of significant interest because of its formation from many Fe^{III} minerals as a result of dissimilatory iron reduction. Magnetite is also a significant corrosion product of Fe metal in suboxic and anoxic conditions, and will likely play a significant role in corrosion of iron waste containers holding uranium-containing spent nuclear fuel.

We investigated the reduction of U^{VI} and Hg^{II} by magnetites of varying Fe^{2+} contents, expressed as the ratio $x = Fe^{2+}/Fe^{3+}$. The Fe^{2+} content of magnetite can vary from maghemite, where $x = 0$ to stoichiometric magnetite, where $x = 0.5$. Previous work by our group has shown that the Fe^{2+} content of magnetite can dramatically influence the rate of nitrobenzene reduction by magnetite, and we hypothesized that magnetite stoichiometry would also influence the rates and extent of U^{VI} and Hg^{II} reduction. For Hg^{II}, we further investigated the influence of chloride on Hg^{II} reduction.

In previous work, discrepancies exist regarding the extent of U^{VI} reduction by magnetite. We observed that varying the initial Fe^{2+} content in the magnetite significantly influenced the extent of U^{VI} reduction by magnetite. Stoichiometric and partially oxidized magnetites with an Fe^{2+}/Fe^{3+} ratio (x) greater than 0.38 reduced U^{VI} to U^{IV} in UO_2 (uraninite) nanoparticles, whereas with more oxidized magnetites ($x < 0.38$) and maghemite ($x = 0$), only sorbed U^{VI} was observed. We further show that aqueous Fe^{II} is capable of recharging the reducing capacity of these oxidized magnetites. Mössbauer spectroscopy provided-direct evidence that the reduction of U^{VI} to U^{IV} is coupled to oxidation of Fe^{2+} in the magnetite.

For Hg^{II}, we have also observed an effect of magnetite stoichiometry on the extent of Hg^{II} reduction. Using a trapping solution to capture volatile Hg^0 indicates that more reduction occurred with magnetites containing higher Fe^{2+} contents (i.e., more stoichiometric). For near-stoichiometric magnetite ($x = 0.48$), XAS results indicate that surface associated Hg is Hg^0, regardless of whether chloride is present. In the absence of chloride, we also observed that most of the Hg^{II} was reduced to Hg^0 with the lower stoichiometric magnetites ($x = 0.28$ and 0.38). Interestingly, though, in the presence of chloride, the lower stoichiometry magnetites did not reduce Hg^{II} all the way to Hg^0. Rather, Hg was stabilized as Hg^I in calomel (Hg_2Cl_2). Our results suggest that magnetite stoichiometry and the ability of aqueous Fe^{II} to recharge magnetite are important when evaluating the potential for reduction of Hg^{II} and U^{VI} in the subsurface.

Understanding and Controlling Precipitation Reaction Fronts in Subsurface Environments: The Idaho National Laboratory SBR Scientific Focus Area

INL SFA (Laboratory Research Manager: Michael McIlwain)

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The theme for the Idaho National Laboratory (INL) Scientific Focus Area (SFA) is "Understanding and Controlling Precipitation Reaction Fronts in Subsurface Environments." We are investigating how the spatial and temporal distribution of biogeochemical reactions in the subsurface depends on the spatial and temporal relationships between sources and sinks for reactants, which can be characterized on molecular, microscopic or macroscopic scales. In the context of environmental remediation, we know that mineral forming reactions can be used to modify the mobility of contaminants via chemical complexation or by modifying permeability and flow paths. However, the engineering challenge is to understand how reactions can be propagated in complex heterogeneous media to produce the desired outcomes with respect to the target contaminants. The INL SFA research program addresses a number of challenging and interrelated topics: the transport and mixing of reactants at multiple scales in porous and fractured media; relationships between fundamental reaction rates and macroscopically observed and modeled volume-averaged reaction rates; the complex behavior of systems where physical processes affecting transport and reactant mixing are tightly coupled to chemical and biogeochemical processes; and novel methods that can be used to noninvasively monitor mixing-reaction fronts in subsurface environments.

During the past year, the INL SFA conducted multiple experimental and modeling campaigns to investigate reaction fronts. The major effort was on a model system for calcium carbonate precipitation and strontium immobilization where dissolved calcium and strontium are present in the pore solution, and carbonate ion is produced *in situ* at solid grain surfaces via the enzymatically catalyzed hydrolysis of urea. Prior to constructing the experiments, model simulations of the coupling of the important physical and chemical processes were performed at the continuum scale, to develop hypotheses and as an aid to the experimental design. Guided by the simulation results, we then conducted packed porous-media column experiments using immobilized urease enzyme to experimentally observe how reaction-front products and calcium carbonate precipitation are distributed along the flow path as a function of reactant fluxes and spatial distributions. Spectral induced-polarization measurements were made along the column to evaluate whether this technique can be used to track the changing position and composition of chemical reaction fronts. In parallel with the column experiment series, another extensive experimental and modeling campaign focused on the dynamics of calcium carbonate and calcium phosphate precipitation within chemical gradients controlled by diffusive mixing of reactants, in the absence of advection. These experiments were conducted within gel media. A third series of experiments examined the effect of $Ca^{2+}:CO_3^{2-}$ ion ratios on precipitation kinetics and strontium co-precipitation in constant composition solution reactors.

Currently the INL SFA is transitioning a major fraction of its efforts to another model for generating *in situ* mixing and $CaCO_{3(s)}$ mineral precipitation; specifically, we are studying the reaction fronts induced by parallel flow of reactant solutions in meso-scale 2-D flow cells with homogeneous and heterogeneous media. Simulation and experimental efforts are under way.

Urease-Mediated Precipitation of Calcium Carbonate in Porous Media Columns

INL SFA (Laboratory Research Manager: Michael McIlwain)

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As part of the Idaho National Laboratory (INL) Scientific Focus Area (SFA) an experimental campaign is being conducted to study reaction fronts within porous media where one of the reactants is generated *in situ*. The model reaction system under investigation is calcium carbonate precipitation with carbonate ion generated *in situ* by the enzymatic hydrolysis of urea. Calcium carbonate precipitation driven by microbially mediated urea hydrolysis has been proposed for a variety of applications in porous media, including ^{90}Sr remediation by coprecipitation in calcite, and improvement of the shear strength of soils. The objectives of the SFA experimental campaign are to : (1) understand how a precipitation reaction front that involves *in situ* reactant production and changes in system permeability can be propagated spatially and temporally, (2) generate data that can be used in the development of a numerical model with the capacity to simulate the tight coupling between chemical and physical processes, and (3) observe how changes in electrical resistance can be correlated to physical and chemical changes associated with the reactions.

Flow-through columns were packed with granular silica gel as the porous medium. Urease enzyme was installed in a central region of the columns, by adsorption to the silica gel, as an analog to a system where microbial urease activity has been stimulated. Solutions containing urea were introduced to first characterize the reaction front for urea hydrolysis. These were followed by solutions containing urea, calcium, and strontium. The chemical reaction fronts for urea hydrolysis and carbonate mineral precipitation were monitored by direct sampling of the aqueous phase within the column and by electrical resistance (conductivity and capacitance) measurements along the column. A pH sensitive dye that was added to the injected solutions made it possible to optically monitor pH changes in the column, using an automated image capture system with backlighting and reflected light. The mobility or sequestration of Sr^{2+} and Ca^{2+} was determined by analysis of the composition of fluid samples and by destructive sampling of the solid media at the end of the experiments. Bromide tracer tests were conducted at the beginning and end of the experiment to characterize changes in physical properties of the columns.

In general, the spatial distributions of the soluble ureolysis products and of $CaCO_{3(s)}$ were correlated with the location of the enzyme in the column. When the enzyme activity was highly concentrated within a limited zone, $CaCO_3$ precipitation was sufficient to reduce column permeability, resulting in a reduction of flow and upstream migration of the precipitation zone. In columns where the enzyme was more uniformly distributed over a larger section, the extent of the precipitation zone was correlated to flow rate (precipitation was distributed farther downstream with higher flow), but little reduction in flow was observed in spite of obvious cementation of the granular media. Precipitation appeared to be concentrated at grain-grain contact points with no apparent mineral deposition on adjacent silica surfaces. It appears that once heterogeneous nucleation occurred, growth was concentrated at the initial nucleation sites, and further nucleation is suppressed by lowering the local saturation state. Although the reacting solution (containing Ca^{2+} and HCO_3^-) appeared to be in intimate contact with $CaCO_3$ within the enzyme zone, the saturation state did not necessarily go to equilibrium; sampling of the pore fluid during the experiment showed that the solution remained supersaturated with respect to $CaCO_3$ phases within, and downstream of, the enzyme/precipitation zone.

Mineral Precipitation Mixing Zones and Chemical Gradients: Experiments with Diffusion-Controlled Mixing, Variable Ion Activity Ratios, and Model Simulations

INL SFA (Laboratory Research Manager: Michael McIlwain)

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Mixing zones for the components of a mineral precipitation reaction are characterized by gradients in the activities of the reactants. These chemical gradients determine local ion activities, activity products, and activity ratios, which through their impacts on nucleation and growth kinetics can influence the spatial distributions and physical and chemical characteristics of the solid reaction products. Furthermore, the spatial distribution of mineral products in porous media can be dynamic, since changes in porosity due to precipitation will be coupled to changes in reactant transport and therefore to the structure of the chemical gradients. This makes the linking of the fine-scale structure of precipitation reaction fronts to volume-averaged rates of reactions, as described by continuum-scale models, extremely challenging. As a practical example, one can imagine that small amounts of precipitate mass deposited in particular locations can result in solid and solution phase product distributions (as observed from field samples) that are very different from the predictions of continuum scale models that rely on volume averaging at larger scales.

We have conducted experiments in double diffusion cells where reactants (calcium and either carbonate or phosphate) mix in gel or granular media through diffusive transport. The objective is to observe transients in mineral precipitate distributions that can: (1) influence long-term outcomes of reactions in the affected region, and (2) be used to test the ability of models employing volume averaging to predict changes in local properties of the system, such as extent, rates and products of reactions. Analysis of the transient nature of the precipitation events is aided by digital time-lapse photography and spectral decomposition methods.

In the diffusion cells, precipitation band formation occurs largely as expected (analogous to Liesegang patterns). The general results for the Ca^{2+}/CO_3^{2-} system are distinctly different from the Ca^{2+}/PO_4^{3-} system. In the phosphate system, nucleation and growth of precipitate occurs in multiple narrow bands composed of relatively densely packed, fine grained material. In the carbonate system, the $CaCO_3$ crystals are distributed within a broader region, and individual crystals are more widely dispersed. Differences indicate the relative influences of rates of nucleation, growth, and diffusive transport of reactants. A fully coupled, fully implicit reactive transport simulator developed at the INL has been used to capture some of the features of the transient precipitation events observed in the experiments. In particular, the simulations have predicted changes in the width of precipitation zones and asymmetry in the chemical conditions that evolve on either side of the precipitation zone.

A separate series of experiments focusing on the influence of $Ca^{2+}:CO_3^{2-}$ ion activity ratios on the rates of $CaCO_{3(s)}$ precipitation has also been completed. These experiments were conducted in batch reactors under constant composition conditions where ion activity ratios were varied, but the saturation state was held constant ($\Omega = 12.8$). Precipitation rates were found to vary by a factor of 2 over the range of ion activity ratios studied, with the maximum precipitation rate observed for an ion activity ratio ( ) value of ~ 3.

Investigation of Spectral-Induced Polarization (SIP) Signatures of Hydroxide Adsorption and Mineral Precipitation in Porous Media

INL SFA (Laboratory Research Manager: Michael McIlwain)

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There is growing interest in the application of spectral-induced polarization (SIP) for noninvasive detection and characterization of spatiotemporal variations in the physicochemical properties of the grain surface-fluid interface of porous media that result from biogeochemical processes, e.g., microbially enhanced mineral precipitation. The SIP technique measures the magnitude and phase shift (φ) of the received voltage relative to the injected current over a selected frequency range and provides information on the properties of both the pore fluid chemistry and the grain surface.

As part of the geophysical component of the Idaho National Laboratory (INL) Scientific Focus Area (SFA), the ability of SIP to provide qualitative and quantitative information on the evolution of calcium carbonate precipitation driven by enzymatically mediated urea hydrolysis in porous media columns has been evaluated. The application of geophysical sensing could facilitate an improved understanding of the chemical reaction fronts associated with *in situ* urea hydrolysis and carbonate mineral precipitation in porous media, as well as provide a valuable tool for noninvasively monitoring the progress of these reactions in an engineering application.

In a flow-through column experiment, extracellular urease was immobilized in a defined region within a matrix of highly porous silica gel (surface area ~500 m^2/g). Chemical reaction fronts associated with ureolytically driven calcite precipitation were monitored by simultaneous SIP measurements and direct sampling of ion concentrations, pH, and fluid conductivity along the column. We observed distinct phase shifts (φ) (4 ± 0.5 mrad) associated with changes in hydroxyl ion concentration during urea hydrolysis in the enzyme zone, with phase decreases when carbonate mineral precipitation occurred. In a separate study focused on the reactions of the hydroxyl ions, SIP and geochemical measurements were made on a saturated column composed of sequential zones of Ottawa sand and silica gel in order to: (1) observe whether adsorption of hydroxyl ion contributes to changes in complex conductivity, and (2) to determine whether changes in solution chemistry follow changes in surface chemistry in porous media (or vice versa). A column was packed with Ottawa sand in both the bottom and top sections, and silica gel in the middle part of the column. The experiment started with a pH 7 NaCl solution (50 mM) flowing through the column at 10 ml/min, followed by higher pH NaCl solutions (pH 8 and pH 10) applied at the same rate. Significant phase increases (from 3.5 to 4.5 mrad) were observed in the silica gel coinciding with an increase in the pH of the influent. The pH of the pore fluid in the sand sections increased with increased influent pH, but no significant changes of pH in the silica gel were observed, due to its high hydroxide adsorption capacity. The diffusive relaxation time and chargeability increased, but subsequently decreased. Although changes in complex conductivity were only observed on synthetic high surface area material, this work encourages further investigation on the correlation between complex conductivity and surface chemistry, and may ultimately provide valuable noninvasive information on carbonate precipitation reaction fronts.

LBNL Sustainable Systems Scientific Focus Area

LBNL SFA (Laboratory Research Manager: Susan Hubbard)

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The scale and complexity of the DOE stewardship responsibility motivates the LBNL Sustainable Systems Scientific Focus Area (SFA) overarching mission, which is to improve predictive understanding of subsurface flow and transport relevant to remediation and long-term environmental stewardship. Our SFA is organized around the following three key challenges, where fundamental (hydrological, biological, geochemical) processes, their couplings, and their macroscopic manifestations are quantified as is needed to improve our predictive understanding of flow and transport processes in heterogeneous and hierarchical subsurface systems.

1. The “Unraveling Biogeochemical Pathways” Challenge focuses on quantifying critical and interrelated microbial metabolic and geochemical mechanisms. One key scientific goal is to determine if there are diagnostic biomolecular signatures indicative of important aquifer biogeochemical processes that can be used to (1) help discriminate between direct (enzymatic) and indirect (abiotic) oxidation-reduction processes relevant to bioremediation and (2) inform and constrain reactive transport models even when geochemical field measurements do not reveal all relevant processes. The four hypotheses associated with this Challenge focus on chromium *in situ* reductive immobilization and reoxidation processes and are being advanced in parallel with field-scale biostimulation experiments at the chromium-contaminated Hanford 100 Site.
2. The “Evolution of Pore Structures and Flowpaths” Challenge focuses on developing a predictive understanding of couplings and feedbacks between microbially facilitated biogeochemical transformations and aquifer flow characteristics, the impact of such feedbacks on overall system behavior, and the discovery of diagnostic signatures of critical system transitions or “tipping points.” The three hypotheses associated with this Challenge are being advanced through integrated tasks that are aligned with and leverage on field experiments that are being conducted at the uranium-contaminated Rifle, Colorado, site by the Rifle IFRC team.
3. The “Predicting Contaminant Mobility at the Plume Scale” Challenge explores the impact of a migrating pH gradient and the use of a “reactive facies” concept as an organizing principle to integrate laboratory and field information about properties and mechanisms as needed to make reliable and computationally tractable predictions of U and iodine mobility at the plume scale and over long timeframes. This Challenge also explores the necessary level of detail required for adequate predictions of plume mobility. Four hypotheses are tested through interrelated laboratory, field, and numerical experiments conducted at the contaminated Savannah F-Area, where enhanced attenuation is a desired closure strategy. The pore-to-plume scale research is being carried out in collaboration with EM-supported SRNL scientists and the Advanced Simulation Capability for Environmental Management (ASCEM) team.

Several crosscutting scientific themes and a common investigative approach facilitate fertilization across the Sustainable Systems SFA. Recognition of subsurface complex subsurface behavior (which includes emergent processes and feedbacks) is common to all of the SFA challenges, which motivates the quest to explore fundamental processes and their interactions, as well as to identify diagnostic (or integrative and often macroscopic) signatures of system responses. Successively larger-scale transitions are considered in each of the three SFA Challenges, which span molecular to plume scales. The SFA investigative approach iterates between laboratory- and field-scale experimentation and observation, hypotheses testing and refinement, all within the context of the reactive transport model. Reactive transport modeling plays an integrative role in the SFA: it allows for transfer of parameters, concepts, and processes across scales, providing a link between the fundamental and system-level research, and it is used as a means to formally address emergent phenomena as a result of fundamental process coupling and the hierarchical nature of the complex subsurface. Improving the predictive understanding of subsurface system behavior is pivotal for sustainable management of subsurface resources and problems; it represents a critical need for environmental science, DOE environmental stewardship, and other DOE mission areas.

Flow-through Column Experiments and Modeling of Cr(VI) Reduction Under Various Electron-Accepting Conditions at Hanford 100H

LBNL SFA (Laboratory Research Manager: Susan Hubbard)

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Remediation of chromium contamination frequently involves reduction of the toxic and soluble hexavalent form, Cr(VI), to the relatively harmless and mostly immobile trivalent state, Cr(III). Microbially mediated Cr(VI) reduction at the Hanford 100H area was investigated by flow-through column experiments. The objective of this study was to identify the biogeochemical reactions that control *in situ* chromium reduction under different dominant electron-accepting conditions, i.e., nitrate-, Fe(III)-, and sulfate-reducing conditions. Replicate columns packed with natural sediments from the site were eluted with artificial groundwater containing lactate (5 mM) and Cr(VI) (5 mM) and kept under anaerobic conditions. Sulfate and nitrate solutions were added to selected columns to promote specific electron-accepting pathways. Iron and manganese oxides were present in all the column sediments. Effluent concentrations of substrates and metabolites were analyzed regularly over a 1-year period using ICP-MS and IC. X-ray absorption spectroscopy was used to analyze the Cr phases formed during the experiment, while pure culture Hanford isolates were investigated under batch conditions to gain a detailed understanding of microbial metabolism. Biogeochemical reactive transport modeling was performed to link the various experimental results and gain further insights into the reaction mechanisms and Cr(VI) bioreduction rates.

In the nitrate-added columns, a strong correlation between denitrification and Cr(VI) reduction processes was observed and was in agreement with the results obtained in batch experiments with a denitrifying bacterium isolated from the Hanford site that have shown that Cr(VI) reduction is cometabolically linked to denitrification.

Two of the sulfate-added columns evolved to complete lactate fermentation to acetate and propionate. These fermenting columns showed a complete removal of injected Cr(VI), visible precipitation of sulfide minerals, and a significant increase in effluent Fe and Mn concentrations. Cell suspensions with a fermentative bacterial isolate that was the dominant bacterium in these columns support that this strain may be directly responsible for Cr(VI) reduction.

Analysis of micro X-ray absorption spectra showed a shift in solid state Cr from primary minerals (e.g., chromite and/or Cr-bearing micas in the untreated sediments) to mixed phase Cr-Fe hydroxides, i.e., $Cr_{1-x}Fe_x(OH)_3$. The new Cr phases were most abundant in both the denitrifying and fermenting columns, but were also seen in the low-lactate-utilization columns. The spectral similarity of the new Cr phases from the different electron-accepting conditions implies a strong structural similarity between the precipitated Cr phases.

Overall, experimental and modeling results suggest that Cr(VI) reduction was most efficient under fermentative conditions. In these columns, Cr(VI) reduction occurred through a complex network of microbial reactions that included fermentation, sulfate reduction, and possibly Fe(III) reduction. Cometabolic Cr(VI) reduction in the denitrifying columns was less efficient on an electron-equivalent basis, with model simulations suggesting that biomass growth completely depleted influent ammonium, which could explain a change in observed reduction rates.

Metagenomic and Meta-Transcriptomic Analysis of Chromate-Reducing Aquifer Microbial Communities from the Hanford 100H site

LBNL SFA (Laboratory Research Manager: Susan Hubbard)

H.R. Beller, H.C. Lim, R. Han, U. Karaoz, E.L Brodie (PI)—*LBNL*

We are developing the use of biomolecular signatures as part of the LBNL SFA challenge entitled "Unraveling Biogeochemical Reaction Networks Mediating Sustained Chromium Reduction", which focuses on *in situ* reductive immobilization of Cr at DOE's Hanford 100H site. By applying a high-throughput approach that uses metagenomic and meta-transcriptomic data, we hope to identify highly expressed genes in a specific microbial community under conditions of interest, without requiring any *a priori* sequence information or assumptions about what processes might be occurring. A key scientific goal is to determine if there are diagnostic biomolecular signatures indicative of important aquifer biogeochemical processes that can be used to (a) help discriminate between direct (enzymatic) and indirect (abiotic) oxidation-reduction processes relevant to bioremediation and (b) to inform and constrain reactive transport models even when geochemical field measurements do not reveal all relevant processes. We are in the process of collecting metagenome and metatranscriptome sequence information from various experimental systems (including flow-through columns and batch microcosms inoculated with Hanford aquifer sediment or groundwater, bacterial isolates from the flow-through columns, and biomass present in groundwater) under conditions relevant to *in situ* chromate reduction at Hanford 100H. Here, we focus on Hanford microcosm studies.

To characterize functional changes in an aquifer-derived, chromate-reducing microbial community as it transitions successively through electron-accepting conditions relevant to the Hanford subsurface, we inoculated anaerobic microcosms with groundwater from the Cr-contaminated Hanford 100H site and supplemented them with lactate and electron acceptors present at the site (e.g., nitrate, sulfate, and Fe(III)). Metagenomic "snapshots" were taken during denitrification, sulfate and Fe(III) reduction, and nitrate-dependent oxidation of Fe(II) and reduced S compounds. We conducted Illumina paired-end sequencing, assembled with abyss-pe and minimus2, and initially annotated using MG-RAST. cDNA samples for metatranscriptome sequencing represented mRNA enriched using a new RT-subtractive hybridization method.

Overall sequences in contigs greater than 150 bp totaled 15 to 30 Mb, with N50s ranging from about 600 to 2600 bp and maximum contig lengths between 35 to 100 kb. Based on metagenome data, both phylogenetic and subsystem diversity were lowest in the initial denitrification phase (with lactate as the electron donor) as compared to other electron-accepting conditions. As expected, denitrification genes were enriched during this period with *ß-Proteobacteria* (*Comamonadaceae, Rhodocyclaceae*) dominating and novel ß-proteobacterial *nir*S transcripts detected in the meta-transcriptome. Sulfate reduction-associated genes were enriched over time with *Firmicutes* (*Peptococcaceae*) dominating. Divergent *dsr*A/B transcripts were also detected. Genes associated with lactate and pyruvate metabolism were also detected in both the metagenome and meta-transcriptome.

Regarding identification of site-specific biomolecular signatures, the observation of divergent sequences for important functional genes suggests a potential advantage of this approach—namely, that it can identify important genes that may not be detected using more traditional characterization techniques, including PCR-based methods and *a priori* functional microarrays.

Isotopic Studies of Microbial Processes Affecting Chromate Reduction in Groundwater at the Hanford 100H Site

LBNL SFA (Laboratory Research Manager: Susan Hubbard)

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Hexavalent chromium, Cr(VI), is a widespread, toxic, carcinogenic, and mutagenic groundwater contaminant. As part of the LBNL Sustainable Systems SFA, we are examining the potential for *in situ* microbial reduction of Cr(VI) to relatively insoluble Cr(III) as a means of Cr immobilization in groundwater at DOE's Hanford 100H site. Stimulation of the native microbial community was initially tested using HRC, a commercially available, slow-release, poly-lactate compound. Following encouraging results, the initial injection was followed by a second, smaller HRC injection. Over the past year, three lactate injections have been conducted at the 100H site as part of an effort to better understand the complex biogeochemical reaction networks that mediate chromate reduction in the Hanford 100H subsurface.

Parallel to the field experiments, a series of anaerobic, flow-through column experiments were conducted using 100H sediments and artificial Hanford groundwater containing lactate and various electron acceptors relevant to the site (e.g., nitrate or sulfate). As a part of these studies, isotopic analyses were conducted to track the progress of microbial reactions and the extent of Cr(VI) reduction. These included using lactate with both natural-abundance and ^{13}C-enriched isotopic compositions to track the fate of the electron donor in both laboratory and field experiments. In addition, the δ^{53}Cr of dissolved chromium was monitored to identify differences in Cr isotope fractionation potentially related to different microbial metabolic processes.

For the column studies, a 2-week pulse of ^{13}C-labeled lactate was added to the columns to quantify low levels of activity (<1% lactate respiration) in some of the columns. In addition, throughout the 18-month duration of the column experiments, δ^{13}C values of DIC (Dissolved Inorganic Carbon) and DOC (Dissolved Organic Carbon) and δ^{53}Cr values of chromium were monitored to examine changes in the isotope signatures related to changing metabolic conditions (e.g., the onset of sulfate reduction). Cr isotopic fractionation occurred under each of the column conditions, but there were differences in fractionation factors. The observed range of fractionation was between 1 and 3‰, with one column (sulfate added - nonfermentative) switching from 1‰ to 3‰ after ~25 days. Under denitrifying conditions, 2‰ fractionation was observed. Under fermentative conditions, Cr fractionation stabilized at 1‰. These results are being compared to pure-culture studies with bacteria isolated from the Hanford 100H aquifer.

During field experiments, ^{13}C-labeled lactate was added to the groundwater to stimulate microbial activity. In all cases, the lactate was quickly consumed. Dissolved oxygen and nitrate in the groundwater (background nitrate concentrations ~0.4 mM) were quickly depleted following lactate addition, and the δ^{13}C of DIC in the groundwater increased to values approximating the δ^{13}C of the ^{13}C-labeled lactate added to the groundwater. The remaining lactate was fermented to form acetate and propionate despite the presence of sulfate at near background levels (~0.6 mM). This slow response of sulfate reducers was observed in earlier field experiments and in the laboratory column experiments. In addition, dissolved methane in isotopic equilibrium with the injected lactate was observed in the injection well, indicating zones of highly reducing conditions within the aquifer.

Geochemical, Biological and Geophysical Processes Accompanying Electron Donor Addition to Rifle, Colorado Aquifer Sediments during a Large-Scale Column Experiment

LBNL SFA (Laboratory Research Manager: Susan Hubbard)

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We present initial results from a large-scale column experiment designed to bridge the gap between field-scale experiments and small column experiments and advance our understanding of the isotopic and geophysical signatures of bioremediation geochemistry. Field-scale experiments generally have difficulty achieving adequate sampling and characterization, whereas small-scale flow-through experiments miss important heterogeneity and larger scale effects. Our study also seeks to test the hypothesis that the cumulative impacts of remediation-induced biogeochemical transformations will lead to changes in flow characteristics that impact remediation efficacy.

The 1 m long, 10 cm diameter column was packed with previously unamended sediments from the Rifle aquifer and scaled to the same average pore-water velocity as the field site to provide a direct analog to the first meter downgradient from an injection gallery at the Rifle site. An artificial groundwater solution was flowed though the column for 116 days to establish steady state prior to addition of 10 mM ^{13}C-labeled acetate for an additional 43 days. Aqueous samples were collected daily from the column effluent and from a series of side ports fitted along the length of the column at 10, 30, 50, 70 and 90 cm from the influent. Measurements included pH, conductivity, major anions and cations, total dissolved organic and inorganic carbon, Fe(II) and HS^-, stable isotopes of hydrogen, oxygen, sulfur, calcium and carbon, radiogenic isotopes of strontium and uranium, and extractions of DNA and RNA from planktonic and sediment-associated microorganisms for analysis of microbial community composition and activity. Solid-phase analyses included destructive sampling of sediments for total metals, organic and inorganic carbon, sulfide and elemental sulfur, and isotopes of sulfur, calcium, and carbon. Both rigid sintered-glass porous beads and micro-columns packed with Rifle sediment were imaged using x-ray micro-tomography before insertion and after recovery to quantify changes in porosity due to mineral precipitation. To monitor changes in permeability and porosity, multiple falling head and breakthrough curve measurements were performed during the experiment. In addition, electrodes were installed along the length of the column for geophysical monitoring of changes in complex resistivity signals.

Preliminary results indicate successful reproduction of biogeochemical reactions observed during field-scale amendments. Iron reduction was detectable 4 days after acetate addition, followed by sulfate reduction at 18 days. Sulfur isotopes show an average fractionation factor of 12‰, and cation concentrations indicate substantial carbonate precipitation. Conservative tracer breakthrough curves correspondingly indicate a drop in porosity of ~8% over the course of acetate injection. Electrical resistivity data agree nicely with measured fluid conductivity, and the changes of phase and imaginary conductivity correlate well with iron and sulfate reduction phases, confirming complex resistivity signal as a proxy of system transitions. Overall, planktonic microbial biomass (DNA) peaked at 3 weeks of acetate injection; however, analysis of individual side ports showed that activity (RNA) was bimodal, with a first peak at 3 weeks followed by a subsequent increase at the 50 and 70 cm ports at week 7.

Understanding the Multiscale Dynamics of Biostimulation-Induced Clogging in the Near-Well Bore Environment

LBNL SFA (Laboratory Research Manager: Susan Hubbard)

L. Yang, J. Ajo-Franklin, S. Molins-Rafa, D. Silin, A. Van Hise, K.H. Williams (PI), C.I. Steefel, S.S. Hubbard—*LBNL*

Previous work at the Rifle IFRC site has suggested that large reductions in permeability accompanying organic carbon addition can greatly impact the region within or immediately adjacent to the injection boreholes. To understand this extreme near-wellbore clogging, a study focusing on the characterization and modeling of near-wellbore clogging associated with the 2008 Rifle biostimulation experiment was initiated and is in progress. To sample the physical and sediment geochemical properties of the impacted wellbore system, one of the injection wells (CD-52) was overcored following the 2008 campaign. The field samples are being supplemented by laboratory experiments characterized with 3D microtomography and interpreted with pore-scale modeling to understand and quantify the physical and geochemical changes associated with biostimulation.

Analysis of the overcored material (annulus as well as nearby sediments) includes identification of secondary minerals using conventional and synchrotron x-ray diffraction along with other synchrotron-based methods (microXANES) and quantification of both secondary minerals (e.g., total inorganic and organic carbon, total sulfide, and elemental sulfur) and biomass (e.g., function-specific qPCR and phospholipid fatty acid content). Initial efforts have focused on the relatively clean overcore sand that forms the annulus of the wellbore, since secondary mineral products and biomass found in this zone can be considered as the result of biostimulation. Preliminary analyses indicate solid-phase Total Inorganic Carbon (principally calcite) and Total Organic Carbon (biomass) averaging 0.1% and 0.2%, respectively. These data supplement geophysical logging used to determine mineral accumulation (via changes to formation density) as a means for more mapping the extent of pore clogging over a larger area in 3D. Estimates of total Eubacterial biomass within the recovered annual material ranged from 3E07 to 8E08 cells/gram, with iron- and sulfate-reducing bacteria and methanogens constituting the predominant phylogenic groups. In all samples analyzed, qPCR resilts indicate that methanogens were the more abundant phylotype. These results corroborate detection of methane within adjacent wellbores and suggest occlusion of pore throats by entrapped methane bubbles may supplement biomass and mineral precipitation in reducing local permeability. Assessment of time-lapse water level, temperature, and well-test data collected within the injection wells were performed to quantify the hydrodynamics associated with wellbore clogging. Time-lapse geophysical tomography were used to image across injection holes during field experimental perturbations and these show a highly preferential release and flow of tracer into the surrounding annulus and sediments.

The data from the time-lapse geophysical survey and wellbore geochemical and microbiological characterization are being used as constraints in mesoscale continuum reactive transport modeling (~10 cm to 1 m scale). This modeling in turn builds on a set of experiments and modeling conducted at the pore scale that include flowthrough acetate amendment experiments characterized by 3D microtomography. These experiments will be interpreted with a newly developed pore-scale modeling approach based on Direct Numerical Simulation that accounts for dynamic interactions between fluid flow, pore space evolution, and reactive transport. Simulations on small-scale geometries show that dissolution and precipitation reactions affect the pore space non-uniformly, and a simple porosity-permeability correlation may not describe the complex dynamics of the pore space evolution.

Quantifying the Impact of Biogeochemical Transformations on Aquifer Flow Properties: Field Studies at the Rifle IFRC Site

LBNL SFA (Laboratory Research Manager: Susan Hubbard)

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Research activities conducted as part of the Lawrence Berkeley National Laboratory's (LBNL) Sustainable Systems Science Focus Area (SFA) at the IFRC site in Rifle, CO, are designed to integrate geochemical, geophysical, hydrological, and modeling studies to enhance our understanding of dynamic pore scale processes and their impact on aquifer properties and sustainable remediation practices. Performed in 2010, the Rifle "Super 8" field experiment was designed to investigate uranium mobility during acetate amendment and under conditions of varying alkalinity. A variety of experimental approaches were used, including introduction of reactive and conservative amendments (e.g., sodium acetate, sodium bicarbonate, sodium bromide, D_2O), groundwater sampling for geochemical and microbial constituents, lithological characterization, geophysical and hydrological wellbore testing, and surface and cross-borehole electrical resistivity/induced polarization monitoring.

Injection wells were configured such that downgradient locations could selectively be exposed to sodium acetate (ca. 5 mM), sodium bicarbonate (ca. 40 mM) or a combination of the two. Distinct conservative tracers (NaBr and D_2O) were used to discriminate the acetate and bicarbonate plumes within the flowcell. LGR, Inc.'s Liquid Water Isotope instrument, was used to quantify D_2O breakthrough, with this work supported jointly by DOE's SBR program and LBNL's SFA research program. To assess the fate of bicarbonate and acetate in groundwater, the abundances and $d^{13}C$ values of total dissolved inorganic carbon (DIC) were monitored. The DIC in monitoring well CU04-6 (unimpacted by acetate) was slightly depleted relative to the deuterium tracer, possibly indicating some loss of bicarbonate to precipitation of carbonate minerals. In CD-02 (impacted solely by acetate), the concentration of DIC increased by ca. 3 mM relative to background, and the $d^{13}C$ values decreased by 6‰ following injection of acetate, indicating input of inorganic carbon from microbial respiration of acetate. Monitoring wells impacted by both the bicarbonate and acetate injections exhibited intermediate effects.

Electrical resistivity tomography (ERT) data collected from the surface and between boreholes were used to track the spatiotemporal distribution of conductive injectates within the aquifer. Borehole induced polarization (IP) data were used to delineate regions of mineral precipitation (e.g., calcite) and microbial activity accompanying the bicarbonate and acetate injections. The anomalous IP response was most pronounced in regions impacted solely by bicarbonate. The response was generally consistent with laboratory measurements performed as part of the LBNL SFA program, which have documented large increases in the phase response during calcite precipitation. The effect is attributed to temporal variations in the calcite surface charge structure as the Ca-$CaCO_3$ system transitions from one of disequilibrium to equilibrium. Such results can supplement and extend aqueous geochemical data obtained from monitoring wells to regions where direct samples do not exist. Information regarding the spatial distribution of the electrical field and its relation to conductive tracers and mineral precipitates can supplement reactive transport models and inform the heterogeneous delivery of injectates with the aquifer. Although hydrological impacts were minimal during the 2010 experiment, the prolonged amendment period planned for 2011 (and its presumed negative impact on flow properties) represents an excellent opportunity to assess the now-validated LBNL SFA methodologies undertaken as part of the "Super 8" field experiment.

Estimation and Modeling Approaches for the Improved Understanding of Subsurface Properties and Processes Associated with Uranium Biostimulation

LBNL SFA (Laboratory Research Manager: Susan Hubbard)

J. Chen, M. Kowalsky—*LBNL*; L. Li, *Penn State U.*; K. Williams, C.I. Steefel, S.S. Hubbard (PI)—*LBNL*

In this work, we have developed approaches for integrating a variety of hydrological, geochemical, and geophysical data to improve the understanding of subsurface properties and processes occurring during biostimulation at a uranium-contaminated site in Rifle, Colorado. The site was formerly a uranium ore processing facility and is now a U.S. Department of Energy (DOE) Integrated Field Research Challenge (IFRC) site. In recent years, it has been the subject of several biostimulation experiments in which an electron donor (acetate) was introduced into the contaminated groundwater, with the expectation that it would react with competing electron acceptors, including U(VI), Fe(III), and sulfate. The goal of bioremediation at the site is to facilitate the reduction of U(VI) to U(IV) by Fe-reducing bacteria, thereby immobilizing uranium and preventing its transport to the nearby Colorado river. The sequence of biogeochemical reactions, the structure of microbial communities, and the nature of mineral precipitates and biomass that accumulate during biostimulation have been examined. However, the role that the heterogeneity of physical, geochemical, and biogeochemical properties plays in uranium bioremediation is poorly understood, and it constitutes a key component of the LBNL SFA "Evolution" Challenge.

In the first of three complementary studies, we consider hydrological inverse modeling of bromide tracer test data collected during biostimulation experiments to obtain 2D and 3D estimates of the permeability, and estimates of the porosity (assumed uniform). Several parameterization techniques, including a geostatistical approach and an approach that allows for additional characterization data to be incorporated, are tested and applied to the field data. The second study uses multiple realizations of the estimated permeability, along with borehole aqueous geochemical data, to explore how small-scale (modeling scale at tens of centimeters) properties and rates impact overall field-scale (tens of meters) remediation efficacy. The spatial distribution of permeability and solid-phase mineral (i.e., Fe(III)) distribution are found to play a critical role in uranium bioreduction at the field scale. Because the spatial and temporal distribution of aqueous geochemistry and terminal electron acceptors may provide additional constraints (over borehole geochemical measurements) for improved reactive transport modeling, in the third study, we focus on developing a hierarchical Bayesian model to estimate the spatio-temporal distribution of aqueous geochemical parameters associated with *in situ* bioremediation, using surface spectral induced polarization (SIP) data and borehole geochemical measurements. The SIP data are first inverted for Cole-Cole parameters, including chargeability, time constant, zero-frequency resistivity, and dependence factor, at each pixel of 2D grids, using a previously developed stochastic method. The inverted Cole-Cole parameters are then linked to borehole aqueous measurements for deriving possible petrophysical models. Overall, the approaches we present are leading to a better understanding of the feedbacks between physiochemical heterogeneity and bioremediation at the field scale.

Adsorption of U(VI) onto F-Area Sediments from the Savannah River Site

LBNL SFA (Laboratory Research Manager: Susan Hubbard)

W. Dong, J. Wan (PI), T. Tokunaga—*LBNL*; M. Denham, *SRNL*; J. Davis, *LBNL*

The Savannah River Site (SRS) was a major DOE facility for plutonium production during the Cold War. Waste plumes containing low-level radioactivity and acidic waste solutions were discharged to a series of unlined seepage basins in the F-Area of the SRS from 1955 to 1989. Although the site has gone through many years of active remediation, the groundwater remains acidic, and the concentrations of U and other radionuclides are still significantly higher than their maximum contaminant levels (MCLs). The overall objective of the multidisciplinary LBNL/SFA research team is to understand the current and predict the future contaminant fate and transport in the F-Area, SRS. As part of this effort, we completed two tasks within this review period. (1) Obtain data on U(VI) adsorption onto representative F-Area sediment samples through laboratory equilibrium experiments. (2) Develop site-specific surface complexation models (SCMs) that can describe U(VI) adsorption behavior in the F-Area, SRS. These models are needed for the planned plume-scale reactive transport modeling.

Laboratory batch experiments were conducted to evaluate U adsorption behavior over the pH range of 3.0 to 9.5. Ten sorbent samples were selected, including six contaminated sediment samples from three boreholes drilled within the plume and along the groundwater flow direction, two uncontaminated (pristine) sediment samples from a borehole outside of the plume, and two reference minerals, goethite and kaolinite (previously identified as the dominant minerals in the clay-size fraction of the F-Area sediments). Sorption experiments show that goethite and kaolinite largely control U partitioning behavior, and thus are the minerals useful for identifying reactive facies in these sediments. The batch experiment data show that in comparison with the pristine sediment samples, U(VI) adsorption onto contaminated sediments have shifted adsorption edges toward lower pH by about 1.0 unit at acidic pH conditions (from pH ≈ 4.5 to pH ≈ 3.5).

Our modeling results show that using existing SCMs of U(VI) adsorption onto goethite and kaolinite, a component-additivity approach successfully predicted U(VI) adsorption onto uncontaminated SRS sediments. However, application of the same SCMs to contaminated sediments resulted in underestimates of U(VI) adsorption at acidic pH conditions. These results suggested that the long-term acid-leaching process might have altered the surface properties of the original sediments. The model sensitivity analyses pointed out possible alterations caused by acid-leaching including: (1) mineral surface charge (e.g., PZC of goethite); (2) new stronger adsorption sites (e.g., formation of stronger reactive surfaces or ternary surface complexes); and (3) exposure of more mineral surfaces, thereby increasing the number of available surface sites. In addition, a nonelectrostatic, two-reaction surface complexation model with generic binding sites was calibrated to describe the average U(VI) adsorption behavior of the contaminated sediment samples. Further studies are needed to understand the effects of acid leaching on the surface reactivity of the sediments.

Geophysical Reactive Facies Characterization, Isotopes, and Reactive Transport Modeling at the Savannah River Site, F-Area

LBNL SFA (Laboratory Research Manager: Susan Hubbard)

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Developing a predictive understanding of subsurface contaminant plume evolution and natural attenuation capacity is hindered by the inability to tractably monitor and characterize controlling flow and transport properties over field-relevant scales. To this effect, the concept of reactive facies is being explored as a means to improve the reliability of reactive transport models for predicting the migration of contaminants at plume scales. The reactive facies concept builds on the nonrandom distribution of lithologic facies associated with depositional processes and aims at identifying different groups of sediments that have unique and linked distributions of properties that influence reactive transport (such as effective surface area, mineralogy, texture, and permeability). Because geophysical attributes can often be used to distinguish various sediments, the aim is also to use geophysical methods to identify and spatially distribute reactive facies and their associated parameters.

We test the reactive facies concept within the uranium and acid-contaminated Atlantic Coastal Plain sediments that underlie the Savannah F-Area Basin, in South Carolina. At this site, we have collected and analyzed historical data, core samples, geophysical well logs, and crosshole ground-penetrating radar and seismic datasets, and have assessed their associated properties and relationships. Our analysis suggests that there are two reactive facies that have unique mineralogy, texture, surface area, and geophysical signatures, and that the combination of GPR attenuation, GPR velocity, and seismic velocity provides the most useful information about reactive facies distribution at the F-Area. We use the developed correlations within a Bayesian framework to integrate the dense geophysical information with the sparse core-based measurements.This procedure yields high resolution (0.25 m by 0.25 m) estimates of reactive facies and their associated uncertainties along the 2D tomographic transects at the local field scale. Our comparison of the geophysically obtained reactive facies spatial distribution with independent laboratory and field data suggests that the method is a reasonable approach. Comparison of well logs, crosshole, and recently collected surface seismic and electrical datasets also suggests the potential for extending the local-scale reactive facies estimation methodology that we describe here to more plume-relevant scales, as is needed to parameterize reactive transport models for prediction of plume evolution.

Isotopic studies are being conducted to complement laboratory and geophysical investigations of reactive facies, and to investigate flow patterns and potential preferential flow pathways beneath the site. Vertically within the plume, both chemical variation and systematic isotopic variations (U isotopes, $^{87}Sr/^{86}Sr$, $d^{15}N$-$d^{18}O$ of nitrate) with depth delineate upper (0-15ft) and lower (15-35 ft) zones within the upper aquifer above the Tan Clay. Together, the data suggest that the lower zone represents the effect of the original contamination plume, while the upper zone represents mostly the inflow of fresher water contaminated by infiltration from the contaminated vadose zone. This model will be tested with stable isotope analysis of depth-discrete water samples. As the plume expands laterally, we have been able to observe the early effect of contamination at well FAW-1, with the greatest extent of contamination at the top of the aquifer, but decreasing with depth. Over a two-year period, significant U isotopic shifts were observed at several wells, indicating migration of U from up-gradient portions of the plume.

Exploratory geochemical and reactive transport modeling is used to assess reactions controlling pH at this site, and effects of heterogeneities in permeability and distribution of reactive minerals on pH and U migration behavior. Investigated mechanisms include the sorption and/or exchange of H+ with the sediments, as well as the precipitation of Al silicates, hydroxides, and sulfates. Simulations indicate that H+ sorption/exchange could buffer pH at the site for long periods of time. Al mineral reactions could also strongly buffer pH. Simulations results are sensitive to the relative rates of mineral precipitation and dissolution, to the relative rates of reaction versus recharge, and to the type of H+ and U sorption models and parameters.

A New Method to Attenuate U(VI) Mobility in Acidic Waste Plumes Using Humic Acids

LBNL SFA (Laboratory Research Manager: Susan Hubbard)

J. Wan (PI), W. Dong, T.K. Tokunaga—*LBNL*

Acidic uranium (U) groundwater plumes have resulted from acid-extraction of plutonium during the Cold War and from U mining and milling operations. A sustainable method for *in situ* immobilization of U under acidic conditions is not yet available. Here, we propose to use humic acids (HA) for *in situ* U immobilization in acidic waste plumes. Using Savannah River Site (SRS) and Oak Ridge Site (ORS) sediments, batch equilibrium experiment results show that HA adsorption onto sediment surfaces is quick, strong, and practically irreversible at $pH < 5.0$. The mineral-adsorbed HA strongly complexes and effectively immobilizes U(VI) under acidic conditions. Results from column experiments show that injection of HA into contaminated SRS sediments caused rapid U immobilization. Subsequent leaching with 100 pore volumes of simulated groundwater over 200 days at pH 3.5 to 6.0 did not release detectable concentrations of U. In comparison, control columns under identical conditions (without HA addition) released 95% and 42% of the total inventory at pH 3.5 and 4.5, respectively.

These results demonstrated that HA-treatment has potential as an *in situ* remediation method for immobilizing U(VI) in acidic plumes. As proposed remediation reagents, HA (refractory decomposition product of biological materials, dead plants and organisms) are very resistant to further biodegradation and persistent in the environment. HA are nontoxic, abundantly extractable from many environments, and are water-soluble, so that they can be easily introduced into the subsurface. Additional research is needed, including determining long-term stability of the immobilized uranium, and the effects of competing ions, such as Ca^{2+}, Mg^{2+}, Al^{3+}, and NO_3^-, which can be present at elevated concentrations in some plumes. An *in situ* field test needs to be conducted to further test this method.

Environmental Transport of Plutonium: Biogeochemical Processes at Femtomolar Concentrations and Nanometer Scales

LLNL SFA (Laboratory Research Manager: Annie B. Kersting)

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This SFA program is designed to better understand the biogeochemical processes that control plutonium (Pu) transport at environmentally low concentrations and field scales. The major challenge in predicting the mobility and transport of Pu is determining the dominant geochemical processes that control its behavior in the subsurface. The reaction chemistry of Pu (i.e., aqueous speciation, solubility, sorptivity, redox chemistry, and affinity for colloidal particles, both abiotic and microbially mediated) is particularly complicated. Its migration is known to be oxidation-state dependent and facilitated by transport on particulate matter (i.e., colloidal particles). Despite the recognized importance of colloid-facilitated transport, little is known about the geochemical and biochemical mechanisms controlling Pu-colloid formation and association.

The objective of this program is the identification and quantification of the biogeochemical processes that control the fate and transport of Pu at picomolar to attomolar (10^{12}–10^{18} mol/L) concentrations. Our program is composed of five research elements that are aligned with processes likely to affect Pu transport at environmentally relevant concentrations: (1) binary sorption to low-site-density, high-affinity surface sites (e.g., surface defects), (2) stabilization of Pu surface complexes on mineral colloids by natural organic matter, (3) surface precipitation of Pu polymers (nanocolloids), (4) co-precipitation with colloids as a result of mineral alteration, and (5) direct and indirect microbial interactions with Pu and colloids.

Here, we highlight this year's results and next year's effort. Accomplishments include:

- Successful measurement of Pu at ultra-trace levels using CAMS. Pu-goethite sorption experiments were performed to aqueous concentrations below 5×10^{-17} M, equivalent to the detection of 3×10^{6} atoms in a 100 mL sample. This CAMS capability will allow us to readily perform laboratory experiments that cover the range of environmental Pu concentrations of interest.
- Demonstrated Pu detection using NanoSIMS at concentrations as low as 0.7 ppm Pu on goethite. This is well below the concentrations at which Pu nanocolloids were observed by TEM and surpassed expectations outlined in the SFA proposal.
- Identified Pu nano-colloids lattice distortion when sorbed to goethite resulting in the formation of a bcc, Pu_4O_7 structure. This distortion results from an epitaxial growth of colloidal plutonium on goethite and provides new insight into the molecular-scale behavior of Pu and how that may facilitate its transport in the subsurface.
- Imaged nanometer-scale Pu distribution on Hanford sediments located 60 feet beneath a disposal crib using the NanoSIMS. Additional work to identify the mineral association of Pu will greatly help us understand how Pu moves in the subsurface.
- •Applied *ab initio* modeling to the dominant Pu monomer under neutral solution conditions ($Pu(OH)_4$) and benchmarked a range of theories in preparation for binary sorption calculations.
- Cultured bacteria from radiologically contaminated NNSS (formerly NTS) groundwater, obtained pure isolates, performed 16S rRNA sequencing, and began harvesting EPS for use in ternary sorption experiments.
- Determined the stability constants of Pu(IV), Th(IV) and Np(V) binding with humic acid

Ab initio Electronic Structure of $Pu(OH)_4$: Comparison Between Density Functional and Multireference Theories

LLNL SFA (Laboratory Research Manager: Annie B. Kersting)

P. Huang (PI), M. Zavarin, A.B. Kersting—*LLNL*

Currently available spectroscopic techniques do not readily allow for the atomic-scale characterization of metal ions and complexes at low concentrations (i.e., $< 10^{-10}$ M). This is particularly problematic for highly insoluble species such as Pu(IV), where little direct information is available on the chemical behavior at environmental conditions. To this end, we have embarked on a program of *ab initio*, atomic-scale simulations for Pu complexes in aqueous environments. However, the electronic structure of actinide complexes poses numerous difficulties. Accurate treatments of actinide complexes often require sophisticated correlated-wave-function techniques whose cost grows rapidly with the number of electrons, and are only practical for small systems. Large-scale dynamical simulations of condensed phase phenomena typically rely on the use of density functional theory (DFT); however, current DFT approximations for exchange-correlation (Exc) can yield large errors for actinides due to the presence of localized f-electrons. We report benchmark studies for the monomeric $Pu(OH)_4$ complex employing a range of *ab initio* techniques, including single-reference approaches (unrestricted Hartree-Fock, UHF; second-order Møller-Plesset perturbation theory, MP2), correlated-wave-function approaches (complete active-space self-consistent field method, CASSCF; multi-reference perturbation theory, MRPT) and density functional theory. We find that single-reference methods such as MP2 provide a reasonable description of ground state geometries and energies, and an explicit inclusion of multiconfigurational effects involving the Pu 5f-electrons is not essential. However, DFT with common semilocal and hybrid approximations for Exc performs poorly for $Pu(OH)_4$.

We explore the use of the DFT+U method as a simple way to improve on the DFT description of on-site Pu f-electron correlations. Using small hydrated cluster models, $Pu(OH)_4(H_2O)n$, we determine an *ab initio* parameterization of DFT+U suitable for the aqueous-phase simulation of $Pu(OH)_4$. With this DFT+U parameterization, we carry out periodic, *ab initio* molecular dynamics simulations of $Pu(OH)_4$ in a bulk water environment, in order to determine the aqueous solvation structure around $Pu(OH)_4$.

ORNL SFA: Biogeochemical and Molecular Mechanisms Controlling Mercury Transformation in Contaminated Environments

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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The ORNL Science Focus Area program takes a systems approach, examining processes from the molecular scale to field scale to obtain a comprehensive understanding of key controls on Hg cycling in contaminated environments. In the last 12 months, we made significant progress in (1) setting up field sites, (2) understanding the role of natural dissolved organic matter (DOM) in mercury (Hg) complexation and redox transformation, (3) surveying both bacteria and archaeal communities at sites and (4) determining molecular mechanisms of Hg-ligand interaction and intramolecular Hg handoff within enzymatic proteins.

Field studies provide key information on major chemical species and processes involved in Hg biogeochemical transformations in water and sediment along a longitudinal transect of East Fork Poplar Creek (EFPC). We found that dissolved methylmercury concentration is positively correlated with the aromaticity and molecular weight of DOM. Preliminary results of creek-sediment and interstitial-porewater vertical profiling suggest that the creek margin is the primary source of MeHg to the surface water of EFPC, but other observations make this interpretation unclear (see poster by Brooks et al.).

Studies of the biogeochemical controls on rates and mechanisms of Hg speciation and bioavailability have revealed key roles played by DOM, including recent findings that DOM plays a dual role facilitating concentration-dependent Hg(II) reduction and Hg(0) oxidation (Gu et al., 2011). We show that the redox states of sulfur in DOM and the DOM:Hg ratio critically influence the transformation of Hg, and thereby the potential microbial production of toxic MeHg. In addition, small ligands, functional groups, and their steric arrangements on aromatic compounds are found to be important factors influencing not only mercury microbial uptake (see poster by Schaefer et al.), but also photo-chemical redox transformation (see poster by Gu et al.).

Using 454 16S rDNA pyrosequencing, we found that archaeal communities have a significant increase in community diversity in MeHg vs. Hg(II) at contaminated areas (Porat et al., 2010). The bacterial community structure and diversity were strongly influenced by Hg(II), MeHg, and U(VI) (Vishnivetskaya et al., 2011) with *deltaproteobacteria* and sulfate reducers strongly trending towards Hg(II) and MeHg, particularly for those closely related to *Desulfobulbus propionicus* (see poster by Moberly et al.). In pure culture studies, the genome of the known methylating bacteria, *Desulfovibrio desulfuricans* ND132 was sequenced (Brown et al., 2011), to elucidate the genes responsible for Hg methylation (see poster by Hurt et al.). We have created a random Tn5 transposon mutant library with established assays. So far, we have obtained 3070 constructs; 905 have been assayed, with 45 possibly decreased in methylation. A second-generation FGA is being tested for quantifying all *mer* genes. Duplicate meta -genomes, -transcriptomes, and -proteomes from the field are being done to examine the structural and functional differences of these sites and comprehensively understand methylation and demethylation at this site.

Combining small-angle X-ray scattering (SAXS), and molecular dynamics (MD) simulations, we found that a single Hg ion can trigger a structural change in MerR. This change is propagated to its operator DNA, causing an underwinding of the double helix, which leads to transcription of mer genes by RNA polymerase (Guo et al., 2010). The multiheme protein OmcA is known to be involved in dissimilatory electron transfer to mineral surfaces. Results from neutron reflectometry studies suggest that OmcA attaches to hematite with a preferred orientation that maximizes interaction with the mineral. SAXS data show changes in the molecular shape of OmcA depending on the redox states of OmcA (Johs et al., 2010). SAXS and MD are also used to study the handover of Hg(II) from the N-terminal domain to the catalytic domain of the mercuric reductase, MerA (see poster by Johs et al.). Progress on Hg-ligand complexation calculations and protein solubility optimization for x-ray crystallization for MerR are described in the accompanying poster by Parks et al.

The SFA program has produced 24 peer-reviewed journal publications, of which 16 are on Hg biogeochemistry, and has also generated ~50 conference abstracts, presentations, and invited talks.The SFA team organized two special sessions on “Biogeochemical Controls on Mercury Transformation and Global Cycling” at the Goldschmidt Conference in June 2010, in Knoxville TN, and a special session on “Mechanisms of Microbial Mercury Methylation and Resistance” has been approved for the ICMGP, July 24-29, 2011, at Halifax, Nova Scotia, Canada.

Site Biogeochemical Processes and Microcosm Studies (Hg SFA at ORNL)

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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Site investigation and geochemical modeling provide key information on major chemical species and processes involved in mercury (Hg) biogeochemical transformations in water and sediment along a longitudinal transect of lower East Fork Poplar Creek (EFPC) in Oak Ridge, TN.

In the past year, three sites were established for long-term detailed characterization of the site biogeochemistry in surface water, interstitial pore water, and creek sediments. Similar measurements are being conducted on the adjacent floodplain sediments. Two of the sites are on the Hg-contaminated EFPC, and one is located on a nearby reference stream that receives only atmospheric Hg fallout. Additionally, longitudinal surveys were conducted along EFPC at multiple locations at the same quarterly sampling interval. At all sites, early results showed essentially uniform water-quality parameters with depth into the creek sediment in the center of the creek channel, whereas steep redox gradients were established in the fine-grained deposits along the creek margin. Consistent with these observations, dissolved methymercury (MeHgD; passes a 0.2 µm size pore) concentration increased with depth at the creek margin. There was no correlation between inorganic Hg and MeHg, but the distribution of total Hg and total MeHg was correlated to sediment texture where higher concentrations were associated with finer texture. Although this may give the impression that the creek margins are the primary source of MeHg to the surface water of EFPC, two other observations make this interpretation uncertain: (1) areas in the sediment with MeHg porewater concentration greater than the surface water do not appear to be hydraulically connected to the surface water, and (2) in a benthic flux chamber study, positive MeHg flux into the surface water was measured in the center channel but not at the creek margin. Further investigations are currently under way, including measurements of Hg methylation and MeHg demethylation rates using intact cores of creek and floodplain sediments.

Both the quantity and quality of dissolved organic matter (DOM) changed with increasing downstream distance in EFPC—the DOM concentration increased and transitioned to higher molecular weight material with greater aromaticity. These changes are at least partially related to downstream changes in land cover in the watershed, which transitions from heavily developed industrial and urban at the headwaters to mixed evergreen and deciduous forest near the mouth of the creek. Dissolved (passes 0.2 µm size pore) inorganic Hg was not related to DOM quantity or quality, but MeHgD was positively correlated with DOM aromaticity and molecular weight. Additional studies are being conducted using ultrafiltration to further examine the relationship between DOM and Hg dynamics in this system.

Due to the volume of data generated, a data management system was adopted for archival and retrieval purposes. The system helps to achieve consistent reporting of field and laboratory results, including metadata, and facilitates organization and retrieval of results for analysis.

Understanding the Mechanisms and Geochemical Controls on the Speciation and Transformation of Mercury

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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The oxidized mercuric ion, Hg^{2+}, has been generally considered as the species that forms complexes with dissolved organic matter (DOM). Although dissolved elemental mercury, Hg(0), is also widely observed in sediments and water, reactions between Hg(0) and DOM have rarely been studied in anoxic environments. In this study, DOM isolates of various origins were pre-reduced either chemically or biologically to simulate natural anoxic environments and subsequently studied for their reactions with either Hg^{2+} or Hg(0). We found that DOM, particularly the reduced humic acid (HA), strongly interacts with dissolved Hg(0) through thiolate-ligand induced reactions. The binding capacity between Hg and DOM complexation varied with different DOM isolates and diminished when DOM became oxidized. The reduced DOM isolates are also capable of rapidly reducing Hg(II) species to gaseous Hg(0) under anaerobic conditions. The purgeable Hg(0) was found to increase with increasing DOM initially, reach a peak value, and then to decrease at higher levels of DOM due to the formation of Hg-DOM complexes. This phenomenon is explained by the dual functional role of DOM in the reduction and complexation of Hg, since the reduced DOM usually contains a higher reducing than binding (thiol ligands) equivalent. This role played by DOM is likely important in influencing mercury species transformations and methylmercury formation, particularly in anoxic sediments and water, where both microbial reduction of DOM and methylation occur.

Photochemical reduction of Hg^{2+} is also one of the most important pathways of elemental Hg(0) production in natural and contaminated waters, and plays a key role in Hg cycling. This redox transformation is affected by aqueous ionic species, among which the aromatic moieties of DOM are thought to be the most important because of their light-absorbing characteristics. However, the exact mechanism by which DOM mediates the photochemical reduction of Hg(II) is not clear, owing to incomplete understanding of the structural arrangement and stoichiometry of reactive functional groups in DOM. Using DOM analogs of salicylic acid, 4-hydrobenzoic acid, anthranilic acid, 4-aminobenzoic acid, and phthalic acid, we show that the photochemical reduction rates of Hg(II) are influenced not only by the neighboring functional groups but also their positioning on the aromatic benzene ring structure. The reduction rate increases in the order: anthranilic acid > salicylic acid > phthalic acid in response to changes in the neighboring functional groups from amine, hydroxyl, to carboxyl on benzoic acid. The substitution position of the functional groups affects reduction rates in the order: anthranilic acid > 4-aminobenzoic acid and salicylic acid > 4-hydroxybenzoic acid. These observations shed light on the role of DOM in Hg photoredox transformation and geochemical cycling.

Mercury Methylation: Genetic and Physiological Determinants of Methylmercury Production in Sulfate-Reducing Bacteria

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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In mercury (Hg) contaminated soils and waters, anaerobic bacteria are responsible for converting Hg to toxic and bioaccumulative methylmercury (MeHg). MeHg production has primarily been confirmed in subsets of dissimilatory sulfate-reducing bacteria (DSRB) as well as dissimilatory iron-reducing bacteria (DIRB). In order to determine the diversity of this activity, eight *Desulfovibrio* species have been tested for the first time and four were found capable of methylation, bringing the total to 11 out of 23 tested. Phylogenetically, there is no obvious pattern to the capability, with the rates varying by up to 10-fold among the methylators. One of the weaker methylators, *D. africanus*, was characterized for methylation and differential gene expression over the growth curve due to extreme pleiomorphology at different growth phases. No obvious gene patterns were found, although several candidate genes have been identified and are being tested. The model organism to date for this work has been *Desulfovibrio desulfuricans* ND132--chosen for its high methylation rate. Methylation rates by ND132 are strongly dependent on the Hg concentration and medium chemistry, but under optimal conditions, in the absence of sulfide N132, can methylate about 30% of 10 ng/ml $HgCl_2$ during batch culture growth. Some small thiol ligands enhance the amount of MeHg produced, but these are different from the thiols that enhance methylation in *Geobacter* spp. ND132 is one of only two mercury methylating sulfate-reducers to have its genome sequenced (JGI). The genome contains 3478 candidate protein-encoding genes with 65.2% G+C and a total size of 3.8Mb, but was left in 1 scaffold and 6 contigs. We have undertaken completing the genome, and to date have filled 4 of the 6 gaps that were due to both sequencing errors and hard GC stops. To elucidate the genes responsible for mercury methylation, we have developed a facile genetic manipulation system via conjugation in ND132 to create a random Tn5 transposon mutant library, as well as a screening assay, and both have been adapted for high throughput. The initial target is 5,000 mutants, but up to 10,000 may be required. Currently, 3,352 mutant constructs have been created, and >900 have been assayed for loss of methylation, with 47 showing decreased methylation to some degree. Decreased methylation in these 47 will be validated using stable isotopes ICP-MS and sequencing. If validated, these candidate genes will be deleted using targeted mutagenesis in order to confirm they are involved in Hg methylation. Through this methodology the responsible genes and pathways for Hg methylation in ND132 may well at last be identified.

Mercury Methylation: Microbial Communities Involved in Hg Transformations (ORNL Hg SFA, Microbial Genetics, and Transformations)

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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M Podar, M.M. Drake, A.V. Palumbo, D.A. Elias (PI)—*ORNL*

In microbial transformation and genetic studies, we examined microbial communities involved in mercury methylation in streams contaminated from past operations in the Y12 plant at Oak Ridge. In collaboration with the Field Task of the Hg SFA program, we investigated Hg methylating microbial communities in Hg-contaminated streams and background sites. Initial microcosms suggest active methylation downstream, with this activity being stimulated by sulfate and inhibited by molybdate. Water and sediment samples were analyzed for the microbial community complement phylogenetically via 454 pyrosequencing of the 16S rDNA gene V4 region. We hypothesize that: (1) there is a greater diversity of genes related to pollutants at the contaminated sites; (2) a lower overall phylogenetic diversity is present at these sites, (3) some groups of microorganisms will correlate with areas contaminated with Hg and/or methylmercury (MeHg), (4) specifically, the number of *Deltaproteobacteria* (the group involved in methylation) will positively correlate with MeHg concentrations. Analysis of 60 samples revealed pronounced phylogenetic and functional differences related to seasonal trends. Geochemical principal component analysis of several sites showed that one area, Bear Creek, was substantially different due to the presence of U(VI) and nitrate, and this was reflected in the microbial community that was mostly devoid of Proteobacteria. Virtually all of the microbial communities in the other five sites trended towards dissolved Hg. Further, such a correlation of the 454 data with geochemistry at the phylum and genus level showed that some Hg methylating bacteria, such as *Geobacter* spp., do not correlate with either Hg or MeHg. However, both the Delta- and Epsilon- Proteobacteria, as well as *Verrucomicrobia,* all trended towards dissolved Hg, and *Desulfobulbus* spp. strongly trended towards MeHg. This is significant in that *Desulfobulbus propionicus* is a known Hg methylator. Methylation and demethylation are being investigated with the type strain under several different culturing regimes to coordinate differences in growth with these activities to point to biochemical methylation pathways. Enrichment and isolation using propionate and sulfate resulted in pure cultures that did not methylate, but these activities are being repeated. A second generation functional gene array with >1000 mer gene sequences is being deployed along with consensus *merA,B* qPCR primers, while we work towards 6 meta –genomes and -proteomes.

Visualizing Intramolecular Interactions of the Mercuric Reductase (MerA) Catalytic Core with Its Hg(II) Shuttling Metallochaperone Domain (NmerA)

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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S.M. Miller, *UCSF*; L. Liang, *ORNL*

As an integral part of the ORNL SFA, this task investigates subcellular processes, including mechanisms of mercury trafficking, mercury-ligand interactions, as well as enzyme-catalyzed reactions involved in bacterial mercury resistance and methylation. Many microbes possess the ability to deal with heavy metal toxicity through elaborate metal-resistance mechanisms. One well-known example, bacterial mercury resistance, is mediated by the *mer* operon, which encodes specific genes involved in the transfer and transformation of toxic Hg(II) species. The mercuric reductase MerA is a key component of the *mer* operon. MerA is an NADPH-dependent flavin-disulfide oxidoreductase that catalyzes the reduction of Hg(II) to Hg(0), which is relatively inert and passively diffuses from the bacterial cell. Here, we combine experimental biophysics and computer simulation techniques to investigate structural features important for Hg(II) transfer in MerA. All MerA proteins consist of a homodimeric catalytic core domain, and many also possess an N-terminal metallochaperone-like domain NmerA, which is tethered to the core by an ~30 amino acid linker of unknown fold. Prior studies using separately expressed NmerA and core domains showed that NmerA acquires Hg(II) from other *mer* proteins such as the organomercurial lyase, MerB, and the membrane transport protein, MerT, and delivers it to the MerA catalytic core for reduction. Here, we have applied small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS) and molecular dynamics simulations to characterize the interactions of NmerA and the core in full-length MerA in solution. Our data reveals the extent of spatial sampling of the two NmerA domains relative to the homodimeric catalytic core and identifies the interdomain docking orientation that occurs during transient handoff of Hg(II) from a pair of cysteine residues on NmerA to a pair of cysteines on the C-terminus of the catalytic core.

Molecular Structure and Dynamics of Mercury Biotransformations

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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As an integral part of the ORNL SFA, this task investigates subcellular processes, including mechanisms of mercury trafficking, mercury-ligand interactions, and enzyme-catalyzed reactions involved in bacterial mercury resistance and methylation. The initial focus has been on the biomolecular structure and function of several key proteins and enzymes that confer mercury resistance in bacteria and impact mercury speciation and bioavailability in the environment.

The expression of mercury resistance genes in the *mer* operon is controlled by the metalloregulator MerR at the level of transcription. Prior *in vivo* and *in vitro* biochemical work showed that Hg(II) binding by MerR induces a large conformational change that underwinds the operator-promoter DNA leading to transcriptional initiation. Although structures exist for several activated MerR-family regulators, there is no structure for MerR itself or any nonactivated MerR-family protein. Using small-angle X-ray scattering (SAXS) and molecular dynamics simulations, we showed (Guo et al., JMB, 2010) that Hg(II) binding transforms apo-MerR from a flattened, compact state into an elongated barbell. MD simulations indicate large amplitude motions of the DNA-binding domains on the nanosecond time scale, which might propagate allosteric changes near the MerR metal-binding site to the DNA-binding site. We constructed a model of MerR bound to its operator-promoter DNA, MerOP, and are doing MD simulations to optimize this model in light of genetic and DNA footprinting data. The optimized model will be used for MD simulation to obtain kinetic and thermodynamic parameters of the Hg(II)-induced transition from repression to activation, and depict how Hg-binding site occupancy is communicated over a distance of 28 Å to the DNA-binding sites. We have overcome previous solubility issues with MerR by screening many conditions, including pH, salts, and other additives (see accompanying poster by Smith et al.). Optimized solubility resulted in successful crystal growth of Hg(II)-MerR and Hg-free-MerR for X-ray diffraction data collection at APS.

The mercuric reductase MerA reduces Hg(II) to Hg(0). Using SAXS and molecular dynamics simulations, we studied the structure and dynamics of full-length MerA to examine the behavior of its N-terminal metallochaperone domain relevant to Hg(II) transfer from mer pathway proteins or from Hg(II)-damaged cellular proteins directly to the MerA active site for reduction (see poster by Johs et al.).

Bacterial Hg(II) reduction also occurs at low levels as a side reaction of dissimilatory iron reduction in Fe-reducing bacteria such as *Shewanella* and *Geobacter*. We hypothesize that this reduction is done by outer-membrane multiheme cytochromes whose natural role is in moving electrons to external Fe(III). We purified the decaheme outer-membrane cytochrome OmcA from *Shewanella oneidensis* MR-1 and determined its solution structure using SAXS. Neutron reflectometry also revealed it assembles as a monomolecular layer on a hematite surface (Johs et al., Biophys. J., 2010). Reduction *in vitro* showed direct electron transfer from OmcA to Hg(II). Ongoing studies measure the Hg(II) reducing capacity of OmcA *in vivo*. As a membrane protein, OmcA is a challenge for X-ray diffraction structure determination. We improved protein solubility with a low ionic strength buffer, and explored several crystallization techniques including capillary counter-diffusion, vapor diffusion, seeding, and cofactor/additive screening. By applying these techniques, we aim to overcome previous issues with crystal morphology. Diffraction data collection and efforts to solve the structure of OmcA are currently under way.

Quantum chemical density functional theory (DFT) calculations can yield quantitative understanding of Hg(II) speciation and ligand-exchange reactions. While presently applying these to the *mer* locus proteins and small Hg(II)-ligand complexes, we are also moving toward applying them to acellular components of natural organic matter (NOM) to describe Hg(II) exchange among NOM and other ligands. Future efforts of this SFA Task will use structural biology and computer simulations to study macromolecular dynamics, enzyme reaction pathways, and routes of mercury transfer to the cell to understand biomolecular Hg(II) trafficking in impacted ecosystems.

Active Transport and Methylation of Hg(II) in Anaerobic Bacteria

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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One key factor controlling the production and accumulation of methylmercury in environments such as the East Fork Poplar Creek (EFPC) ecosystem at the U.S. DOE Oak Ridge Reservation, is the species of Hg(II) available for uptake by methylating bacteria. Little is known regarding the mechanism of Hg(II) uptake by these organisms, in part because of the inherent difficulty in measuring the intracellular Hg concentration. Using the model Hg methylating bacteria *Geobacter sulfurreducens* and *Desulfovibrio desulfuricans* ND132, we demonstrate that Hg(II) uptake occurs by active transport. We also show that Hg(II) uptake by *G. sulfurreducens* is highly dependent on the characteristics of the thiols that bind Hg(II) in the external medium, with some thiols promoting uptake and methylation. and others inhibiting both. The Hg(II) uptake system of *D. desulfuricans* has a higher affinity for Hg(II) than that of *G. sulfurreducens* and promotes Hg(II) methylation in the presence of stronger complexing thiols. Our results raise the question of the physiological function of the underlying Hg(II) cellular uptake system, and point at Hg(II) complexation by thiols as an important factor controlling Hg(II) methylation in anaerobic environments.

Role of Microenvironments and Transition Zones in Subsurface Reactive Contaminant Transport: The PNNL SFA

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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The PNNL Scientific Focus Area (SFA) is investigating critical Hanford and basic subsurface science issues through integrated, multidisciplinary, science-theme focused research on the role of microenvironments and transition zones in the reactive transport of technetium (Tc), uranium (U), and plutonium (Pu). The long-term goals of the SFA are to develop: (1) an integrated conceptual model for microbial ecology in the Hanford subsurface and its influence on contaminant mitigation, (2) a fundamental understanding of chemical reaction, biotransformation, and physical transport processes in microenvironments and transition zones, and (3) quantitative biogeochemical reactive transport models for Tc, U, and Pu that integrate multiprocess coupling at different spatial scales.

Cohesiveness and integration are achieved by focus on Hanford-relevant contaminant scenarios and three scientific themes: biogeochemical electron transfer mechanisms, pore-scale reactive transport and upscaling; and microbial ecology and field scale biogeochemistry. The theme areas traverse multiple scale domains and involve laboratory, numerical, and field research. The SFA relies strongly on the Environmental Molecular Sciences Laboratory (EMSL) for fundamental science capabilities and high performance computing, and the Hanford Integrated Field Research Challenge (IFRC) site as an essential location for samples, conceptual model development, down-hole biogeochemistry studies, and the evaluation of up-scaling approaches and integrative models. The IFRC site and its associated environment in Hanford's 300A contain multiple transition zones that are the focus of current and future research, including: a dramatic redox interface, a fluctuating water table, a spatially dynamic zone of groundwater-river mixing within the aquifer, and a hyporheic zone where groundwaters discharge to surface water.

Notable scientific accomplishments have been made.

- An in-depth molecular ecology investigation was completed on a 33 m subsurface sediment sequence in Hanford's unconfined aquifer that contains a redox transition zone.
- A functional outer membrane redox protein was crystallized and its structure determined. Experimental and modeling studies were completed to evaluate its reaction mechanisms and kinetics when localized at the organism surface.
- The mechanisms of Tc heterogeneous redox reactivity were identified using unique model mineral systems and transition zone sediments varying in Fe speciation.
- The variation in uranium surface complexation and mass-transfer parameters were evaluated in Hanford sediments from the subgrain to intact core scale, to understand process and parameter scale dependencies.
- The biogeochemical function of a multifacies redox transition zone was revealed by microbiologic studies of core samples and down-hole experiments in IFRC wells.

Scale-Dependence of Intragranular Diffusion

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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Some observations of diffusive contaminant release from intragranular pores deviate significantly from analytical solutions for diffusion out of a sphere. This research examines how a specific phenomenon–low connectivity of the intragranular porespace–may cause that deviation. The research is specifically motivated by contaminant release at the Hanford 300 Area, although the issues and how we address them are broadly applicable.

Our initial modeling used a pore-scale network model of a single grain, with diffusion modeled using random walk (RW) methods. We examined how contaminant release, starting from equilibrium, was affected by low pore connectivity. As connectivity decreased, the release curve shifted to later times, as expected. But as connectivity approached the percolation threshold, the shape of the release curve also changed, becoming more like the observed "deviant" curves. Specifically, the low-connectivity curves showed increased early-time release and much slower late-time release. For example, the first 10% of a contaminant could be released slightly faster from a low-connectivity grain than from a high-connectivity grain, but the last 10% could be 3 or 4 orders of magnitude slower.

The pore-scale networks were mapped pore-by-pore to examine reasons for the observed release patterns. At connectivities near the percolation threshold, accessible porosity and tortuosity varied strongly with both distance from the grain's exterior, and even small changes in connectivity. These changes in both accessible porosity and tortuosity agreed with scaling relationships of percolation theory.
Because the pore-scale model uses Monte Carlo techniques, with each realization requiring up to several days, we developed a faster finite difference (FD) model. The FD model uses percolation scaling relationships to parameterize the accessible porosity and diffusivity as functions of both distance from the exterior, and the connectivity's proximity to the percolation threshold. It also handles the infinite cluster separately from the edge-accessible finite clusters; this increases complexity and time, but improves the match. We ran some new RW simulations with both in- and out-diffusion, with the out-diffusion starting from several nonequilibrium points. Over a wide range of connectivities and nonequilibrium points, the FD model closely matched the RW model.

With a self-consistent and physically plausible explanation for the observed deviations from the analytical solutions, we are now seeking experimental evidence to determine whether low intragranular pore connectivity actually obtains in Hanford sediment. If it does, then future work may include (1) parameterizing the FD model with measured grain-size distributions, intragranular porosities, and diffusivities, (2) adding grain coatings to the model, and (3) adding geochemistry to the model in the form of sorption sites and equilibrium reactions.

Plutonium Speciation in Hanford Sediments and Redox Transformations Induced by Fe(II)

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

A. Felmy (PI), *PNNL*; S.D. Conradson, *LANL*

The overall objectives of this project are to characterize the different chemical forms of plutonium (Pu) in the sediments at the Hanford site, how these different chemical forms respond to changes in redox conditions, and what impact such changes have on the potential for future subsurface transport. Key scientific questions include: What is the mechanism of reaction of aqueous Fe(II) at the surface of insoluble Pu(IV) compounds found at Hanford? How do naturally occurring Fe(II) compounds found at Hanford influence this reactivity? What influence does the formed Fe(III) reaction product have on the solubility of the Pu(IV) compounds? Specific activities include: (1) obtaining and archiving samples from "high value" PNNL SFA secondary field sites with Pu contamination at Hanford, (2) detailed molecular level characterization of the chemical form of Pu in the sediments, and (3) experimental electron transfer (PNNL SFA Theme 1) laboratory studies of the redox transformations of these different chemical forms in response to changes in the chemical form of Fe(II).

Unique high Pu containing sediment samples have been obtained as a function of depth from the Hanford 200 west area and the chemical form of the Pu in these sediments, characterized by a combination of XAFS spectroscopy, TEM, and NanoSIMS (conducted by A. Kersting at LLNL). The results show that the chemical form of Pu changes as a function of depth in the sediments. For example, XAFS spectroscopy has determined that the surface sediments are dominated by the presence of PuO_{2+x} which occurs in two distinct forms; as large distinct micron-sized particles that apparently were disposed directly from Z-plant operations, and as very small particles that apparently formed as the acidic waste was neutralized by interaction with the soil. In contrast, XAFS analysis of the chemical form of the Pu in a deep silt layer (60-65 ft below the ground surface) does not show the Pu-O or Pu-Pu distances characteristic of PuO_{2+x} indicating the Pu is associated with other components of the sediment. NanoSIMS analysis of individual sediment particles is currently being performed to identify the specific Pu associations with Al, Fe, P, and F. Samples of uncontaminated sediments from the silt layer have also been sent to Clemson University (B. Powell) for Pu adsorption studies.

In our laboratory studies, we have obtained the first experimental data verifying the importance of the Fe(III) reaction product in the reduction of PuO_{2+x} by Fe(II). Specifically, we have examined the increases in PuO_{2+x} reduction induced by the formation of the Fe(III) reaction products goethite, hematite, and magnetite. These results indicate that the reduction of insoluble Pu(IV) species to soluble Pu(III) species can be dependent upon the nucleation and growth of Fe(III) reaction products of different thermodynamic stability. In the course of these experiments, we also were able to significantly improve the standard procedure for determination of Pu oxidation states by solvent extraction.

Characterization of the Intragranular Water Regime in Hanford 300A Sediments: Pore Volume, Surface Area, and Mass-Transfer Limitations

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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Transport of U(VI) in the Hanford 300A uranium plume is limited by sorption to mineral surfaces. Laboratory experiments with sediments from the 300A have demonstrated that desorption of the adsorbed U pool is slow, with batch-scale desorption experiments requiring hundreds to thousands of hours to reach equilibrium. It is hypothesized that this kinetic limitation is primarily the result of intragranular/intra-aggregate diffusion; diffusion of U within grain fractures, clay aggregates, and clay coatings on sediment grain surfaces. A complete understanding of this kinetic control is limited by both physical and chemical complexities, including pore-network heterogeneity, complex aqueous and surface speciation of uranium, and effects of anion exclusion on the diffusion of U and U-complexing ions. The objective of this work is to characterize the intragranular diffusion regime within Hanford 300A sediments (including pore sizes, pore surface areas, and mass-transfer rates) using multiple techniques that remove the complexities of aqueous and surface chemical speciation.

Tracer uptake and release experiments were performed on 300A North and South Process Pond sediments using the nonreactive tracers tritium and bromide. Batch and column-scale experiments were designed to achieve a sufficiently high resolution with each tracer to probe the intragranular pore network. These data were modeled using a distributed-rate mass-transfer scheme to extract diffusion parameters (intragranular pore volume and mass-transfer coefficients) that can later be applied to a sorptive-diffusion model for U. Tritium-hydrogen exchange on surface hydroxyls was accounted for by conducting additional batch experiments on sediment that was vacuum dried after reaction. The complementary ("wet" and "dry") techniques allowed for the simultaneous determination of intragranular porosity and surface area using tritium. Batch and column results with tritium were mutually consistent and highly complementary, with batch experiments exhibiting greater sensitivity to relatively fast diffusion and column experiments exhibiting greater sensitivity to slower diffusion. Comparison with nitrogen gas adsorption results suggests that the intragranular pore space probed using tritium includes both "micropores" (< 2 nm diameter) and "mesopores" (> 2 nm). Whereas the tritium results suggest a high degree of heterogeneity in the diffusive regime, results using bromide indicate a narrower range in diffusion rates and a smaller intragranular pore volume, consistent with anion exclusion of bromide by negatively-charged pore spaces.

Spatial and Temporal Dynamics of Microbial Community Structure within the Saturated Zone of the 300 Area, Hanford Site

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

X. Lin, D. Kennedy, A. Plymale, A. Konopka (PI)—*PNNL*

Relatively little is known about the structure and function of subsurface microbial communities at DOE's Hanford Site, particularly in the sensitive Columbia River near-shore environments. Characterization of microbial biomass levels and their activity, as well as phylogenetic and functional diversity, has been an essential element for developing conceptual and mechanistic models of how the microbial community might impact the fate and transport of mobile contaminants such as U(VI) and Tc(VII). Here, we summarize research that has been performed within the Hanford IFRC field site to relate changes in microbial community composition (MCC) and functional activities to physical, chemical, and hydrological conditions identified by other researchers.

We analyzed 21 sediment samples recovered primarily from well C6209 that was drilled down to the basalt (52 m bgs), hence encompassing the saturated zone and basalt flow-top beneath the 300 Area. Approximately 8000 near full-length bacterial and archaeal 16S rRNA gene sequences were analyzed across geological strata that included the transition from unsaturated vadose zone to the saturated sediment ("smear" zone), the oxic coarse-grained Hanford formation, fine-grained oxic and reduced Ringold Formation sediments. Microbial community structure and richness varied substantially across the different geological strata. Bacterial OTU richness was highest (>700) near the water table and in the upper Hanford formation, containing a greater diversity of phylotypes belonging to α-proteobactera, Bacteroidetes, and Actinobacteria than other depths. Microbial communities from just below the smear zone were characterized with higher proportion of candidate division NC10 and Acidobacteria. The oxic Ringold sediments harbored the broadest set of divisions and were dominated by Nitrospira, GAL15, and Chloroflexi. The richness declined to less than 50 in the deeper reduced zones, which were dominated by Proteobacteria and one or two other distinct phyla (e.g., Dehalococcoides at 39 m).Quantitative real time PCR detected the highest relative abundances of dsrA gene and *Geobacteraceae* in a redox transition zone near the Ringold oxic-anoxic interface. The nosZ gene was prevalent in the upper Hanford formation and in the upper reduced Ringold sediment. Most nosZ sequences were affiliated with Ochrobactrum anthropi and Achromobacter sp. Although biomass levels in Hanford formation gravels were modest (250-1000 pg phospholipid g-1 sediment), analysis of 3H-Leucine assimilation activity in groundwater or bacteria desorbed from sediments suggested bacterial doubling times of 10 h or less.

Horizontal distribution of the subsurface microbes was assessed by pyrosequencing ~68 samples from 27 wells in IFRC field. Results suggested horizontal shift of MCC from inland IFRC towards the river, with higher proportions of NC10, GAL15, and Acidobacteria OTUs in the east side (close to the river) and more abundant OTUs belonging to deltaproteobacteria, Alcaligenaceae, and Methylobacteraceae in the west side.

Temporal dynamics in groundwater were analyzed in ~400 samples collected over 10 months from 9 wells screened at 3 distinct strata (10 m, 13 m, 17 m). SSU rRNA gene pyrosequencing analyses revealed clear seasonal and vertical patterns. For example, Limnohabitans increased with depth and from winter to summer months. Highest abundances of Nitrosopumilus (ammonia-oxidizing archaea), Geobacter, Sulfurimonas (sulfur oxidizer), and putative methane oxidizers (e.g., Methylotenera and Crenothrix) were found at 17 m in winter. In shallow wells, MCC significantly shifted during a period of Columbia River water intrusion; OTUs belonging to Actinobacteria ACK-M1 increased from undetectable to 30% of total sequences at the highest water level, and then declined back to undetectable within 2 weeks.

Ecological analyses thus emphasize elevated microbial diversity, functionality, and substantial heterotrophic activity in the redox transition zone, and the fluctuating hydrological gradient significantly impacts the spatial/temporal dynamics of the microbial community in the Hanford unconfined aquifer.

Pore-Scale Reactive Transport and Upscaling of Uranyl Sorption/Desorption Kinetics

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

C. Liu (PI), J. Shang, S. Kerisit—*PNNL*; R. Ewing, *Iowa State U.*; J. Zachara, *PNNL*

This research investigates pore-scale coupling of transport processes with uranyl sorption/desorption reactions in intragranular diffusion and intergranular flow domains, to establish upscaling relationships and parameters by which to scale uranyl sorption/desorption reactions—from the molecular to grain scale, and from the grain scale to flow media. Molecular dynamics models were developed to simulate uranyl species diffusion in intragranular pore domains that host uranyl in Hanford sediments, and to calculate species-specific diffusion coefficients and the kinetic rate of surface uranyl species transformation. N_2 sorption/ desorption and stirred flow-cell reactors were used to provide insights into the intragranular pore structures and connectivity in sediments; pore-scale models in intragranular and intracoating domains were developed to simulate the coupling of uranyl sorption/desorption reactions and diffusion, and to derive upscaling theory and parameters to describe apparent grain-scale uranyl adsorption and desorption kinetics. Column experiments and intergranular pore scale simulations were performed to investigate the manifestation of complex coupling of grain-scale kinetics and pore-scale flow and diffusion in flow domains.

Our results showed that the molecular rate of uranyl surface complexation reactions in intragranular domains was fast, with a rate constant at an order of 10^4 s^{-1}. The apparent rate of uranyl adsorption/desorption, however, decreased to 10^{-3} s^{-1} at the grain scale, and further decreased to 10^{-5} (for sorption) to 10^{-6} (for desorption) s^{-1} in flow domains that contained field-textured sediments. Pore-scale reactive transport simulations revealed that the decrease in the rate constants from the molecular to the grain scale could be explained by coupled diffusion and local equilibrium surface complexation reactions in the intracoating domain, which has long been suspected as an important uranyl host location in the Hanford sediments. A two porosity, distance-dependent diffusivity model had been derived as an upscaled model to describe the grain-scale uranyl adsorption/desorption kinetics, based on extensive simulations of diffusion in the intragranular domains with complex pore structure and connectivity. The upscaled model contains parameters that can be either estimated from microscopic properties in the intragrain domains or treated as macroscopic fitting parameters that reflect microscopic complexity. The decrease in the rate of uranyl adsorption/desorption from the grain scale to the flow domain was caused by the pore-scale heterogeneity of pore and grain size distributions, which affected local flow residence times and chemical conditions that collectively affected local uranyl adsorption/desorption rates, as well as the overall transport flux of uranyl species in porous media. The slower rate of uranyl desorption (compared to adsorption) in the field-textured sediments was attributed to the pore-scale mass transfer between the faster and slower flow domains, and the short-term irreversibility of the grain-scale adsorption/desorption processes along the mass-transfer paths.

Isolation and Characterization of Metal Redox-Transforming Microorganisms from the Hanford Site 300 Area

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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This research focuses on isolating and characterizing Fe redox-transforming microorganisms from the Integrated Field Research Challenge (IFRC) Hanford 300 Area, which may directly and/or indirectly influence radionuclide mobility within the Hanford subsurface. The objectives of this project are to obtain Hanford Site microorganisms and characterize their phylogeny, metal biotransformation rates, electron donor utilization, and the identification of putative terminal reductase proteins (i.e., *c*-type cytochromes) involved in Fe redox reactions. The study of Fe redox-transforming microorganisms from the Hanford Site will advance our understanding of the field-scale microbial diversity, distribution, and activity under Theme Area 3 of PNNL's Scientific Focus Area (SFA). The integration of this data with the "Molecular and Microscopic Electron Transfer Processes" SFA Theme Area 1 will generate a new understanding of how to describe and predict radionuclide mobility at multiple scales of observation.

To enrich and isolate relevant microorganisms able to reduce Fe(III)-oxides either directly or indirectly, sediment samples from the aerobic Hanford formation, the oxic/anoxic transition of the upper fine-grained Ringold Formation, and the underlying Ringold Formation lower mud and Unit E facies were collected for microcosm construction. Microcosms were amended with Hanford 300 Area groundwater or synthetic groundwater that closely matched site geochemistry. Efforts to obtain indigenous Fe(III)-reducing microorganisms from groundwater have also been initiated using BioSep beads recovered from two Area 300 boreholes and used as inocula for establishing microcosms. For a more comprehensive characterization of the diversity of Fe(III)-reducing organisms native in Area 300 groundwater, isolation chip (ichip) environmental cells, specifically designed to aid in the cultivation of not-yet-cultured microorganisms, were also deployed in IFRC wells. To stimulate the growth and activity of subsurface microorganisms capable of metal redox transformations, active microcosms were transferred to a defined minimal medium amended with synthetic Fe(III)-oxide, Mn(III/IV)-oxide, sulfate, nitrate, ferric citrate, fumarate, or elemental sulfur (S^0) provided as terminal electron acceptors and one of fourteen different electron donors. Ichip *in situ* enrichments were initiated using an Fe-bearing phyllosilicate as a solid-phase terminal electron acceptor.

The microcosm studies demonstrated that four organic acids (acetate, lactate, succinate, and pyruvate) and five alcohols (ethanol, glycerol, butanol, propanol, and methanol) supported the reduction of Fe(III)-oxide. Interestingly, the responsiveness to the suite of electron donors varied between sediment samples from the three distinct redox zones, indicating differences in microbial community structure in each strata. Fe(III)-oxide reduction was also influenced by biotic interactions with S-compounds including sulfate, which is a component of Hanford groundwater. Current efforts use enrichment cultures to elucidate the biotic and abiotic interactions between Fe(III)-reducing and sulfate-reducing bacteria and the reduced products Fe(II) and sulfide.

Several *Geobacter* isolates were obtained from microcosms amended with different electron donor and electron acceptor combinations. Interestingly, the isolates shared highly similar 16S rRNA gene sequences suggesting that enrichment with different substrates yielded similar *Geobacter* strains. All of the *Geobacter* isolates were affiliated with the recently described *G. toluenoxydans* and *G. daltonii*, a clade within the Geobacteraceae characterized by its ability to couple Fe(III) reduction to the oxidation of monoaromatic compounds. During FY11, we will complete the isolation efforts and continue physiological and phylogenetic analyses of selected metal-biotransforming isolates from the Hanford subsurface. Biotransformation and growth studies will use relevant metal-containing mineral phases and/or radionuclides, and organisms of interest will be selected for genome sequencing through JGI's small-scale microbial genome sequencing program.

Geochemical Investigations of Redox Effects within the Hanford Site SFA

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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The Field Scale Microbial Ecology and Biogeochemistry Theme focuses on an area that is stratigraphically confined to three formations: underlying Columbia River basalt flows, intermediate fluvio-lacustrine Ringold sediments, and uppermost catastrophic flood deposits of the Hanford formation. The upper Ringold and the Hanford sediments are the environmentally accessible and impactful stratigraphic components. The Hanford formation was deposited by Pleistocene floods, and consists of mixed exogenous sandy gravels and locally derived scour from the Ringold formation. Spatial and temporal variations in floodwater carrying capacity caused the differential deposition of particulates throughout the formation, resulting in textural variations and local zones of fine-grained clays, silts, and sands within coarser gravels and sands. Rises and falls of river stage in the proximal Columbia River cause forcible changes in hydraulic gradient that drive the advection of groundwater solutes across fine and coarse lithologic boundaries.Wells within the 300 A IFRC site were used to evaluate the hypothesis that fine-grained intervals within the stratigraphic column could harbor microbial populations in environments that were sufficiently sequestered to support anaerobic metabolism.

A series of passive multilevel samplers were deployed across known fine-to-coarse lithologic boundaries at the top and bottom of the Ringold formation's uppermost mud. Aqueous and dissolved-gas samples were analyzed for the metabolically sensitive solutes O_2, NO_3-, NO_2-, SO_4^{2-}, Mn^{2+}, Fe^{2+}, HS^-, N_2O, H_2, and CH_4. (However, not all analytes were determined for all samples.) Across the gravel-mud boundary from the Hanford to the Ringold formation, the concentrations of SO_4^{2-}, Mn^{2+}, NO_2-, H_2, and CH_4 increased with depth, while the concentrations of O_2 and NO_3- decreased. At the mud-gravel boundary within the Ringold formation, HS- increased with decreasing depth (i.e., upward, into the mud). Fe^{2+} and Mn^{2+} were present throughout the sampled Ringold formation, and H_2 and CH_4 increased with depth near a gravel-mud boundary deeper in the section. Anaerobic metabolism within the fine portions of the Ringold formation apparently supported anaerobic microbial populations that actively consumed available electron acceptors. The presence of H_2 and CH_4 suggested that perhaps fermentation reactions drove a supply of electron donors, and the contrast in HS^- and SO_4^{2-} gradients across the lithologic boundary suggested that advection existed from the Ringold into the Hanford formation.

Continued, more comprehensive sampling and analysis of vertical lithologic boundaries, as well as lateral exploration of these interfaces in the extended river-forced environment, will allow a better understanding of microbial ecology within the SFA.

Reactions of Tc with Fe(II) in Anoxic Hanford Sediments

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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Technetium-99 (^{99}Tc) is a fission product of uranium-235 and plutonium-239 that has been introduced to the Hanford site environment as a result of the leakage and disposal of tank waste. Technetium valence state is a crucial factor determining Tc mobility in terrestrial environments, and its potential uptake into the food chain. Under oxic conditions, Tc exists as the pertechnetate anion (TcO_4^-) which is weakly sorbed. Because of its mobility and long half-life ($t_{1/2}$=2.13•10^5 years), Tc is a high-risk contaminant. To date, over 400 Ci of Tc has been released into the vadose zone at Hanford, with risk for transport to the nearby Columbia River. The reduced form of technetium, Tc(IV), is stable in anoxic environments, typically existing in the form of insoluble $TcO_2 \bullet nH_2O$ and related compounds. The mechanisms of Tc(VII) to Tc(IV) reduction are the subject of active research, because of their implications for subsurface migration and remediation.

The objectives of this PNNL SFA research are to: (1) investigate abiotic electron transfer reactions between Fe and Tc in mineralogically heterogeneous sediment obtained from a subsurface redox transition zone, including their reaction products and kinetics; (2) identify the reactive ferrous mineral forms and associated reaction networks that control Tc speciation; and (3) develop a generalized, "Fe(II)-speciation-driven" kinetic reaction model for Tc.

Technetium(VII) reduction experiments were performed with anoxic sediments collected from Hanford's unconfined aquifer (~18 to 55 m bgs) within the Columbia River corridor. Chemical extractions, X-ray diffraction (XRD), electron microscopy (SEM and TEM), and Mössbauer spectroscopy were applied to characterize the Fe(II) mineral suite, which included Fe(II)-phyllosilicates, pyrite, magnetite, and siderite. The Fe(II) mineral-phase distribution differed between the sediments. Technetium(VII) reduction occurred in all sediments, and Tc(IV) was immobilized as sorbed $TcO_2 \bullet nH_2O$-like clusters. The reaction time differed significantly between the sediments (8-219 d), with the rate being influenced by Fe(II) phase speciation. Mössbauer analysis of the Tc-reacted sediment revealed that Tc(VII) was partially reduced by siderite. Ongoing experiments seek to understand the role of Fe(II) carbonates in Tc(VII) reduction. Reduction experiments will be conducted with synthetic and natural siderites, and with ferrous hydroxy carbonate to study Tc reduction kinetics at different chemical conditions. The residual sediment suspensions are used to investigate Tc(IV) distribution and association with Fe phases. Post-reaction solids are analyzed by autoradiography and SEM to identify Tc-containing particles for further mineral identification by micro-XRD.

The Biochemistry of Electron Transfer to the Microbe-Mineral Interface

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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A key question regarding electron exchange between a bacterium and extracellular electron acceptors or donors is the mechanism of electron transfer across the outer membrane. We have developed a conceptual model as a framework in which we propose that the multi-heme cytochrome “wires” can embed within an outer-membrane porin sheath to form electrically conductive complexes. These porin-cytochrome complexes can then interact with various biochemical termini on either side of the outer-membrane, depending on their physiological function (e.g., Fe(III) reduction or Fe(II) oxidation). Our objectives are to solve the molecular structure of this complex, reconstitute it into liposomes, study electron transfer to a range of minerals in these artificial membrane systems, and prove that this model applies widely in environmental systems where it is necessary to elucidate the molecular properties of the cytochrome-porin complexes and the termini that interact with them, over a range of environmentally relevant bacterial species.

In addressing these broad objectives, we have: (1) obtained a 3-Angstrom crystal structure of a deca-heme Fe(III) reducing terminus of an outer-membrane wire cytochrome-porin wire (MtrF) and used this to explain how electron transfer at the microbe-mineral interface can be direct protein-to-mineral or mediated by soluble electron shuttles such as flaving; (2) incorporated a porin-cytochrome complex into proteoliposomes, enabling us to examine electron exchange with different mineral surfaces, for example hematite, goethite, or birnessite, and study the effectiveness of different outer membrane deca-heme termini in these processes; and (3) undertaken biochemical, spectro-potentiometric, and structural studies on a range of outer-membrane electron transport systems to understand functional commonalities and differences.

Recovery of Phyllosilicate Fe(II)-Oxidizing Microorganisms from Hanford 300 Area Sediments

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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In this project, we are examining the potential for lithotrophic microorganisms to catalyze the oxidation of solid-phase Fe(II)-bearing phyllosilicate phases in Hanford 300 Area sediments. Such phases are abundant in Hanford and other DOE-relevant subsurface environments, and may have an important impact on the speciation and stability of risk-driving contaminants such as U and Tc. However, their ability to serve as an energy source for microbial lithotrophic metabolism, and the influence of such metabolism on phyllosilicate reactivity, is virtually unknown. Recent studies with a previously described lithotrophic Fe(II)-oxidizing, nitrate reducing enrichment culture (Shelobolina et al., submitted) showed that specimen biotite (a Fe(II)-bearing mica) and reduced smectite phases could serve as electron donors for autotrophic growth. This same culture was able to oxidize a significant fraction (~20%) of the 0.5M HCl-extractable () Fe(II) content of reduced sediments from the fine-grained unit of the upper Ringold Formation at Hanford 300 Area.

Our current work focused on recovery of phyllosilicate Fe(II)-oxidizing organisms from Hanford 300 Area subsurface sediments and groundwater. The presence of a well-defined redox boundary near the top of the Ringold Formation (~18 m depth), characterized by major changes in Fe redox speciation, sulfide content and redox reactivity with Tc(VII) (Peretyazhko et al., submitted), suggests that phyllosilicate-associated Fe redox transformations are ongoing. This in turn led us to hypothesize the presence of microorganisms capable of phyllosilicate Fe(II) oxidation. Enrichment and isolation procedures were applied to sediment and groundwater samples using three kinds of Fe(II)-bearing phyllosilicates: (1) Bancroft biotite, (2) chemically reduced NAu-2 smectite, and (3) the fine silt/clay size fraction of reduced Ringold material, which is dominated by smectite-illite. Enrichment cultures from reduced Ringold sediment were established using previously collected core materials, while enrichment cultures from groundwater were obtained through a five-month *in situ* incubation campaign employing i-chips (diffusion chambers) developed by S. Epstein (Nichols et al., Appl. Environ. Microbiol., 2010). Fe(II)-bearing phyllosilicates were added to diffusion chambers as an electron donor. Several strategies were utilized to isolate Fe(II)-oxidizing microorganisms from the resulting enrichment cultures. To isolate mixotrophic microorganisms, enrichment cultures were diluted to extinction on an aerobic heterotrophic medium. Isolated colonies were transferred to either an lithoautotrophic Fe(II)-oxidizing medium or Fe(II)-oxidizing medium containing 0.2 mM acetate as a carbon source. To isolate lithoautotrophic organisms, enrichment cultures were serially diluted in an lithotrophic medium with aqueous Fe(II) as the sole energy source. The highest positive dilutions were serially diluted again, and this procedure was repeated three times. Only a small number of isolates could grow lithotrophically for three transfers, and all were 99% similar in 16S rRNA gene sequences to *Bradyrhizobium japonicum*. Strains of *B. japonicum* could also repeatedly grow aerobically on biotite or basaltic glass provided as the source of Fe(II), and anaerobically with nitrate as the electron acceptor and reduced NAu-2 smectite as the electron donor. Several organisms which could grow by Fe(II) oxidation in the presence of 0.2 mM acetate as the carbon source were also recovered, including strains of *Brevundimonas mediterranea*, *Bosea thiooxidans*, *Ralstonia solanacearum*, and a *Comamonadacea* strain.

Recovery of these phyllosilicate Fe(II)-oxidizing organisms will facilitate understanding the impact of solid-phase Fe redox metabolism on contaminant (e.g., U and Tc) mobility in Hanford 300 sediments, and provide model cultures for studies of the molecular mechanisms of solid-phase Fe(II) oxidation.

Molecular Structure and Electron Transfer Properties of Microbial Outer-Membrane Cytochromes

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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As part of the PNNL SBR SFA's Molecular and Microscopic Electron Transfer Processes Science Theme, this line of research seeks to understand rates and molecular mechanisms used by microbial multiheme c-type cytochromes (c-Cyts) for mediating extracellular Fe valence transformation, an important process affecting the availability and supply of reactive Fe(II) in the subsurface. This includes an understanding of: (1) how c-Cyts interface with and achieve molecular configurations that enable facile electron exchange with solid phase Fe-containing minerals, and (2) how the c-Cyts-containing protein complexes interact with each other and work collectively for facilitating rapid electron transfer across the microbial cell envelope. Complementary laboratory experiments and molecular modeling are being used to characterize c-Cyts of Hanford-relevant and model microorganisms, such as those of the Mtr pathway and their homologues.

Synchrotron x-ray diffraction measurements conducted by external co-principal investigator David Richardson at the University of East Anglia have revealed a unique molecular structure for a crystallized outer-membrane redox protein from an iron-reducing bacterium. The MtrF structure has been determined at a resolution of ~3 Å. Results show that MtrF is folded into four distinct domains: domains I and III each contain seven anti-parallel β-strands folded together to form a split-β barrel structure, while domains II and IV each bind five tightly packed hemes. The four domains fold together, so that the penta-heme domains II and IV are packed to form a central core with the two split-β barrel domains I and III flanking either side. The ten hemes of MtrF are organized into a unique "wire cross," in which a staggered 65 Å octa-heme chain (hemes 10,9,8,6,1,3,4,5) transects the length of the protein through domains IV and II and is crossed at the middle by a 45 Å tetra-heme chain (hemes 2,1,6,7) that connects the two split β-barrel domains I and III. This wire cross is made up of a lower order organization of two triads of parallel hemes (hemes 3,4,5 and hemes 8,9,10) that lie perpendicular to a quartet of parallel hemes. Each heme is within 7 Å of its nearest neighbors, in principle permitting rapid electron transfer among the hemes.

Taking advantage of this experimentally determined MtrF structure as major scientific opportunity, molecular simulations are being carried out to understand for the first time the basic dynamic functioning of and rate controlling factors for intraprotein electron transfer. Large-scale molecular dynamics (MD) simulations of the MtrF structure in explicitly described solvent water have been performed on the EMSL's massively parallel supercomputer. Using the technique of thermodynamic integration, relative redox potentials of all possible intraprotein electron transfer steps have been directly computed with MD, which shows that upon possible electron entry into heme 5, only a 0.84 kcal/mol (1.4 kT) activation free-energy barrier for electron transfer to heme 4 opposes otherwise net thermodynamically downhill heme-to-heme conductance to hemes 2, 7, and 10 at the protein-environment interface. It is therefore proposed that heme 5 is the input site of electrons from partner proteins MtrDE up the electron transport chain, and that MtrF transfers electrons down the chain directly to Fe(III)-oxides via highly solvent-exposed heme 10. Domains I and III are thought to be involved in binding and reduction of flavin and soluble metals, ostensibly via hemes 2 and 7, respectively. Quantum mechanical calculations of the electronic coupling for heme-to-heme electron transfer kinetics prediction are under way. The combined experimental and computational simulation activity is collectively providing a comprehensive understanding of bacterial outer-membrane c-Cyt functioning at the level of individual heme redox potentials, heme-to-heme electron transfer rates across the protein, and insights into the dynamic effects of protein fluctuation and solvent reorganization on overall electron transfer conductance.

Tc(VII) Reduction by Synthetic and Natural Hanford Mixed-Valent Iron Oxides

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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As part of the PNNL SBR SFA's Molecular and Microscopic Electron Transfer Processes Science Theme, this line of research seeks to understand rates and molecular mechanisms of contaminant Tc(VII) and U(VI) reduction by Fe(II)-bearing iron oxides naturally present in sediments at the Hanford Site and elsewhere. Because of their reactive Fe(II) content, spinel-type iron oxides such as magnetite (Fe_3O_4) are key mineral phases that can affect the form and stability of redox-active contaminants. Natural magnetites are typically highly impure with titanium, and structural Fe(III) replacement by Ti(IV) yields a proportional increase in the relative Fe(II) content in the metal sublattice to maintain bulk charge neutrality. This variability of the lattice Fe(II) density in principle adjusts the total reducing capacity of the resulting titanomagnetite ($Fe_{3-x}Ti_xO_4$) minerals, and is therefore central to overall redox reactivity. In the absence of surface passivation, magnetites have been shown to spontaneously reduce and thereby effectively immobilize ^{99}Tc(VII), a major risk-driving contaminant at Hanford, to less soluble Tc(IV) in the form of a mixed Fe(III)-Tc(IV) co-precipitate. However, prior surface passivation can occur as a result of exposure to oxygen from the atmosphere or oxygenated water. This process progressively consumes structural Fe(II) both by oxidation to Fe(III) and by replacement with cation vacancies, leading to a relatively Fe(II)-deficient surface tending towards titanomaghemite ($Fe^{3+}(Fe^{3+},Ti^{4+},Fe^{2+},\square)_2O_4$). The Hanford subsurface also contains microenvironments in which oxygen-scavenging mineral reactions and microbial activity outcompete oxygen replenishment, resulting in reduced zones where there is potential for restoring reducing equivalents to "titanomaghematized" surfaces by exposure to aqueous Fe(II). This project is studying the reversibility of this process with respect to Tc and U redox reactions and the impact of mineral containing Fe(II) reducing equivalent accessibility and restorability on reaction rate and extent.

Natural magnetite particles were isolated from sediments at Hanford's central plateau by ferromagnetic separation. Successful procedures were developed, using EMSL facilities, to synthesize compositionally controlled $Fe_{3-x}Ti_xO_4$ bulk powders and nanoparticles to provide high-surface-area pristine material for comparative studies. The Fe(II), total Fe and Ti content of the $Fe_{3-x}Ti_xO_4$ samples were determined by acid digestion, ferrozine assay, and ICP-MS. Characterization also involved *in situ* XRD, Mössbauer spectroscopy, XANES/EXAFS, and *ex situ* techniques such as TEM, EPMA and XMCD. This analytical suite enabled analysis of the Fe(II)/Fe(III) ratio both in the bulk mineral and in the upper few nanometers of the surface, before and after reaction with pertechnetate solution. It also provided information on local coordination, bond distances, and magnetic ordering, allowing discrimination of different possible reactive pools of ferrous iron, including octahedral and tetrahedral structural Fe(II) in the lattice and in other discrete phases. Ti(IV) enters octahedral sites in bulk $Fe_{3-x}Ti_xO_4$ powders (~10 µm), up to $x = 0.94$ and Fe(II) increases with Ti-content, entering the tetrahedral site when $x\ ^{3}\ 0.4$. $Fe_{3-x}Ti_xO_4$ nanoparticles (~10 nm) accept structural Ti(IV) into the octahedral metal sublattice with concomitant increases in lattice Fe(II) content up to $x = 0.35$; higher values up to $x = 0.6$ yield discrete amorphous Fe(II)/Ti(IV) phases on particle exteriors. Titanomagnetite constitutes a significant component of the natural magnetic particles (<125 µm) with a composition ~ $x = 0.15$. Reaction of $Fe_{3-x}Ti_xO_4$ nanoparticles with 10 and 30 mM Tc(VII) solution yielded fast exponentially decaying reduction kinetics with rates that increased with increasing Fe(II)/Fe(III) ratio, as a function of Ti-content. Beyond oxidized titanomagnetite (titanomaghemite), no crystalline reaction products were detected. We have developed a mechanistic reaction model involving the reduction of Tc(VII) by Fe(II) at the nanoparticle surface to form a Tc(IV)/Fe(III) phase, followed by replacement of reducing equivalents by migration of Fe(II) from the bulk spinel structure to the surface, with the concurrent injection of cationic vacancies for charge balance. This reaction process was quantitatively linked to mass- and electron-balanced changes in the $Fe_{3-x}Ti_xO_4$ nanoparticles. Oxidation of $Fe_{3-x}Ti_xO_4$ nanoparticles inhibits Tc(VII) reduction, but the reduction potential can be readily restored through the replacement of reducing equivalents in the mineral structure by exposure to Fe(II)-containing solutions. Bulk $Fe_{3-x}Ti_xO_4$ powders also reduce Tc(VII) to Tc(IV) and the measured reduction rate for $x = 0.15$ is the same order of magnitude as that for the natural sample from the Hanford site. These measurements point to a picture of condition-sensitive dynamic exchange of reducing equivalents between bulk lattice Fe(II) and more accessible surface-associated Fe(II) that is dictated by the chemical potential of Fe(II) built into the solid, a property that in turn depends on the nature and content of aliovalent metal substitution impurities. Contaminant reduction kinetics therefore appear to be strongly dependent on the availability and relative proportions of these Fe(II) pools.

Modeling Pore-Scale Processes to Darcy-Scale Phenomena

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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Many processes of critical importance in subsurface biogeochemistry are most naturally defined, and can be best understood, at micron to millimeter scales. In porous systems, this corresponds to the pore scale, at which individual sediment grains and pore spaces are explicitly resolved. However, predictions of the long-term fate and transport of contaminant plumes at DOE sites must be made over scales of tens to hundreds of meters or larger. Such simulations rely on a continuum- or Darcy-scale representation of porous media. A grand challenge in subsurface science is overcoming this "tyranny of scales," which currently limits us to the use of empirical models with calibrated or fitted parameters at the field scale. Relating processes that occur in microenvironments (pore-scale) to phenomena observed at field scales (Darcy-scale) in a way that more directly incorporates mechanistic information is a focal point of PNNL SBR Scientific Focus Area (SFA) research under Theme Area 2 (Pore scale reactive transport and upscaling).

We are exploring the use of pore-scale experimentation and modeling as a vehicle for quantitatively transferring microscale information to macroscale simulations. We have developed and adapted two pore-scale simulation techniques (Computational Fluid Dynamics or CFD and Smoothed Particle Hydrodynamics or SPH) to simulate flow and transport at the pore scale in large three-dimensional volumes using high-performance computing resources. To bridge the gap between pore-scale processes and field-scale heterogenous facies distributions, we aim to perform simulations over cm- to dm-scale volumes (representative elementary volumes) with pore-scale resolution and process descriptions. We have performed the first known simulation of pore-scale flow and tracer transport on a column-scale domain, based on an intact core collected at the Hanford IFRC site and characterized using x-ray microtomography by other SFA investigators. We compare the results of our tracer simulation with observations of tracer breakthrough in laboratory experiments conducted on the same core. We also present results of pore-scale simulations in synthetic porous geometry that incorporate diffusion into the solid phase (intragranular diffusion). The results of these simulations are cast in terms of two upscaled models: (1) a multirate mass-transfer model, and (2) a waiting time distribution model. Diffusive mass transfer also occurs in IFRC sediments between macropores and microporous aggregates or coatings. We have developed and tested a multiscale hybrid model that couples pore-scale simulation of macropore flow and transport with continuum approximation of diffusion in microporous regions. Our novel capability to solve pore-scale models on continuum-scale domains positions us to develop a field-scale simulation (based on the Hanford IFRC site) in which pore-scale models are used directly to quantify model forms and parameters at the hydrofacies scale. We are now initiating that activity in conjunction with investigators in Theme Area 3 (Field-scale microbial ecology and biogeochemistry). The IFRC site is influenced by daily and seasonal river stage fluctuations that cause fluctuating water table gradients. We have developed methods to study effects of fluctuating flow direction on solute transport at the pore scale, with numerical volume averaging to quantify dispersion, and are testing those in 2D model systems; these will be expanded to fully 3D systems in the near future. We are also developing simulations of micromodel experiments by other SFA investigators, including experiments involving microbially mediated metal reduction.

Identification and Characterization of Microbial Proteins Important for Molecular Biogeochemical Electron Transfer Reactions

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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As part of the PNNL SBR SFA's Molecular and Microscopic Electron Transfer Processes Science Theme, this research project focuses on establishing a molecular-level understanding of key microbial protein-mediated Fe(II/III) redox reactions in the subsurface, and their constitutive relationships to redox transformation of U(VI/IV), Tc(VII/IV), and Pu(IV/III). Redox proteins of Hanford-relevant and model microorganisms are being investigated using complementary laboratory experiments and modeling to elucidate the electron transfer reactions that affect the availability and supply of reactive Fe(II). The results of these studies will contribute to resolution of PNNL SBR SFA hypotheses pertaining to the role of subsurface microenvironments and transition zones as dominant regions of contaminant oxidation-reduction reactions at Hanford.

We identified and characterized UndA-HRCR6, a *c*-type cytochrome (*c*-Cyt) of a *Shewanella* strain isolated from the Hanford Reach of the Columbia River (HRCR). In *S. oneidensis* MR-1 (MR-1), UndA-HRCR6 (1) restored an outer membrane cytochrome-deficient mutant's ability to reduce ferrihydrite to 40% of that for the wild type, (2) increased extracellular formation of UO_2 associated with the outer membrane and extracellular polymeric substances in a U(VI) reduction assay and (3) was secreted to the extracellular environment by the bacterial type II secretion system. UndA-HRCR6 was purified from the membrane fraction following its overexpression in MR-1 cells. Purified UndA-HRCR6 possessed 11 hemes and reduced ferric chelates *in vitro*. Collectively, these results show that UndA-HRCR6 is an outer membrane endecaheme *c*-Cyt and can serve as an extracellular metal reductase with functions similar to that of MR-1 outer membrane *c*-Cyts MtrC and OmcA. UndA-HRCR6 has been crystallized, and efforts for determining its molecular structure are under way.

The structure of MR-1 MtrF (an MtrC homologue) was determined at a resolution of ~3 Å. Results showed that MtrF was folded into four distinct domains in which Domains I and III were putative flavin-binding sites and Domains II and IV bound 10 hemes. It is proposed that Domain II interacts with solid and aqueous metal substrates, and Domain IV physically interacts with MtrDE complex. To test the latter prediction, MtrDEF were co-expressed in MR-1 cells. Following their co-expression, MtrDEF co-purified, indicating that, like MtrABC, they form a complex. The interaction regions among MtrDEF are being investigated by chemical cross-linking in conjunction with identification of the cross-linking sites by mass spectrometry. The biochemical, thermodynamic and electrochemical properties of MtrC, MtrF and OmcA were also compared under identical conditions by using UV-visible spectropotentiometric titrations, electron paramagnetic resonance and protein film voltammetry to identify common and distinct features of these *c*-Cyts.

To identify microbial proteins important for Fe(II) oxidation, the sequenced genomes of neutrophilic Fe(II)-oxidizing bacteria *Dechloromonas agitate* RCB, *Gallionella ferruginea* ES-2, and *Sideroxydans lithotrophicus* ES-1 were surveyed for homologues of the genes encoding microbial proteins that were known to be involved in extracellular electron transfer reactions. Results revealed that all bacterial genomes possessed MtrAB homologues. In MR-1, MtrAB form a tight protein complex involved in the transfer of electrons across the outer membrane. To characterize their roles in Fe(II) oxidation, the genes encoding MtrA homologues from these bacteria were cloned, and the MtrA of *S. lithotrophicus* ES-1 (SL-MtrA) was purified. Initial characterization of purified SL-MtrA focused on its ability to oxidize Fe(II) and on comparison of its biochemical properties with that of MR-1 MtrA.

Determining Individual Mineral Contributions to U(VI) Adsorption in a Contaminated Aquifer Sediment: A Fluorescence Spectroscopy Study

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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As part of the PNNL SBR SFA's Molecular and Microscopic Electron Transfer Processes Science Theme, this research focuses on determining individual mineralogical contributions to U(VI) adsorption in a fine-grained vadose zone aquifer sediment from the Hanford 300 area (SPP1 GWF), by a combined approach of liquid helium temperature (LHeT) laser-induced time-resolved fluorescence (TRLIF) spectroscopy and chemometric analysis.

Uranium is a major subsurface contaminant at the Hanford site and many other U.S. DOE facilities. Subsurface sediments are heterogeneous mixtures of mineral phases, and evidence has suggested that multiple uranium hosts exist. For the large majority of contaminated soil and sediment sites where the overall uranium concentration is low (< 25 ppm), the concentration of dissolved uranium in the aqueous phase is determined by its surface complexation to various mineral phases. Knowledge of the surface complexed species and the host mineral phases (surface speciation) can support a robust understanding of the adsorption processes for geochemical modeling.

In this work, batch adsorption experiments of U(VI) on the sediment and a series of reference minerals--montmorillonite, illite, Michigan chlorite, North Carolina chlorite, California clinochlore, quartz and synthetic 6-line ferrihydrite (6L-Fh)--that represent the mineralogical constituents of the sediment were performed at field-relevant uranium concentrations (5×10^{-7} M and 5×10^{-6} M) in synthetic groundwater (pH 8.1). Surface-area-normalized K_d values displayed the affinity series of 6L-Fh > North Carolina chlorite ≈ California clinochlore > quartz ≈ Michigan chlorite > illite > montmorillonite. The high affinity of chlorite and its weathering products, such as ferrihydrite, for U(VI), implies that chlorites and associated phases may play important but as yet undocumented roles in U(VI) adsorption in the field. LHeT TRLIF spectra were recorded for both the wet U-bearing solid pastes and the supernatants, and chemometric analysis was performed on the resulting spectra. The fluorescence spectra of U(VI) adsorbed on the reference minerals displayed three characteristic patterns: (1) on quartz and SPP1 GWF, a weaker first vibronic band appeared at ~ 481 nm and followed by overlapping bands at 499 nm, 520 nm and 541 nm; (2) on montmorillonite, North Carolina chlorite, California clinochlore, and Fithian illite, the first band at ~ 481 nm became much stronger and followed by a set of almost evenly spaced bands at ~ 501 nm, 522 nm and 544 nm; and (3) on 6L-Fh and Michigan chlorite, only weak and unresolved spectra that were red-shifted relative to the others were observed. Such weak spectra likely resulted from fluorescence quenching by Fe.

Both time-resolved spectra and asynchronous 2D correlation analysis of the sediment spectra at different delay times indicated that two major adsorbed U(VI) species were present in the sediment that resembled U(VI) adsorbed on quartz and phyllosilicates. Simulations of the normalized fluorescence spectra confirmed that the U(VI) speciation in the sediment was best represented by a linear combination of U(VI) adsorbed on quartz (90%) and phyllosilicates (10%). This suggests that a Component Additive model might be a workable approach in this case, given proper adjustment of the surface site densities, reactive surface area, and/or surface complex stability constants for the individual phases. The low fluorescence quantum yield for U(VI) adsorbed on phyllosilicates implied that its fractional contribution to U(VI) speciation may be much higher than 10%.

Spectral comparison with literature data suggested that U(VI) exists primarily as inner-sphere complexes on quartz and as surface U(VI) tricarbonate complexes on phyllosilicates. Since the spectral motif of uranium in SPP1 GWF is common to many Hanford sediments, the present work offers the prospect of more realistic surface complexation modeling in these complex sediment assemblages. Phyllosilicates are important in U(VI) adsorption and retention in most subsurface sediments containing a measurable silt and clay content. The similar, uranyl-tricarbonate-like spectra on the different phyllosilicates studied suggest a common type of surface U(VI) adsorption site in weakly basic groundwater.

Investigating Effects of Coupled Biogeochemical and Hydrodynamic Processes on Contaminant Fate and Transport in Model Pore-Scale Microenvironments

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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Microenvironments and transition zones dominate the subsurface biogeochemical cycling of key contaminants at the Hanford site, with strong effects resulting from the coupling of chemical reactions, physical transport, and microbiological processes. Understanding the impact of pore-scale environments (i.e., spatial heterogeneity, chemical gradients, and redox potential) is essential for modeling contaminant fate and transport in the subsurface. The driver for biogeochemical processes at the pore-scale changes from macroscopic advection to microscale diffusion, and this has a significant effect on the retention of soluble, highly mobile contaminants. This project is focused on developing a pore-scale understanding of factors that control the reduction of the highly soluble radionuclides pertechnetate $[Tc(VII)O_4^-]$ and uranyl $[U(VI)O_2]^{2+}$ to sparingly soluble $[Tc(IV)O_2.H_2O]$ and $[U(IV)O_2.H_2O]$ by microbes and mineral phases relevant to the Hanford subsurface.

Micromodels with a range of pore structures, multiple inlet/outlet ports, and surfaces sputter coated with Fe(III) oxides (i.e., hematite) are used to study coupled reactive transport processes at the pore scale. Cell colonization and growth in pore spaces of 180 µm has been demonstrated with *Geobacter sulfurreducens* using fluorescent protein expression vectors and microscopy, while effluent geochemistry has shown the complete consumption of electron donor (acetate) across the anaerobic zones. Fe-cycling has been simulated by creating mixing zones between aerobic and anaerobic groundwater containing aqueous Fe(II) in the micromodel with multiple inlets. Fe K-edge X-ray Microprobe (XMP) and X-ray absorption spectroscopy (XAS), carried out on 20-ID at the Advanced Photon Source, showed that Fe(II) precipitated as an amorphous Fe(III) oxide upon contact with aerobic groundwater. Fe(III) oxide was subsequently colonized by *G. sulfurreducens* cells, and microbially induced reductive dissolution of the Fe(III) to Fe(II) occurred in the presence of a humic analogue (AQDS). *G. sulfurreducens* cells were also found to colonize and reduce crystalline Fe(III) oxides in hematite-coated micromodels.

The Fe-containing micromodels will be used in experiments to assess the effect of the *G. sulfurreducens* cells and the mixed Fe(II)/Fe(III) mineral phase, on Tc(VII) and U(IV) reduction. The reduced Tc(IV) and U(IV) phases will subsequently be exposed to oxygenated meteoric water to assess potential differences in the susceptibility of the reduced radionuclide phases to re-oxidation. XMP and XAS will be used to map the distribution, oxidation state. and structural environment of Fe, U and Tc in the micromodels. Preliminary experiments to map U precipitates in the micromodels showed that they are associated with the biomass in the pore spaces. Micromodels with heterogeneous pore structures will be used in these experiments to investigate the effects of advection-dominated versus diffusion-dominated flow regimes on contaminant reduction and re-oxidation. Future experiments will also focus on the enrichment of key bacterial species from Hanford groundwater using micromodels. By flowing groundwater through Fe(III)-containing micromodels, we aim to enrich strains that carry out Fe(III)-reduction in the Hanford subsurface. Subsequent molecular analysis of enriched biomass will aid in identifying such species.

Through flexibility in micromodel design, combined with advanced characterization techniques, a range of pore-structures with distinct physical, chemical, and biological properties can be probed to provide a better understanding of subsurface biogeochemical redox processes occurring in *dynamic flow-through systems at the pore scale*--and their impact on the fate and transport of key contaminants.

SLAC SFA Project Overview: Biogeochemical Processes Governing the Speciation, Dynamics, and Stability of Uranium in Reduced Aquifers

SLAC SFA (Laboratory Research Manager: John Bargar)

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The biogeochemistry of U(IV) is of critical importance to uranium fate and transport in reduced aquifers. Manipulation of the redox state of uranium, from the U(VI) state to the relatively immobile U(IV) state, has been intensively studied as a means to attenuate uranium in groundwater, and there is increasing evidence that naturally reduced U(IV) plays important roles in the maintenance of large and persistent uranium plumes. The chemical and physical forms of U(IV), as well as the biogeochemical processes by which they form and transform, profoundly influence U(IV) behavior in contaminated aquifers. In spite of their importance, our understanding of the fundamental biogeochemical factors controlling U(IV) behavior in aquifers is nascent. The identity of U(IV) species that occur in aquifers, their evolution with time and changing aquifer conditions, their relationship to microbial community structure, and their rates of transformation are poorly known.

The SLAC SFA program is using a suite of laboratory and innovative field-based approaches that combine microbiological, x-ray, and microscopy tools to develop a deeper and fundamental understanding of U(IV) speciation and dynamics in aquifers and their relationship to biogeochemical conditions and Fe redox cycling. This work has been accomplished by focusing on the following research questions: (1) What are the fundamental compositional, structural, and microbiological controls over the stability of biogenic nano-uraninite—a product of biological U(VI) reduction—in aquifers? Because its composition and thermodynamic and kinetic parameters are relatively well defined, uraninite also provides a useful proxy for other, less well-characterized forms of U(IV) in the subsurface; (2) What other forms of U(IV), such as monomeric complexes, are produced by biological U(VI) reduction, and what controls their production relative to uraninite? (3) What is the occurrence, nature, and stability of these and similar U(IV) species in time and space in naturally and artificially reduced aquifers? and (4) What is the impact of (bio)geochemical iron oxide structure, composition, and redox cycling on uranium attenuation?

Work in the past year has produced a molecular-scale structural model for the uraninite-water interface, a deeper understanding of the biogeochemical “switch” that determines whether biogenic uraninite is precipitated by bacteria in the lab and field, molecular-scale structural models for poorly ordered environmental Fe (oxy)hydroxides and for uranium incorporated in Fe oxides. This work has shown that monomeric U(IV) complexes are critical to uranium fate and transport in sediments reduced in the field. Starting in FY 2012, we will use similar approaches, augmented with microbial community analyses and in-well and laboratory diffusion-limited reactor studies, to elucidate time-dependent molecular-scale structures and compositions of U(IV) complexes and their stability and fate in biostimulated as well as naturally reduced aquifers. By characterizing structure-speciation-reactivity-microbial community relationships and linked redox processes over a hierarchy of scales from angstroms to centimeters, we are developing new conceptual paradigms and quantifying rate/stability parameters that provide the basis for improved biogeochemical models of subsurface uranium fate and transport.

Speciation and Dynamics of Reduced Uranium in the Old Rifle Aquifer

SLAC SFA (Laboratory Research Manager: John Bargar)

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The chemical and physical forms of U(IV), as well as the biogeochemical processes by which they form and transform, profoundly influence the behavior of uranium in reduced contaminated sediments. Obtaining this information for sediments biostimulated *in situ*, i.e., in the field, has been one of the most important and difficult scientific challenges in the field of uranium bioremediation. Direct, frequent, and robust characterization of U(IV) species in reduced field sediments has been difficult because of the low concentrations of uranium present (typically < 10 ppm), and the cost and/or difficulty of drilling to recover large sample sets. Historically, the product of stimulated bioreduction of U(VI) to U(IV) has been assumed to be uraninite (UO_2). However, recent laboratory studies have shown that biologically reduced U(IV) can also occur as a number of different monomeric complexes, and monomeric U(IV)-Fe associations have been observed in surged bioreduced fines from the Oak Ridge site. These reports raise the question as to the relative importance of monomeric forms of U(IV) and of uraninite in biostimulated aquifers. There is no published systematic spectroscopy/microscopy investigation of uranium speciation in whole sediments (i.e., all size fractions and mineralogical associations present) that were biologically reduced *in situ*. As a result, the U(IV) products obtained in reduced aquifers are poorly known, a significant hindrance to anticipating and accurately modeling the long-term fate of U(IV) in the subsurface. We have developed an *in situ* technique for studying uranium reduction dynamics following electron-donor amendment to aquifers at the Rifle IFRC. This technique uses in-well columns to obtain direct access to sediment U(IV) species, evolving microbial communities, and trace and major ion groundwater constituents. Whole sediments from these *in situ* columns are being examined using x-ray and electron microscopy, x-ray absorption spectroscopy, and chemical digestions. In all cases, which include Fe-reducing and deep sulfate reducing conditions, EXAFS measurements showed that U(IV) was bonded to oxygen atoms, even when physically associated with iron sulfides. In sediments that had received electron-donor amendments in previous Rifle IFRC field experiments (2007-2008), repeated biostimulation with acetate in 2009 resulted in the production of predominantly or exclusively monomeric U(IV) complexes, a fraction of which transformed into uraninite in the aquifer over the subsequent 12-month period. Preliminary analyses of recently *in situ* acetate-stimulated sediments that had not previously received electron-donor amendment suggest the production of both monomeric U(IV) and uraninite.

This work establishes the importance of monomeric U(IV) complexes in subsurface sediments at the Rifle site and provides a conceptual framework in which previously observed U(IV) reduction products can be related. These experiments also establish that U(IV) species are dynamic in aquifers and can undergo nonoxidative transformation reactions. Future experiments will focus on the rates, mechanisms, and products of these U(IV) transformations.

Fundamental Research on the Structures and Compositions of Environmental Monomeric U(IV) Complexes

SLAC SFA (Laboratory Research Manager: John Bargar)

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In situ immobilization of uranium in a reduced form is the goal of bioremediation approaches at the Old Rifle IFRC and other U-contaminated sites. Reduced uranium is obtained through direct enzymatic as well as the abiotically mediated reduction of U(VI). Laboratory studies have recently shown that, depending on the geochemical conditions, the U(IV) product may either be crystalline nanoparticulate uraninite or monomeric complexes diffusely distributed on cells and biopolymers. We have expanded on this previous work in several ways, including additional structural characterizations of monomeric complexes on biomass and mineral surfaces, the development of extraction techniques that can distinguish between uraninite and monomeric U(IV), and the characterization of the stability of monomeric U(IV) complexes *in aquifers*. We have characterized the structures and composition of U(IV) derived from the enzymatic reduction of U(VI) using coupled U L_{III} and P K edge EXAFS and FTIR spectroscopic measurements. The emerging conceptual model for U(IV) associated with biomass appears to involve the binding of U(IV) to phosphato moeities on the cell biomass.

Monomeric U(IV) is also produced as a result of the interaction of U(VI) with phosphate-bearing Fe(II)-containing phases. In particular, biogenic vivianite, an Fe(II)-phosphate phase, catalyzes the reduction of U(VI) to a sorbed monomeric U(IV) species. A similar product is obtained when U(VI) is reacted with magnetite onto which phosphate has been pre-sorbed. Interestingly, magnetite reduced U(VI) to UO_2 in the absence of phosphate. Thus, phosphate appears to preclude the precipitation of uraninite and to play a critical role in the formation of monomeric U(IV). Our current understanding of the formation of monomeric U(IV) probably represents only a fraction of the processes leading to that product in the subsurface. We have initiated complementary experiments to characterize the sorption affinity and molecular-scale structures of U(IV) adsorbed to the surfaces of minerals such as alumina, silica, and montmorillonite.

In order to study the processes controlling the formation of monomeric U(IV), we have developed a method to preferentially extract monomeric U(IV) from mixtures with UO_2. This method allows the quantification of the contribution of monomeric U(IV) to various samples, including those produced in controlled laboratory systems or in field sediments. A significant finding revealed by this method is that even in systems that appear (based on spectroscopic results) to contain primarily UO_2, monomeric U(IV) is measurable. Thus, we expect that mixtures of U(IV) products will be formed in the subsurface and their relative reactivity will need to be characterized to accurately predict the long-term stability of U(IV) in remediated sediments. To investigate this point, mixtures of monomeric U(IV) and UO_2 were deployed in the subsurface at the Rifle IFRC during sulfate reduction. A transformation of monomeric U(IV) (but not UO_2) was observed by spectroscopy over time. This result suggests the reactivity of the monomeric U(IV) fraction and underscores the importance of further investigations.

The general themes that emerge from the study of monomeric U(IV) and its formation and reactivity in the subsurface are: it appears to form readily, it is probably a mixture of several species, and it is more labile than nanoparticulate UO_2.

Fe (oxyhydr)oxide Structure and Redox Cycling: Impact on Uranium Fate in Reducing Sediments

SLAC SFA (Laboratory Research Manager: John Bargar)

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Ferrihydrite (Fh), a common, disordered nano Fe-oxyhydroxide that precipitates on microorganisms, biofilms, and sediments at redox transition zones in aquifers, is among the most reactive of natural ferric (oxyhydr)oxides. Reduction of Fh by Fe(II) in the presence of U(VI) results in Fh transformation to more stable phases, generally goethite (Gt) and magnetite (Mt), and the incorporation of uranium into these phases. Prior research suggests that uranium incorporation begins with U(VI) adsorption. The ability of Fh to bind U(VI) and Fe(II) and the subsequent reductive transformation pathways are fundamentally controlled by the molecular-scale structure of Fh. This portion of the SLAC SFA program is focused on developing robust structural models for natural Fh that will enhance our ability to rationalize and predict its transformation pathways and uranium incorporation into the transformation products.

A series of X-PDF and laboratory studies by our group on both natural and synthetic samples have led to a new single-phase structural model for synthetic Fh, which predicts a composition of Fe8.2O8.5(OH)7.4, contains 10-12% tetrahedrally coordinated Fe(III), and indicates the presence of ~18% Fe(III) vacancies in the structure. We recently tested this model on natural Fh and found that natural Al, Si, and natural organic matter (NOM)-bearing Fh samples collected from an acid mine drainage system exhibit structural characteristics that are comparable to those of synthetic Fh. Observed differences between natural and synthetic Fh were related to the incorporation of structural impurities, primarily Al3+, and the surface poisoning effects of silica and NOM present during Fh precipitation. We expect natural Fh forming in soils and groundwater undergoing cyclical redox variations to exhibit similar structural and compositional characteristics. We further posit that cation vacancies and tetrahedral Fe(III) sites play catalytic roles in Fh reductive transformation pathways.

The incorporation of uranium into Fe-(oxyhyd)oxides during their reductive transformation provides a potentially important and recalcitrant reservoir for this contaminant in the subsurface, particularly under oxic conditions. We have studied Fe(II)-driven reductive transformation of Fh under aquifer-relevant geochemical conditions and variable initial U(VI) concentrations to assess the structural mechanisms by which Fe-(oxyhydr)oxides accommodate uranium. Gt and Mt were found to be the dominant transformation products. XAS showed that U(V/VI) was structurally incorporated into Gt at two distinct structural sites: (1) distorted octahedral Fe sites and (2) an interstitial channel-like feature of the Gt structure. We postulate that substitution of U(V/VI) into the Fe-(hydr)oxide structure is accommodated by the creation of Fe(III) vacancies and/or removal of H+. The creation of Fe3+ vacancies is supported by our work showing Fe site vacancies to be important in the Fh structure (the precursor solid phase in these experiments). These results provide a structural basis for understanding uranium attenuation during Fe-(oxyhdr)oxide redox cycling.

Subsurface Biogeochemistry of Nano-Uraninite

SLAC SFA (Laboratory Research Manager: John Bargar)

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The long-term stability of biogenic uraninite in aquifers is critical to the performance of *in situ* bioremediation strategies for uranium-contaminated sites. Stability is influenced by subsurface biogeochemistry, structure, and composition of uraninite, and coupling with pore-scale transport processes. A research approach that spans molecular-scale characterization to field-scale measurements has quantified biogeochemical controls on uraninite stability in subsurface environments.

Field-scale measurements of uraninite stability and dissolution rates were performed at the Rifle IFRC site. Biogenic uraninite was deployed in wells as suspensions in permeable membrane-sealed modules and also as a solid mixed into polyacrylamide gels (i.e., gel pucks). The uraninite was reacted in wells with oxic and suboxic groundwater. Uraninite was still present in both wells after three months. The solids recovered from the membrane-sealed cells were still entirely uraninite, and no UO_{2+x} or U(VI) solids had accumulated. Field-scale dissolution rates were measured by examining the UO_2 loss from the gel pucks. No uranium loss occurred in the suboxic well. In the oxic well, dissolution rates were slower by a factor of two for uraninite that had not had the biomass fraction removed. Biomass-free biogenic uraninite in the oxic well exhibited the fastest dissolution rates measured. However, these rates were still 50-100 times slower than rates determined in well-mixed laboratory experiments. Reactive transport modeling suggests that the slower rates in the field are caused by the modest diffusive limitations to oxygen transfer to the biogenic uraninite inherent to the experiment. The impact of diffusive transport limitations on U(IV) oxidation was confirmed in laboratory experiments with the membrane-sealed modules.

Groundwater contains abundant ions that can moderate U release from biogenic uraninite. Following previous experiments that found that adsorption of Mn^{2+} strongly inhibited the oxidative dissolution of biogenic uraninite, experiments were performed with Ca^{2+} and Zn^{2+}. Calcium only slightly inhibited dissolution, but zinc had a much greater inhibitory effect. Complementary sorption experiments verified that Zn^{2+} adsorbed more strongly than Ca^{2+} to the uraninite surface. The inhibition of dissolution by the cations is orders of magnitude stronger for oxic conditions than for anoxic conditions, which suggests that the adsorbing or surface-precipitating cation is acting to block the access of oxygen to the UO_2 surface.

Ultimately, adsorption, oxidation, and dissolution are controlled by the molecular-scale physical and electronic structure of the uraninite-water interface. Crystal truncation rod (CTR) x-ray diffraction was used to investigate the physical part of this system. Results reveal crystalline overlayers of non-stoichiometric UO_{2+x} up to 20 Å thick on the dry uraninite (111) surface. Addition of liquid water results in thinning and loss of order in the oxidized overlayer, which is consistent with dissolution of the overlayers. The rapid rearrangement of uraninite surfaces to form UO_{2+x} indicates facile disordering and partial oxidation. This high reactivity may help to explain the discrepancy between experimentally observed solubilities of uraninite and those predicted from calorimetric measurements of ΔG_{fm} of UO_2.

Student Abstracts

Contrasting Structure of Microbial Assemblages at Three Subsurface DOE Sites: Evidence from Taxa-Sampling Relationships to Optimize Experimental Design

Student Abstracts

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We characterized three subsurface bacterial assemblages from Hanford, WA; Rifle, CO; and Oak Ridge, TN using a taxa-sampling relationship. This characterization was a necessary first step in a larger reciprocal-transplant experiment of assemblages between sediments. The project was designed to test the contributions of sediment and groundwater geochemistry as well as bacterial community structure, composition and functional potential as factors impacting the assemblages' trajectories and functions during uranium bioremediation. The full-factorial experimental design, including sterile controls, consisted of 192 flow-cells, each with a volume of 15.86 cm^3. Flow-cells primarily consisted of gamma-irradiated sediment incubated with uranyl-acetate, with a small mass of nonsterilized sediment added as seed inoculum. Given the small volume of the flow-cells, it was important to minimize the amount of seed inoculum to avoid confounding the influence of geochemistry. However, it was critical to add sufficient inoculum to account for assemblage heterogeneity and ensure that each inoculum aliquot was representative of the assemblages.

We conducted a taxa-sampling relationship analysis using sieved, homogenized sediment from each site. DNA was extracted from five sediment masses ranging from 1.0 to 3.0 g, in triplicate (15 samples for each sediment), and the assemblages were analyzed using high-density 16S rRNA gene microarrays (G3 PhyloChip). We found no significant differences in detected richness at the operational taxonomic unit (OTU) or subfamily level by sample mass within a sediment type; although mean detected OTU richness differed by site (Hanford: 5,145 ± 663; Oak Ridge: 8390 ± 604; Rifle: 11,508 ± 747) (mean ± 1 s.d.). The degree of compositional overlap was low between the sediments, with 44.4% of 16,584 detected OTUs common to all three sediments. Ordination using presence/absence data at different taxonomic ranks showed that the assemblages in each sediment are significantly different and distinguishable at the phylum-level (Anosim R=0.786, $P < 0.001$). We used a combination of detected richness patterns, taxa-accumulation curves and a measure of β diversity, β_{sim}, to choose a seed inoculum mass of 2.0 g for the reciprocal-transplant experiment. β_{sim} analysis indicated that a 2.0 g mass resulted in seed inoculums that were highly similar (Hanford: 88.8 ± 1.8%; Oak Ridge: 92.2 ± 1.5%; Rifle: 91.7 ± 1.1%) (mean ± 1 s.d.).

Although the assemblages differ markedly in detected richness and composition, use of a taxa-sampling relationship has allowed us to quantify the variability, or patchiness, of the assemblages. This information has enabled us to optimize our experimental design by identifying the minimum inoculum mass required to accurately represent the assemblages. More broadly, these data demonstrate that subsurface assemblages at the three DOE sites harbor a high amount of diversity and significantly differ in structure to the extent that they are distinguishable at the phylum-level.

Construction of a Genetic System To Visualize Bacteria Performing Calcite Precipitation -Based Remediation of Contaminants in Porous Media

Student Abstracts

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The bacterial enzyme urease is common among native aquifer communities and can aid in the co-precipitation of strontium-90 into calcite (calcium carbonate) as a remediation strategy. The enzyme hydrolyzes ammonia from urea (added as a stimulating material), thus increasing pH and creating a shift in the bicarbonate equilibrium towards precipitation of calcium carbonate. In an effort to better understand how the bacteria and the enzyme will use the urea substrate when added, our team designed a study to investigate the spatial relationships and interactions of the bacteria, the calcium carbonate, and the nutrient pathways through a model porous-media reactor. The project's aim is to ultimately exploit the interactions to ensure the most widely distributed coverage of calcium carbonate throughout a network of pore space. To permit direct observation of the physiological health of the calcite-precipitating microbes, the objective of this work is to develop a model microbe that is both ureolytic and will express green fluorescent protein (gfp) that can then be used in our 2D flow-cell reactors set up for the study of calcite precipitation. One of the target microbes, *Pseudomonas aeruginosa* AH298 has a chromosomal gfp that constitutively expresses the fluorescent proteins and does not require an antibiotic to maintain the function. We used this strain as the host for a newly constructed plasmid vector made from PJN105 (part of a pBAD family of vectors) with an arabinose inducible promoter. We inserted a PCR amplification of DNA containing the urease structural genes. The urease genes came from a PUC19 plasmid extracted from the pURE14.8 line, which was constructed to be a urease positive E. coliDH5α. We will also transform an E. coli strain, AF504 which also has a chromosomal gfp constitutively expressed. Our preliminary results show that the newly constructed organism is capable of ureolysis as well as gfp expression. In a urea broth test, arabinose was found to induce transcription of the genes as expected; however, a leaky promoter in PJN105 allows for some mid-level of urease expression all the time, regardless of whether arabinose is present. The next step will be to sequence the roughly 8 kB insert section of the vector that contains the urease structural genes. This new organism will be used in our flow-cell reactors that simulate porous media. The ability to visualize the cells over time while in the flow-cell system will allow investigation of cell health and how the system responds to various flow regimes and amounts of urea added. This information will be incorporated into an overall strategy that will allow aquifer managers to conduct remediation efforts towards the most effective distribution of calcite throughout an aquifer.

Sorption of U(VI) to *G. uraniireducens* and *A. palmae* under Old Rifle Conditions

Student Abstracts

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Microbial reduction as a remediation method for uranium-contaminated Department of Energy (DOE) sites has been explored with promising results (Lovley et al., 1991; Lovley et al., 1993; Suzuki, et al., 2006; Merroun et al., 2008). Although transport models have been improved to include variations in geochemical concentration, reductive microbial processes and adsorption of uranium to minerals (Fang et al., 2005; Yabusaki et al., 2007), they do not incorporate the presence of microbes as possible sorption surfaces that may influence the overall transport of uranium.

Our overall objective is to examine U(VI) sorption to biomass by determining partition coefficients between U(VI) and the microbial species of *Geobacter uraniireducens* and *Acholeplasma palmae*. Once these partition coefficients are obtained, they will be incorporated into a thermodynamic model with the geochemical parameters of the Old Rifle Site.

Preliminary results indicate that U(VI) sorbs more strongly, 1000X, to bacteria at low levels of dissolved inorganic carbon (DIC) and decreases as DIC increases. U(VI) sorption to the surface of *G. uraniireducens* is 10X stronger than to the surface of *A. palmae*, and in high-DIC waters is comparable in strength to reported U(VI)- mineral surface sorption. While the concentration of *G. uraniireducens* during and after remediation results in a relatively small sorption site density, the possibility still persists that sorption to *G. uraniireducens* may retard uranium transport at the geochemical gradient between low O_2, high DIC groundwater and oxygenated, low DIC surface water equilibrated with the atmosphere. Also, *Geobacter* are planktonic during reduction and may travel with groundwater to areas of noniron reducing and lower DIC, thus resulting in high densities of sorbed bacteria, and consequently strong sorption C.

Colloid Transport under Field Conditions in Vadose Zone Lysimeters at the Semi-Arid Hanford Site

Student Abstracts

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The objective of this study is to quantify colloid transport under field conditions at the Hanford site. Specifically, we investigate colloid movement under natural and elevated precipitation. We used lysimeters at the Field Lysimeter Facility at the Hanford 300 Area. The lysimeters are 7.6 m deep and have been filled with Hanford sediments in 1978. Four lysimeters were instrumented with fiber glass wick samplers at 1, 2, 4, and 7 ft below surface to collect pore water. Colloidal tracers (two types of polystyrene spheres and intrinsic Eu hydroxide colloids) were applied to the lysimeter surfaces in March 2009. The polystyrene spheres were 200 nm and 20 nm in diameter, and the EU colloids were 100 nm in diameter. One lysimeter was exposed to natural precipitation, and three other lysimeters received irrigation proportional to the natural precipitation, but in larger amounts. One lysimeter was irrigated with twice the amount of annual recharge at the site, one lysimeter received four times the annual recharge, and one lysimeter received four times the annual recharge plus a simulated Chinook event. Porewater was periodically collected from the fiber glass wicks, and analyzed for porewater chemistry and colloid contents. In August 2010, we also took soil cores to a depth of 30 cm to determine a depth profile for the colloid concentrations. Concentrations of the polystyrene spheres were measured by fluorescent spectrometry and Eu colloids were quantified by ICP-MS. Water contents in the lysimeters were monitored with a neutron probe. Depth profiles of water contents in the different lysimeters showed that during the summer months, the lysimeters dried up close to the surface. The drying was particularly pronounced in the lysimeter that was exposed to atmospheric conditions. A distinct infiltration front could be observed in the irrigated lysimeters during the winter months. The infiltration front moved deepest in the lysimeter that received the Chinook event. The amount of porewater collected by the wicks was, in general, proportional to the amount of irrigation at the surface. Less outflow water was collected during the dry summer months. Analysis of the colloid concentrations in the porewater showed that both polystyrene and Eu colloids were detected down to 2 feet depth so far. Transmission electron microscope images confirmed the presence of all three types of colloids in the porewater. However, the total amount of colloids detected so far in the outflow samples is low (0.1 to 2%). Most of the colloids still remain in the top 30 cm of the sediments.

Understanding the Local Structure of Contaminant Incorporation in Weathered Sediments with Solid State Nuclear Magnetic Resonance

Student Abstracts

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The rate at which radioactive isotopes move through the vadose zone will be a key factor in deciding how to address the remediation of high-level radioactive waste at sites of U.S. plutonium production. Understanding how Cs^+ and Sr^{2+} interact with and are incorporated into native sediments will provide insight into the physical mechanism underlying waste migration. Solid-state nuclear magnetic resonance (NMR) provides an atomic-scale view of changes in native sediments due to interaction with caustic tank leachates and the corresponding incorporation of elements within these neophases.

The effects of simulated caustic waste leachate on sediments native to the Hanford site have been studied by ^{27}Al and ^{29}Si NMR. Dissolution of quartz and kaolinite species has previously been observed with a simultaneous precipitation of several feldspathoid neophases. Multiple-quantum magic angle spinning (MQMAS) NMR experiments provide ^{27}Al spectra of sufficient resolution to confirm the assignment of distinct feldspathoid species. Two of these species have been identified as sodalite and cancrinite in the bulk X-ray diffraction experiments of our collaborators.

While NMR provides direct information detailing the local environments of cations in complex solids, direct investigation of Cs^+ and Sr^{2+} has historically been hindered by the low NMR sensitivity of ^{133}Cs and ^{87}Sr. Recently developed sensitivity-enhanced NMR experiments, including the double frequency sweep (DFS) pulse sequence, have allowed us to observe ^{87}Sr in favorable systems. Planned ^{133}Cs NMR experiments in weathered sediments, building on the advances made in ^{87}Sr NMR, will provide a greater understanding of contaminant incorporation. Extended X-ray absorption fine structure (EXAFS) analyses by our co-investigators have indicated that strontium initially precipitates under simulated weathering in a carbonate-type phase; we have therefore prepared a series of calcium-strontium carbonate solid solutions for further study. Initial ^{87}Sr-NMR investigations have indicated that a strontianite analog phase is formed. Determining the local environment of strontium is complicated by the interaction of the quadrupole moment of ^{87}Sr with surrounding charges; as a result independent sites are not distinguishable in the ^{87}Sr NMR spectra. Electronic structure calculations have helped to provide further insights into these spectra, providing constraints on the degree of ordering present in these materials. It is expected that further instrumental improvements will aid in producing NMR spectra of increasing resolution.

The Effect of Fulvic Acid and Other Organic Ligands on the Sorption of Plutonium to Minerals

Student Abstracts

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Understanding plutonium geochemical behavior is imperative to the development of schemes for remediation of plutonium environmental contamination and accurate assessment of risks posed by the disposal of plutonium bearing wastes. The primary mechanism of plutonium mobility in the environment is subsurface transport. The mobility of plutonium is significantly influenced by redox and complexation reactions. Although the effects of surface-mediated redox reactions on plutonium's subsurface mobility have been previously documented, little has been done to determine the impact of organic ligands on sorption behavior and oxidation states. To adequately predict the behavior of plutonium in the environment, the influence of natural organic matter on plutonium geochemical behavior must be understood. This work primarily investigates the complexation of plutonium with fulvic acid and subsequent influence on sorption to gibbsite. Sorption of plutonium in the presence of desferrioxamine B (DFOB), citric acid, and humic acid was also examined to determine the influence of organic ligand character on plutonium sorption. These ligands are ubiquitous in the environment, and their presence generally drives plutonium to the +4 oxidation state in surface water and groundwater. The ligands will likely readily complex with plutonium, resulting in either increased solubility due to stabilization in the aqueous phase, or increased sorption due to the formation of ternary surface complexes.

Batch sorption experiments involving ternary systems containing plutonium, gibbsite, and one of the studied ligands at a concentration of 5 or 50 mg/L have revealed a definite impact of the ligands when compared with the binary system containing only plutonium and gibbsite. In the binary system, greater than 90% of the plutonium sorbed to the gibbsite. However, the addition of citric acid, DFOB, or fulvic acid caused a significant fraction of plutonium to remain in the aqueous phase, dependent on the pH of the system. The sorption experiments involving humic acid presented a drastic difference when the aqueous phase was separated by centrifugation versus filtration, indicating the possibility of an aggregated humic acid–plutonium particle that could be filtered from solution but not centrifuged. Also, it appears that humic acid may form a ternary surface complex with plutonium at low pH values, leading to increased sorption.

Solubility experiments involving Pu(IV) and fulvic acid were performed in an oxygen-free environment. PuO_2 nanoparticles were filtered from solution, and the aqueous-phase plutonium concentration was measured. Fulvic acid was subsequently added to the system, and an increased aqueous phase concentration of plutonium was observed over a period of two weeks. Upon reaching equilibrium, the data will be used to determine Pu-fulvic acid stability constants.

This work is part of the Transuranic Subsurface Transport Scientific Focus Area project "Environmental Transport of Pu: Geochemical Processes at Femtomolar Concentrations and Nanometer Scales" led by Annie Kersting (PI) and Mavrik Zavarin (Co-PI) of Lawrence Livermore National Laboratory.

Direct Geoelectrical Observations of Mass Transfer at the Lab Scale

Student Abstracts

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The processes governing groundwater contaminant transport are poorly understood due to an incomplete understanding of mass transfer. At many Department of Energy (DOE) sites, tailing behavior-- a long, slow decrease of concentration in time-- is observed that cannot be explained by the classical advection-dispersion transport equation. Tailing behavior can be explained by exchange between a less-mobile and mobile domain. However, the rate and scale-dependency of mass transfer is poorly understood. Standard fluid samples only measure the mobile phase, while electrical resistivity measurements, a function of the total solutes of both the immobile and mobile domain, are used to observe mass exchange between the two domains. The synthesis of co-located bulk electrical conductivity and fluid conductivity measurements can be used to calculate the mass-transfer rate through a temporal-moment analysis. However, measurements of the parameters controlling this exchange have yet to be performed outside of numerical modeling.

This work involves handling the direct-current (DC) resistivity portion of the project, including building of columns, running the experiments, and analyzing the results. Here, we directly measure mass-transfer rates at the lab scale using temporal moments from bulk and fluid conductivity measurements. Column tracer tests are performed on well-sorted sand and the porous zeolite clinoptilolite. The zeolite's internal porous network acts as an immobile domain. Using sodium chloride solution as a tracer, tracer experiments are performed on the two media at various flow rates, with potential electrodes used for electrical geophysics spaced every 2.5 cm on the sides of the columns, in addition to current electrodes placed at the top and bottom to measure bulk conductivity. Fluid electrical conductivity is measured with a flow-through probe at the end of the column. Parametric sweeps are performed in COMSOL Multiphysics by adjusting parameters, including the mobile and immobile porosity and the mass-transfer rate. The simulation results are compared to the measured fluid conductivity of the mobile domain, and the best-fit parameters are determined by the smallest root mean square error between the simulations. Geophysical inversions performed in R3t, a 3D Occam's type solution code, provide a detailed spatial and temporal resolution of tracer movement throughout the column. The mass-transfer rates will be determined using temporal moments from the bulk and fluid electrical conductivity measurements and compared to best-fit results from the parametric sweeps.

Uranium(VI) Adsorption to Manganese Oxides at Conditions Relevant to Contaminated Subsurface Environments

Student Abstracts

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The stability of U(IV) products produced during *in situ* bioreductive immobilization of uranium contamination can determine the success of bioremediation strategies. Manganese is present at appreciable concentration at several uranium-contaminated sites, and coupling of the biogeochemical cycles of uranium and manganese can affect the fate and transport of uranium. Manganese can influence uranium speciation, in ways that may either promote or degrade uranium stability in the subsurface. Manganese oxide (MnO_2), which can be produced biologically even at low dissolved oxygen concentration, can act as a powerful oxidant that accelerates the oxidative dissolution of UO_2 and the oxidation of other U(IV) species. However, MnO_2 is also a strong sorbent for metal cations and can retard the transport of U(VI). An experimental study is under way to improve our understanding of the balance between the antagonistic and beneficial effects of the coupled biogeochemical cycles of manganese and uranium.

Knowledge of U(VI) adsorption to MnO_2 is a critical step in understanding uranium-manganese redox interactions. The adsorption of U(VI) to synthetic δ-MnO_2 and biogenic MnO_2 has been systematically investigated at conditions relevant to subsurface environments. Equilibrium adsorption was measured in batch experiments over a wide range of pH and at varied dissolved inorganic carbonate (DIC) and total U(VI) concentrations. At equilibrium, U(VI) adsorbed strongly to MnO_2 above pH 4. Carbonate had a negative effect on U(VI) adsorption above pH 8 due to the formation of stable nonadsorbing U(VI)-carbonate complexes. Zeta potential measurements provided information on the effect of U(VI) adsorption on the surface potential that helped constrain the possible forms of the adsorbed U(VI). A surface complexation model was developed that could successfully fit the experimentally-measured U(VI) sorption over the full range of water chemistry conditions studied. U(VI)-loaded MnO_2 samples from the adsorption experiments were collected and will be analyzed by X-ray absorption fine structure (XAFS) spectroscopy to determine the molecular-scale structure of the adsorbed U(VI) and the effects of pH and carbonate on the structure.

Building on this knowledge regarding U(VI) sorption to manganese oxides, the next step of the project is to investigate oxidation-reduction reactions involving U(IV) species and manganese oxides. A multichamber reactor will be employed to access the degree to which UO_2 oxidation by MnO_2 requires direct physical contact. Given that soluble Mn(III) can also act as an oxidant in the subsurface, the importance of its availability and mobility to U(IV) oxidation will also be investigated by using a Mn(III) stabilizing ligand such as pyrophosphate.

Is Soil Natural Organic Matter a Sink or Source for Mobile Radioiodine (^{129}I) at the Savannah River Site?

Student Abstracts

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I-129 is one of the three major radiation risk contributors to the public as a consequence of past nuclear reprocessing activities at Department of Energy (DOE) facilities. Elevated levels of ^{129}I are present in the wetland soils of F-Area of Savannah River Site, which used to be an isotope separation facility for the production of nuclear weapons components. The ^{129}I in soils is bound predominantly to soil organic matter (SOM). Measurements of stable ^{127}I and radioactive ^{129}I in humic acids (HAs) and fulvic acids (FAs), obtained by five successive alkaline, two glycerol, and one citric acid-alkaline extraction, demonstrated that these extractable humic substances (HS) together account for 54-56% and 46% of the total ^{127}I and ^{129}I in the soil, respectively. The remainder was likely bound to residual SOM or mineral matter. The iodine content (μg-I/g-C) generally decreased with each subsequent extract, while ^{129}I/^{127}I increased concurrently.

The coincident variations in chemical compositions, aromaticity, functional groups (e.g., aliphatic), degree of humification, relative migration in the hydrophobic interaction column, and molecular weight indicated that: (1) iodine in different HAs was bound to a small-size aromatic subunit (~10 kDa); (2) the large-size subunit (~90 kDa), which likely linked the small-size unit through some weak chemical forces (hydrogen bonds, hydrophobic or electrostatic interactions), determined the relative mobility of iodine bound to organic matter; (3) from the strong correlation between iodine contentand aromaticity in the HAs, it was suggested that iodine incorporation into the SOM via a covalentaromatic C-I bond is the key mechanism controlling iodine behavior in this system. However, this relationship is not universal for all fractions of organic matter, as evidenced from the different slopes of this relationship at the two sampling sites, as well as from different relationships among HAs and FAs. These differences in iodination are due to different SOM molecular sizes, compositions, and availability of preferred iodination sites. I-129 in the soil downstream from the contaminated site and near a wetland dropped abruptly below our detection limit (0.5 pCi-129I/g-soil), which suggests that the high SOM in the plume soil around the ^{129}I-contaminated F-Area might be a natural barrier to scavenge radioiodine released from the nuclear waste repository by forming organo-iodine compounds. Soil resuspension experiments of organic colloids showed that mobile ^{129}I was mostly associated with a low molecular weight amphiphilic organic carrier (13.5-15 kDa). SOM clearly behaves as a sink for iodine at the Savannah River Site. However, this work demonstrates that a small fraction of the SOM can also behave as a potential source, namely as a small fraction that may be readily dispersible under some environmental conditions and presumably release iodine in the organic-colloidal form. This radioiodiinated organo-colloid likely moves with the water and might migrate further into the wetlands. Results from this study provide the geochemical basis for future ^{129}I migration controls, remediation, and/or land-groundwater management strategies [1].

[1] Xu, C., Zhang, S., Ho, Y.-F., Miller, E.J., Roberts, K.A., Li, H.-P., Schwehr, K.A., Otosaka, S., Kaplan, D.I., Brinkmeyer, R., Yeager, C.M., Santschi, P.H. 2011. Is soil natural organic matter a sink or source for radioiodine (^{129}I) at the Savannah River Site? Geochim. Cosmochim. Acta, submitted.

Investigating the Versatile Contributions of *Desulfovibrio vulgaris* Strain, a Sulfate-Reducing Bacterium, to Uranium Bioremediation

Student Abstracts

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In partnership with a team from the University of Michigan, the ASU team is assessing the role of biogenic iron sulfides in the long-term sequestration of uranium (U) by sulfate-reducing bacteria. During the first year of our project, we conducted a series of experiments to evaluate the effects of environmental conditions on the growth of a *Desulfovibrio vulgaris* strain and its ability to produce iron sulfide solids with characteristics desirable for U sequestration. The working hypothesis is that conditions that enhance the production of mackinawite (Fe1+xS) are optimal, since mackinawite is generally considered the most reactive of the iron sulfides. So far, we have found that, over ranges of pH (6.5 to 8.6) and concentration ratios of lactate-to–sulfate (0.5:1 to 1.9:1) and iron-to-sulfate (0.11:1 to 1:1), *D. vulgaris* primarily produces mackinawite.

This research is elucidating that, while different concentrations of lactate, sulfate, aqueous Fe(II), and pH within a certain range do not affect bacterial growth, they can affect total iron sulfide production and crystallinity. Low pH values, such as 5.6, considerably inhibit FeS formation, lowering the yield of iron sulfide. This can be explained by the relatively high solubility of mackinawite at pH below 6. High lactate-to-sulfate ratio (1.9:1), high sulfate-to-iron ratio (1:0.5), or weakly acidic pH (6.5) enhances mackinawite crystallization. Higher degrees of crystallinity typically result in more stable and less redox-reactive solid. From the perspective of biogenically producing redox-reactive iron sulfides for retarding uraninite oxidation and mobilization of U, these crystallinity differences may be important and are being tested by the UM team. In addition, we tested various alternative electron donors, such as hydrogen and pyruvate, and showed that they can enhance biogenic production of mackinawite.

We also found that other reduced iron phases are formed under certain environmental conditions. For example, a lactate-to-sulfate ratio as high as 1.9:1 led to greigite (Fe_3S_4) formation, a generally less redox-active iron sulfide mineral compared to mackinawite. When a high concentration of iron (above 33 mM, in stoichiometric molar excess of biogenic sulfide) was present initially during incubation, formation of vivianite [$Fe_3(PO_4)_2 \cdot 8(H_2O)$] was observed along with mackinawite. This points to the importance of having a molar excess of sulfide compared to Fe(II) for biogenically producing mackinawite rather that other reduced Fe phases under sulfate-reducing conditions.

During the second year, we have initiated a detailed investigation of uranium reduction by the *D. vulgaris* strain. We observed enzymatic uranium(VI) reduction in batch experiments, with 90% removal within 10 hours. *D. vulgaris* reduced U(VI) fastest under sulfate-reducing conditions in absence of aqueous Fe(II). These results implicate chemical reduction of U(VI) by biogenic H_2S in addition to enzymatic reduction, but not by aqueous Fe(II). Thus, the results illustrate the versatile contributions by the sulfate-reducing bacteria to U(VI) reduction by direct (enzymatic) and indirect (chemical) pathways. We are using electron-flow balances to provide insight into the mechanism of U(VI) reduction.

Modeling Community Metabolism during Uranium Bioremediation

Student Abstracts

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During uranium bioremediation at a contaminated site in Rifle, CO, there is an initial bloom of *Geobacter* accompanied by removal of U(VI) from the groundwater, followed by an increase of sulfate-reducing bacteria (SRBs), which are poor reducers of U(VI). Existing analysis suggests that there is competition between *Geobacter* and SRB, and when biologically usable and more energy efficient Fe(III) is depleted, the SRB compete more effectively for the substrate and are able to grow using the available sulfate. However, the dynamics of the microbial competition at this site is not clearly understood, motivating the need for systems-biology tools to understand the associated microbial ecology.

In order to understand the metabolic interactions in microbial communities, we have developed a dynamic genome-scale modeling approach denoted as the Dynamic Multi-species Metabolic Modeling framework. We have previously used this approach to model the community competition among Fe(III) reducers and shown that the trade-off between growth rate and biomass yield under different environments was the decisive factor controlling the microbial competition at that site. In this work, we have used this framework to create a community model of *Geobacter* and SRBs. Batch simulations of community dynamics were used to examine the cause of the late onset of sulfate-reduction using different percentages of starting inoculants of *Geobacter* and SRB. These simulations showed that no matter what the starting conditions, the *Geobacter* were able to dominate the early period of the experiment with very little change in the timing for the onset of sulfate reduction. The field-scale simulation was able to accurately predict the field data and suggests that the observed field data are due to growth-rate dynamics and not due to thermodynamic competition.

Field-scale simulation of iron addition suggests that Fe(III) addition is a potential strategy for continuous uranium removal. We simulated the simultaneous addition of acetate and Fe(III) in both batch and field conditions using the community model. This showed that the addition of Fe(III) allowed *Geobacter* species to maintain its dominance over SRB. We then computationally optimized the rate of iron and acetate addition into the subsurface. We show that it is possible to maintain the uranium concentration below the environmental safety standards.

These simulations show that simultaneous addition of acetate and Fe(III) has the potential to be an effective uranium bioremediation strategy. They also show that computational modeling of the microbial community is an important tool for designing effective strategies for practical applications in biotechnology.

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