

# Lawrence Berkeley National Laboratory

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**Title**

Subsurface Biogeochemical Research (SBR) Contractor-Grantee Workshop--Abstracts

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**Subsurface Biogeochemical Research (SBR)  
Contractor-Grantee Workshop**

**Abstracts**

**March 29–31, 2010**

**JW Marriott Hotel  
Washington, DC**

**Subsurface Biogeochemistry Research**

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## Table of Contents

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Introduction .....	9
SBR Contacts.....	11
Preliminary Agenda .....	13
<b>Abstracts</b> .....	17
<b><i>Grantee-Led Research</i></b> .....	19
Barnett.....	21
Becker .....	22
Beyenal .....	23
Burgos.....	24
Chatterjee (Seliskar) .....	25
Choreover .....	26
Clapp.....	27
Colwell.....	28
Daley .....	29
Day-Lewis .....	30
Elliot .....	31
Finneran .....	32
Flury .....	33
Geiger .....	34
Gorby .....	35
Gregory .....	36
Hayes .....	37
Helms .....	38
Hou.....	39
Hu.....	40
Johnson1.....	41
Johnson2 (Basu) .....	42
Johnson3 (Raddatz).....	43
Kaplan .....	44

Kenna.....	45
Kostka .....	46
Loeffler (Sanford) .....	47
Lovley1 (Yun) .....	48
Lovley2 (Zhuang) .....	49
Li .....	50
Linguist.....	51
Sanford .....	52
Martinez .....	53
Moser .....	54
Moysey.....	55
Murray (Allen-King) .....	56
Myneni.....	57
Nagy.....	58
Newman .....	59
Panikov .....	60
Peyton .....	61
Pfiffner.....	62
Powell.....	63
Prodan.....	64
Rajaram.....	65
Reguera (Cologgi) .....	66
Revil .....	67
Roden.....	68
Ryan.....	69
Sanchi .....	70
Schlautman .....	71
Slater .....	72
J. Smith .....	73
R. Smith .....	74
Steefel (Van Cappellen) .....	75
Stephen.....	76
Stoltz (Chovanek).....	77

Stucki (Day).....	78
Summers1 .....	79
Summers2 .....	80
Tebo .....	81
Tiedje (Yang).....	82
Tratnyek .....	83
Van Nostrand .....	84
Valocchi (Werth) .....	85
Wall .....	86
Weber .....	87
Wildenschild (Iltis) .....	88
Xu.....	89
Yee.....	90
Zhang .....	91
<b>Federal Agency-Led Research.....</b>	<b>93</b>
Curtis.....	95
Davis .....	96
<b>Joint BER-EPA-NSF-Funded Nanoparticulate Research.....</b>	<b>97</b>
Doktycz .....	99
Shang (Liu).....	100
Wan.....	101
<b>SciDAC Research.....</b>	<b>103</b>
Lichtner .....	105
<b>Integrated Field-Scale Subsurface Research Challenges (IFRC) .....</b>	<b>107</b>
Hanford IFRC—Zachara (Overview) .....	109
Hanford IFRC—Christensen .....	110
Hanford IFRC—Fredrickson.....	111
Hanford IFRC—Haggerty .....	112
Hanford IFRC—Lichtner.....	113
Hanford IFRC—McKinley .....	114
Hanford IFRC—Rockhold .....	115
Hanford IFRC—Rubin .....	116

Hanford IFRC—Vermeul .....	117
Hanford IFRC—Ward.....	118
Hanford IFRC—Zachara .....	119
Hanford IFRC—Zheng .....	120
ORNL IFRC—Brooks (Overview) .....	121
ORNL IFRC—Barnett (Savage) .....	122
ORNL IFRC—Green .....	123
ORNL IFRC—Gihring.....	124
ORNL IFRC—Gu .....	125
ORNL-IFRC—Hubbard .....	126
ORNL IFRC—Parker.....	127
ORNL IFRC—Wu.....	128
Rifle IFRC—Long (Overview).....	129
Rifle IFRC—Fox .....	130
Rifle IFRC—Jaffe .....	131
Rifle IFRC—Kerkhof.....	132
Rifle IFRC—Kukkadapu .....	133
Rifle IFRC—Peacock .....	134
Rifle IFRC—Wilkins (Banfield) .....	135
Rifle IFRC—Williams.....	136
Rifle IFRC—Yabusaki .....	137
Rifle IFRC—Yang.....	138
<b>Scientific Focus Areas (SFA) .....</b>	<b>139</b>
ANL SFA—Giometti (Overview) .....	141
ANL SFA—Boyanov.....	142
ANL SFA—Kwon.....	143
ANL SFA—Latta.....	144
ANL SFA—O’Loughlin .....	145
INL SFA—Redden (Overview) .....	146
INL SFA—Gebrehiwet.....	147
INL SFA—Huang .....	148
INL SFA—T. Johnson (Versteeg).....	149
INL SFA—Redden .....	150

LBNL SFA—Hubbard (Overview).....	151
LBNL SFA—Beller .....	152
LBNL SFA—Christensen .....	153
LBNL SFA—Nico .....	154
LBNL SFA—Steefel .....	155
LBNL SFA—Wu .....	156
LBNL SFA—Spycher .....	157
LBNL SFA—Wan .....	158
LBNL SFA—Tokunaga.....	159
LLNL SFA—Kersting (Overview).....	160
ORNL SFA—Liang (Overview).....	161
ORNL SFA—Brooks .....	162
ORNL SFA—Elias.....	163
ORNL SFA—Gu.....	164
ORNL SFA—Kucken.....	165
ORNL SFA—Parks (Johs).....	166
ORNL SFA—Schaefer .....	167
PNNL SFA—Zachara (Overview).....	168
PNNL SFA—Felmy .....	169
PNNL SFA—Lee .....	170
PNNL SFA—Lipton .....	171
PNNL SFA—Liu .....	172
PNNL SFA—Lin .....	173
PNNL SFA—Marshall.....	174
PNNL SFA—Peretyazhko .....	175
PNNL SFA—Qian .....	176
PNNL SFA—Rosso.....	177
PNNL SFA—Scheibe .....	178
PNNL SFA—Rodin (Shelobolina).....	179
PNNL SFA—Shi.....	180
PNNL SFA—Ward .....	181
PNNL SFA—Zachara (Hay) .....	182
PNNL-SFA—Zhang.....	183



SLAC SFA—Bargar (Overview) .....	184
SLAC SFA—Bargar (Overview) .....	185
SLAC SFA—Bernier-Latmani.....	186
SLAC SFA—Glammar .....	187
SLAC SFA—Michel.....	188
<b><i>Student Abstracts</i></b> .....	<b>189</b>
Cologgi.....	191
Iltis.....	192
Jasrotia.....	193
Latta .....	194
Li .....	195
Loganathan .....	196
Murakami .....	197
Salome .....	198
Stucker .....	199
Wu.....	200
Zhang.....	201

**U.S. Department of Energy—Office of Science**

***Subsurface Biogeochemical Research***

**2010 Principal Investigators Meeting**

Welcome to the 2010 Subsurface Biogeochemical Research (SBR) Principal Investigators meeting. The past year has been exciting and productive for your science and for our program. On behalf of the SBR program managers in the Climate and Environmental Sciences Division (CESD), Office of Biological and Environmental Research, we are looking forward to hearing about the progress of each of your projects and your plans for the future.

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

This year, we have a very full agenda that includes a Darcy Award lecture by Dr. Tim Scheibe from the Pacific Northwest National Laboratory (PNNL) and a Birdsall-Dreiss Award lecture by Dr. Susan Hubbard from the Lawrence Berkeley National Laboratory (LBNL) on Monday afternoon.

To accommodate the large volume of posters and the limited evening time for posters, we have added an extra poster session after lunch on Tuesday. While we have compressed the agenda somewhat to include time for the SFA sessions, we have also worked to retain the more relaxed schedule that we implemented last year. We have provided opportunities for discussion and interaction among the formal presentations. We have also provided large blocks of time for the evening poster presentations. Each SBR-funded project will be represented by a poster and at least one of the investigators. We will use these sessions to explore the progress and plans of each project.

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

Best Regards,

Paul E. Bayer

SBR Program Manager



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Contractor-Grantee Workshop  
March 29-31, 2010  
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Preliminary Agenda

**Monday, March 29**

7:00-8:30am     **Registration**

8:30-10:15am   **Accomplishments and the Road Ahead**

8:30-8:45am     **Dr. David Thomassen**, Chief Scientist, Office of Biological and Environmental Research

8:45-9:15m      **Mr. Paul Bayer**, Climate and Environmental Sciences Division

9:15-9:45am     **Dr. David Lesmes**, Climate and Environmental Sciences Division  
**Title:** Complex System Science for Subsurface Fate and Transport

9:45-10:15am   **Dr. Juan Meza**, Lawrence Berkeley National Laboratory  
**Title:** Advanced Simulation Capability for Environmental Management

10:15-10:45am   **Break**

10:45-12:15pm   **Darcy and Birdsall-Dreiss Lecturers**

10:45-11:30     **Dr. Timothy Scheibe**, Pacific Northwest National Laboratory  
**Title:** Darcy Lecture

11:30-12:15     **Dr. Susan Hubbard**, Lawrence Berkeley National Laboratory  
**Title:** Birdsall-Dreiss Lecture

12:15-2:00pm    **Buffet Lunch** (Research Team Meetings)

2:00-5:00pm     **Breakout Sessions:** Microbiology, Biogeochemistry, and Transport (TBD)

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

## **Tuesday, March 30**

### **8:30-10:15am Plenary Session**

- 8:30-8:45am **Dr. Anna Palmisano**, Associate Director, Office of Biological and Environmental Research
- 8:45-9:15am **Dr. Mary Firestone**, U. of California Berkeley  
**Title:** Using Ecological Theory to Evaluate Microbial Community Trajectories During Uranium Bioremediation
- 9:15-9:45am **Dr. Dwayne Elias**, Oak Ridge National Laboratory  
**Title:** Progress in Understanding Mercury Biotransformations: From Molecular Modeling to Microbial Community Dynamics
- 9:45-10:15am **Dr. Yuri Gorby**, J. Craig Venter Institute  
**Title:** Bacterial Nanowires and Extracellular Electron Transfer to Heavy Metals and Radionuclides by Bacterial Isolates from DOE Field Research Centers

### **10:15-10:45am Break**

- 10:45-11:15am **Dr. Haluk Beyenal**, Washington State U.  
**Title:** Microscale Metabolic, Redox and Abiotic Reactions in Hanford 300 Area Subsurface Sediments
- 11:15-11:45pm **Dr. Jon Chorover**, U. of Arizona  
**Title:** Release of Aged Contaminants from Weathered Sediments: Effects of Sorbate Speciation on Scaling of Reactive Transport
- 11:45-12:15pm **Dr. Max Boyanov**, Argonne National Laboratory  
**Title:** The Influence of Ligands on the Formation of Non-uraninite U(IV) Phases During Biotic and Abiotic U(VI) Reduction

### **12:30-3:30pm Poster Session II – Box Lunches Provided**

### **4:00-5:00pm Plenary Session**

- 4:00-4:30pm **Dr. Annie Kersting**, Lawrence Livermore National Laboratory  
**Title:** Science Focus Area: Actinide Transport at Environmental Conditions - Nanoscale and Picomolar
- 4:30-5:00pm **Dr. Don Baer**, Pacific Northwest National Laboratory  
**Title:** New Capabilities at the Environmental Molecular Sciences Laboratory (EMSL) – a DOE/BER Scientific User Facility

### **5:30-8:00pm Poster Session III –Hors d'oeuvres and Refreshments (Cash Bar)**

### **Wednesday, March 31**

#### **8:30-10:00am Plenary Session**

- 8:30-9:00am **Dr. Yi Lu**, U. of Illinois at Urbana-Champaign  
**Title:** Development and Fundamental Understanding of Catalytic DNA Biosensors for On-site and Real-time Detection and Quantification of Radionuclides and Metal Ions
- 9:00-9:30am **Dr. Lee Slater**, Rutgers U. at Newark  
**Title:** Geophysical Characterization and Monitoring of Groundwater/Surface-Water Interaction in the Hyporheic Corridor at the Hanford 300 Area
- 9:30-10:00am **Dr. Andre Revil**, Colorado School of Mines  
**Title:** Advanced Self-potential and Induced Polarization Inversion: Development and Use for Investigating Natural Recharge Processes

#### **10:00-10:30am Break**

- 10:30-11:00am **Dr. Thomas Johnson**, U. of Illinois at Urbana-Champaign  
**Title:** Tracing Contaminant Transformation, Immobilization, and Transport Using Cr, U, and Hg Isotope Ratios
- 11:00 - 12:00pm **Dr. Margaret Torn**, Lawrence Berkeley National Laboratory  
**Title:** Carbon Cycling in Terrestrial Ecosystems: Research Challenges and Opportunities

#### **12:00pm Close-out and Adjournment**





# Abstracts



***Grantee-Led Research***

***Grantee-Led Research***



## Understanding the Subsurface Reactive Transport of Transuranic Contaminants at DOE Sites

*Grantee-Led Research*

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

We know much less about the subsurface fate of Np and Pu than we do of their fellow DOE priority contaminants Cr, Tc, and U. Our primary hypothesis is that transuranics can interact with surfaces in fundamentally different ways than other metals, metalloids, and oxyanions—namely by reductive surface precipitation from undersaturated solutions. We propose to test our hypotheses by studying the sorption of Pu and Np to representative heterogeneous subsurface materials from collaborating field sites at Hanford and SRNL. We will also connect our macroscopic laboratory observations with state-of-the-art synchrotron x-ray speciation studies to develop and quantitatively test our conceptual model.

We are developing our experimental techniques using oxidation state analogues, Th(IV) and U(VI), for Pu(IV) and Pu(VI), respectively. Like Pu(IV), Th(IV) is extremely insoluble over the pH range of natural waters, but the actual values vary widely. Thermodynamic modeling indicates that  $\text{ThO}_2(\text{s})$  is approximately six orders of magnitude less soluble than  $\text{Th}(\text{OH})_4(\text{am})$ . However, at  $\text{pH} > 2.5$ , the dissolution of  $\text{ThO}_2(\text{s})$  is irreversible, and the solubility of Th(IV) becomes controlled by  $\text{Th}(\text{OH})_4(\text{am})$  making sorption and dissolved phase transport an important process. Simultaneously, procedures have been established for preparing solutions of both Np and Pu in desired oxidation states without the addition of reagents that might interfere with subsequent reactions. Np(IV), Np(V), Np(VI), Pu(III), Pu(IV), and Pu(VI) solutions are all available for study.

We will conduct column experiments to study transuranic transport and partitioning between colloidal, dissolved, and immobile-solid phases during steady flow and during periods of transient porewater flow and chemistry. The column experiments will be carried out separately with Pu and Np and their analogues and distinguished on the basis of porewater pH, ionic strength, DOC concentration, and porous media composition. These data will be the first of their kind and critical to advancing the development of a mathematical model suitable for making inferences on Pu and Np mobility within DOE sediments. We will proceed with the theoretical development by modifying our published models for colloid-facilitated contaminant transport to appropriately account for the mass-transfer mechanisms that regulate Pu and Np mobility.

The activities and preparations for the synchrotron radiation (SR) investigations and experiments have been under way since the recent start of the research program (August 2009). We have been planning and preparing for the first preliminary SR experimental characterization of native soil materials by STXM at the ALS-MES Beamline that is planned for the ALS. These are prerequisite STXM characterization experiments for examining these same soils after they have been subjected to sorption with Pu and Np. Similar hard x-ray experiments that will give the microscale plus distributions will be performed at ALS and/or SSRL. The information to be extracted from both sets of soft and hard x-ray experiments are the overall composition, elemental distributions, speciation of the constituents, and the overall associations present. The investigations at the ALS-MES STXM will provide critical information about the importance of colloids in these systems.

## Measuring Channeled Flow in Fractured Bedrock: Application of Harmonic Hydraulic Interference Testing

*Grantee-Led Research*

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

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 that are 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 (1) harmonic interference hydraulic tests and (2) multi-tracer breakthrough tests. These tools will be confirmed at our bedrock research site using surface ground penetrating radar (GPR) to image the fracture aperture distribution and the path of tracer transport. Here, we present initial results of the hydraulic testing.

Field experiments were conducted at the Altona Flat Rock fractured sandstone to establish the value of these tests for establishing interwell connectivity. Sinusoidal head oscillation was induced in one well and the propagation of the pressure pulse through a single fracture was measured in four observation wells. Pressure response was interpreted by nonlinear regression fitting of the hydraulic diffusion equation to drawdown. Hydraulic diffusivity (transmissivity / storativity) inverted from these tests were found to be much more sensitive to local heterogeneity than traditional constant rate (Theis) tests.

Laboratory measurements of matrix diffusion are under way. The 2010 summer field season will focus on GPR measurements of fracture aperture and tracer migration. In the 2011 summer field season, we will complete the multi-tracer diffusion experiments.

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

Grantee-Led Research

H. Beyenal (PI), *Washington State U.*; J. Fredrickson, *PNNL*; J.S. McLean, *J. Craig Venter Institute*;  
B. Cao, *Washington State U.*; P.D. Majors, *PNNL*

We are applying novel microscale experimental approaches to understand how microscale biotic and abiotic reactions and their combined interactions control uranium mobility in the subsurface at Hanford's 300 Area. To initially study biotic and abiotic factors controlling uranium immobilization and remobilization, we prepared flow-through column reactors using core samples from the Hanford 300 Area IFRC site. Uranium was successfully immobilized in all the columns. U L(III) edge XANES (in collaboration with K. Kemner, Argonne National Laboratory via his support from the PNNL FSA) showed that 80-85% of uranium immobilized in organic stimulated sediment samples was in the U(IV) valence state. However, samples from nonstimulated columns contained solid phase-associated U in the U(VI) valence state, likely uranyl adsorbed to metal oxides as revealed by U L(III) edge EXAFS. In addition, Fe and Mn were solubilized from the organic stimulated column, probably due to microbial reductive dissolution.

Microscale physiochemical conditions at the interface of sediment and groundwater in the organic-stimulated sediments from the column reactors were studied using microelectrodes. Redox potential, dissolved oxygen, pH, and H<sub>2</sub> profiles in the sediments within microscale range were obtained under both aerobic and anaerobic conditions. The effects of uranium addition into the system on these physiochemical conditions were also studied. Our results showed that there were significant differences between macroscale and microscale conditions near the sediment-groundwater interface. We found the presence of H<sub>2</sub> in the sediments but not in the bulk phase. There were significant redox profiles between the bulk and in the sediments. In addition, we have developed novel microelectrodes to measure uranyl concentrations and successfully measured uranyl concentration profiles in *Shewanella oneidensis* MR-1 biofilms. NMR (EMSL) was used to monitor spatial and temporal metabolite concentrations and material-transport rates within live biofilms. Using pulsed-field gradient NMR methods, we measured *in situ* two-dimensional effective diffusion coefficient maps of water in *Shewanella oneidensis* biofilms, and obtained surface-averaged relative effective diffusion coefficient profiles. This variation of effective diffusivity by time will be critically important in modeling uranium diffusion near the subsurface. We are currently using NMR to study the live metabolism of biofilms and the metabolic and structural response to the influx of U(VI).

We completed the design of a reactor, which allowed us to poise an electrode surface to a desired potential and directly image cells for quantification. The system was tested by growing biofilms from dispersed single cells on electrodes (as analogs to solid-phase electron acceptors) and quantifying electron-transfer rates on a per cell basis over time. Remarkably, we found that even in the presence of oxygen, single cells on a surface have the ability to transfer electrons to a solid-phase electron acceptor. This indicates that very thin biofilms could generate enough reductive activity to enable dissimilatory metal reduction in subsurface microenvironments even under bulk aerobic conditions. This finding will bring new mechanistic explanations to uranium mobility in Hanford 300 Area sediments.



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

*Grantee-Led Research*

W. Burgos (PI), *Penn State U.*; H. Dong, *Miami U. of Ohio*;  
K. Kemner, *ANL*; J. Zachara, *PNNL*

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.

Because of the high abundance of phyllosilicates as the primary solid-phase redox buffer at all DOE IFRC sites, the fate and transport of U and Cr will likely be controlled by their reactivity with Fe-bearing phyllosilicates. However, thermodynamic redox properties of phyllosilicates (i.e. reduction potential,  $E^0$ ) required to model the fate and transport of U and Cr are currently lacking. Both specimen clay minerals and IFRC clay-sized fractions will be examined, in both simple background electrolytes and synthetic IFRC groundwaters, to provide sufficient understanding of the complex redox processes between phyllosilicates and U and Cr under field-relevant conditions. Our results should improve our ability to model the fate and transport of U and Cr at the field scale.

Our proposed research is based on the following related hypotheses:

- 1) Thermodynamic favorability of phyllosilicate-Fe(II) towards redox-active contaminants (U, Cr, NACs) is determined by Fe(II) concentration and phyllosilicate physicochemical properties.
- 2) Kinetic reactivity of phyllosilicate-Fe(II) towards reduction of U(VI) and Cr(VI) is controlled by the distribution of Fe(II) within each phyllosilicate mineral (surface-complexed, interlayer-exchanged, and structural Fe(II) species).
- 3) Kinetic reactivity of U(IV) oxidation by phyllosilicate-Fe(III) is determined by Fe(III) concentration and speciation within each phyllosilicate mineral.
- 4) Biological reduction of phyllosilicate-Fe(III) followed by reoxidation of phyllosilicate-Fe(II) will irreversibly alter the physicochemical properties and reactivity of iron-bearing phyllosilicates towards uranium and chromium.

We propose to study three specimen phyllosilicate minerals that represent end-members with respect to Fe(III) and Fe(II) content and anticipated reactivity towards U and Cr, and three IFRC clay-sized fractions (two from Hanford and one from Oak Ridge) that represent critically important minerals for each site. We propose to conduct bioreduction experiments with individual specimen phyllosilicate mineral or IFRC clay-sized phyllosilicate fraction inoculated with a single Fe(III)-reducing bacterium. We propose to conduct these experiments with Fe(III)-reducing isolates obtained from Hanford and Oak Ridge sediments. At the end of bioreduction, cell-clay suspensions would be pasteurized, reactive Fe(II) species would be characterized and quantified, and physicochemical properties of the phyllosilicates would be determined. The  $E^0$  values for the phyllosilicates would be measured, and the reactivity of phyllosilicate-Fe(II) towards U(VI), Cr(VI) and nitroaromatic compounds (NACs) would be tested. Bioreduced phyllosilicates would also be used in reoxidation experiments to evaluate the irreversibility of phyllosilicate-Fe(III) to phyllosilicate-Fe(II) to phyllosilicate-Fe(III), and to test the reactivity of phyllosilicate-Fe(III) towards U(IV) (uraninite) reoxidation.

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

Grantee-Led Research

C.S. Seliskar, W.R. Heineman, *U. of Cincinnati*; S.A. Bryan, S. Chatterjee (PI)—*PNNL*

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

The sensor 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  $[\text{ReO}_2(\text{py})_4]^+$ ,  $[\text{ReO}_2(\text{CN})_4]^-$ ,  $[\text{ReO}_2(\text{P}\cap\text{P})_2]^+$  and  $[\text{ReO}_2(\text{S}\cap\text{S})_2]^+$ . These complexes can be readily synthesized from  $\text{ReO}_4^-$  at ambient temperatures and display fluorescence in fluid solution. The chemistry is now being extended to analogous Tc complexes.
- Synthesized lower oxidation-state Tc-complex  $[\text{Tc}(\text{dmpe})_3]^+$  to use as a model compound for developing Tc sensors. It exhibits the important properties of solution fluorescence at ambient temperatures and reversible electrochemistry.
- Fluorescence spectroelectrochemistry initiated for  $[\text{Tc}(\text{dmpe})_3]^+$  and preconcentration at SSEBS with electrochemical modulation of fluorescence signal demonstrated.
- $\text{TcO}_4^-$  shown to preconcentrate in sensor films containing anion exchange polymers and to undergo electrochemical reduction.
- Dual analyte model system developed: preconcentrated  $\text{Pb}^{2+}$  and  $\text{Fe}^{2+}$  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  $\text{Re}(\text{diimine})(\text{CO})_3(\text{halide})$ . These will serve as models for the  $\text{Tc}(\text{diimine})(\text{CO})_3(\text{halide})$  that can readily be formed from  $\text{Tc}(\text{CO})_x(\text{halides})_{6-x}$  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.

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

*Grantee-Led Research*

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Sediments impacted by tank leakage of caustic high level radioactive waste have undergone incongruent silicate mineral weathering concurrent with contaminant uptake. In the current work, we are studying the impact of background pore water (BPW) on strontium, cesium, and iodine desorption and transport in Hanford sediments previously reacted with simulated tank waste leachate (STWL) solutions. Using lab-weathered Hanford sediments (HS) and model precipitates formed during nucleation from homogeneous STWL solutions (HN), we are (1) providing thorough characterization of reaction products over a matrix of field-relevant gradients in contaminant concentration,  $\text{PCO}_2$ , and reaction time; (2) improving molecular-scale understanding of how sorbate speciation controls contaminant desorption from weathered sediments upon removal of caustic sources; and (3) developing a mechanistic, predictive model of contaminant reactive transport under these conditions.

Characterization of STWL reaction products using EXAFS, NMR, EM and quantitative-XRD indicate the importance of STWL reaction conditions on the kinetics of mineral transformation during contact with STWL (native silicates  $\rightarrow$  zeolite  $\rightarrow$  sodalite  $\rightarrow$  cancrinite). The presence or absence of  $\text{CO}_2$  during HN affects solid phase templating and growth of either zeolite X (no  $\text{CO}_2$ ) or sodalite/cancrinite (with  $\text{CO}_2$ ), either of which sequester nitrate, Sr, and Cs into distinct sites. Concentration of  $\text{CO}_2$  also affects calcite weathering in HS, wherein greater dissolution of  $\text{Ca}^{2+}$  in absence of  $\text{CO}_2$  promotes strätlingite formation in the STWL-impacted sediments.

Saturated and unsaturated column experiments are being conducted to assess the kinetics of contaminant desorption from HS and HN products upon leaching with BPW. For sediments weathered at low contaminant concentrations, reactive transport (CrunchFlow) modeling of HS column BPW effluents is consistent with rapid I desorption from a paucity of anion exchange sites, and slower Cs desorption from illitic frayed edge sites on native illitic sediments--whereas Sr desorption is best fit by aluminosilicate dissolution reactions. However, results from HN column experiments and quantitative XRD of BPW-leached HS samples suggest that contaminant desorption from the neoformed zeolites and feldspathoids can also occur via ion exchange with minimal mineral feldspathoid dissolution. Hence, ion exchange reaction or contaminants within the neo-precipitates themselves need to be incorporated into future versions of the transport model.

Hanford sediments reacted for 12 months with various STWL solutions were also subjected to four 24 h dry/wet leaching cycles with BPW. After each drying event, large  $\text{NO}_3$  pulses were observed in effluents, but these were not accompanied by corresponding Si pulses that would signal feldspathoid dissolution. Wetting-drying increased  $\text{NO}_3$  desorption 4-5 fold over continuous flow experiments. Sr and Cs desorption is enhanced in the dry/wet cycle experiments relative to continuous saturated or unsaturated flow, except for cases where  $\text{CO}_2$  was eliminated from the STWL weathering reaction.

Our results to date indicate that STWL composition,  $\text{PCO}_2$ , and reaction time strongly influence contaminant sequestration mechanisms and, therefore, the rate and extent of subsequent remobilization and transport in Hanford vadose zone sediments.

## Evaluation of Membrane-Delivered Hydrogen for Stimulating *In Situ* Reduction of Nitrate and Uranium

*Grantee-Led Research*

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Using laboratory aquifer-sediment column studies and a parallel field-scale groundwater recirculation study, this project is evaluating the feasibility of delivering dissolved hydrogen to the subsurface to reduce and immobilize uranium in groundwater with high nitrate concentrations. The column study is comparing the efficacy of hydrogen and ethanol for: (1) achieving denitrification and uranium reduction/immobilization, (2) avoiding porous media bioclogging, and (3) minimizing U(VI) complexation and stabilization by carbonate. The field study is evaluating hydrogen transfer rates/ efficiencies and associated uranium and molybdenum reduction/ immobilization efficiencies at an *in situ* recovery (ISR) mining site. The research is being driven by the following hypotheses:

1. A sediment column supplied with membrane-delivered hydrogen will achieve significantly higher nitrate and uranium removal rates than a parallel ethanol-fed sediment column, while also resulting in significantly less bioclogging and associated decline in hydraulic conductivity.
2. A sediment column supplied with membrane-delivered hydrogen will have significantly lower carbon dioxide partial pressures and carbonate concentrations than will a parallel ethanol-fed column; the lower carbonate concentrations will make U(VI) less resistant to reduction and immobilization, while making U(IV) more resistant to reoxidation and mobilization.
3. In the field, dissolved hydrogen can be transferred to the subsurface economically, efficiently, and safely using sparging in combination with groundwater injection and extraction wells. This will stimulate growth or uranium- and molybdenum-reducing bacteria without causing aquifer bioclogging.

To test the first two hypotheses, three columns were packed with ORNL sediments and fed uranium-contaminated groundwater spiked with nitrate. To stimulate denitrification and U(VI) reduction/ immobilization, the first column is supplied with membrane-delivered hydrogen, the second with ethanol, and the third with no exogenous electron donor (as a negative control). To test the third hypothesis, approximately six pore volumes at the field site were saturated with dissolved hydrogen (~16 mg hydrogen/L at a hydrostatic head of 340 ft) over a two-month period, while the groundwater was recirculated at a flow of 44 gpm between the injection and extraction wells. A tube trailer (120,000 scf) was used to transfer hydrogen to the aquifer formation at ~1750 scf/day. A bromide solution was added to the injected groundwater at 10 ppm as a tracer.

The column study results, to date, indicate that hydrogen and ethanol promoted rapid denitrification and resulted in equivalent headloss increases across the columns. The continual input of nitrate appeared to inhibit U(VI) reduction and immobilization. Decreasing nitrate loading coincided with decreasing headlosses and accumulation of dark precipitates in the hydrogen- and ethanol-fed columns, but has not yet resulted in significant uranium or molybdenum removal. In contrast, the field study results, to date, show that hydrogen delivery has coincided with a 95% reduction in dissolved uranium concentration for about one pore volume of extracted groundwater. However, after an initial reduction, molybdenum concentrations have rebounded significantly.

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

### *Grantee-Led Research*

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The transport, displacement, distribution, and mixing of one or more reactive agents is required to enhance biological and chemical processes in the subsurface. Biological and chemical reactions that occur at a molecular scale must be translated to the meter scale if such phenomena are to successfully achieve a desired result in porous media. Our objective is to experimentally characterize and computationally describe the growth, evolution, and distribution of microbial activity and mineral formation in porous media that receive two or more reactive amendments. Our model system aims to encourage microbial urea hydrolysis and the associated mineral precipitation ( $\text{CaCO}_3$ ), a process promoted as a means to co-precipitate  $^{90}\text{Sr}$  and other divalent cations. Using this system, our experiments will move through different lab scales to visualize and quantitatively describe the spatial relationships between amendment transport and consumption, stimulating biomass and mineral development that progressively modifies the permeability and heterogeneity of porous media. Initial experiments have examined the feasibility of using different microbes to propagate calcite precipitation, while permitting *in situ* characterization of biomass growth, activity, and mass deposition in one- and two-dimensional micro-model flow cells. Preliminary examination of flow cells using light and confocal scanning laser microscopy and synchrotron-based x-ray tomography has enabled a distinction between porous media solids, biofilm, and pore space. We are testing different mixing scenarios at progressively larger scales and will eventually test heterogeneous porous media and microbes native to a candidate field site. Pore-scale simulations will address upscale and volume averaging of media properties. The research will define the key physical, chemical, and biological processes that influence the form and mobility of DOE priority contaminants (e.g.,  $^{60}\text{Co}$ ,  $^{90}\text{Sr}$ , U) in the subsurface and expand our current understanding of how scaling affects the mixing and delivery of nutrients in biogeochemically dynamic porous media systems.

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

*Grantee-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. We have previously found that U bioreduction and maintenance of low aqueous U concentrations is strongly dependent on the organic carbon (OC) supply rate. Our results also showed that OC supply rate had a significant effect on microbial community structure, while the effect of two different OC types was secondary over the duration of the experiment. The differences between communities attributable to different rates of OC supply diminished through time, despite the fact that different rates of OC supply resulted in different environmental conditions within the columns. Together, these data indicate that microbial communities stimulated for bioremediation may follow predictable trajectories. Based on our prior work, and operating under the premise that microbial communities can be controlled and predicted, as well as the resulting remediation capacity, the objectives of our current project are to: (1) determine if the trajectories of microbial community structure, composition and function following OC amendment can be related to, and predicted by, key environmental determinants; and (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 analyzing three sediments (Oak Ridge, TN; Rifle, CO; Hanford, WA) and their microbial communities using a reciprocal transplant experimental design. Initial characterization of the three sediments show that they vary in mineralogy; particle size distribution; bulk density; base cations; CEC; SAR; iron, manganese, phosphate, and sulfate concentrations; organic and inorganic carbon concentrations; pore-water chemistry; and microbial community size and composition. Flow-through reactors, receiving simulated groundwater at two OC supply rates, are being destructively sampled over a period of 18 months. Microbial community trajectories are being followed using: 16S PhyloChip analysis of community DNA (overall structure) and RNA (active members); GeoChip functional analysis of community DNA (functional potential) and community RNA (active functions); and meta-transcriptome analyses to explore functional capacities not included on extant arrays. Geochemical characteristics of reactor effluents and sediments are being used to model factors influencing microbial community structural and functional trajectories. These analyses will provide a framework for the microbial community ecology underlying subsurface metal remediation at DOE sites.

## Geoelectrical Measurement of Multiscale Mass-Transfer Parameters

*Grantee-Led Research*

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The U.S. Department of Energy (DOE) faces enormous scientific and engineering challenges associated with the remediation of legacy contamination at former nuclear weapons production facilities. Selection and optimization of appropriate site remedies (e.g., pump-and-treat, biostimulation, or monitored natural attenuation) requires reliable predictive models of radionuclide fate and transport; however, current modeling capabilities are limited by an incomplete understanding of multiscale mass transfer—its rates, scales, and the heterogeneity of controlling parameters. At many DOE sites, multiscale mass transfer is evident as long “tailing” behavior, concentration rebound, and slower-than-expected cleanup. Despite the importance of mass transfer, there are significant uncertainties associated with its controlling parameters, for lack of experimental methods to verify and measure it *in situ* or independently of tracer breakthrough. In this work, we are investigating the utility of two geophysical methods—time lapse resistivity and static, complex resistivity—to improve the basic and site-specific understanding of mass transfer. Our research objectives are to (1) develop and demonstrate geophysical strategies to measure mass-transfer parameters spatially and over a range of scales; and (2) provide independent, geophysical estimates of mass-transfer parameters for materials from the Hanford 300 Area.

Our 3-year project began in October 2009. During our first months of funding, we have focused on (1) the design and construction of laboratory setups for time-lapse electrical resistivity tomography (ERT) and complex-resistivity measurements; (2) selection of materials for controlled laboratory experiments; and (3) selection and development of codes for data modeling and analysis.

Our 3-year research plan involves (1) numerical modeling to evaluate the utility of our approaches for different geologic settings and experiment designs; (2) laboratory experiments on synthetic materials and cores from the Hanford 300 Area; and (3) field demonstration experiments at the DOE’s Hanford 300 Area. Our first laboratory experiments this spring involve column tracer tests monitored with ERT, and complex resistivity measurements on cores. Initially, we will focus on materials with well defined mass-transfer parameters, i.e., zeolites and possibly vycor glass. We also aim to examine complex resistivity spectra for a range of mixtures of natural sediments from the Hanford site. After optimizing and verifying our laboratory protocols, we will perform the same experiments on undisturbed cores collected from a low hydraulic conductivity (K) layer present in wells at the Integrated Field Challenge (IFC) well field. In year 2 of our project, we will target this layer in field experiments combining time-lapse geophysical imaging and tracer experiments.

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

*Grantee-Led Research*

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In this proposed research, we plan to test two interrelated hypotheses about Cs sorbed by weathered micaceous materials in subsurface materials from the SRS: (1) that stable cesium has become significantly enriched relative to potassium 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  $^{137}\text{Cs}$ . Quantification of the amounts of stable cesium concentrated in various sites within 2:1 phyllosilicates by natural processes is highly relevant toward understanding the future sorption of  $^{137}\text{Cs}$  by the mica, illite, vermiculite, and HIV phases present in the subsurface at and in proximity to SRS. Sorption and fixation of Cs in the micaceous phases' interlayers potentially lead to increased knowledge to the extent that stable Cs resists exchange with the strong ion exchange cations (Mg,  $\text{NH}_4$ , or even alkyl ammonium compounds) and to the extent that Cs can become fixed over the long term. Such knowledge will help in the development of  $^{137}\text{Cs}$  remediation strategies for the long-term, which is a critical aspect of the SBR goals.

The clay mineralogy of the Vacluse soils collected from surface to 70 inches below surface in SP09 consist of kaolinite and a Al-hydroxyl interstratified vermiculite (HIV). The clay mineralogy of the deeper sections of the 3B core is composed of kaolinite from near surface to ca 100 feet below surface. The clay mineralogy of the deepest samples from the 3B cores are composed of smectite, illite (tr.) and kaolinite. Leach experiments employing various solutions on select soils at the SRS site, and K-Ar dating of leached clay fractions, were performed on select SRS soils (Orangeburg, Vacluse, Blanton).

A long-term experiment has shown that < 20% of the Rb in five SRS soil samples undergoes isotopic exchange over 2 months at room temperature in slightly acidic solution. The degree of Rb isotopic exchange is fairly consistent across the five SRS soil samples. Leaching with strong acid removes K, Rb, and Cs from these soil samples in fairly consistent proportions after the first step in which exchangeable cations are removed. Most of the Rb and Cs, but only about one-tenth of the potassium, in the soil samples is removed by leaching for a few days in 0.5 M  $\text{HNO}_3$  at 70°C. These results, coupled with measurements of radiogenic argon, indicate that most of the potassium in the soil samples is in mica-like interlayers of HIV inherited from Paleozoic muscovite, but most of the Cs and most of the Rb in the soil samples have been fixed in other sites, which are accessible by strong-acid leaching, but only very slowly accessible to isotopic exchange.



## Characterizing the Combined Roles of Iron and Transverse Mixing on Uranium Bioremediation in Groundwater Using Microfluidic Pore Networks

*Grantee-Led Research*

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The primary objective of this work is to characterize the combined effects of hydrology, geochemistry, and biology on the bioremediation of U(VI). Our underlying hypothesis is that 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.

These objectives will be met by testing the following hypotheses: (1) U(VI) will be reduced in the presence and absence of Fe(III), but its reduction rate will be significantly faster in the presence of Fe(III); (2) Adsorbed Fe(II) will oxidize in preference to U(IV), and therefore “buffer” U(IV) re-oxidation; (3) Soluble abiotic reductants (sulfides and hydroquinones) will reduce U(VI) in the presence of Fe(III) (i.e., iron catalysis), but not in its absence, thereby enhancing U(VI) reduction kinetics; (4) A mixed biomass / U(IV) phase will form along the transverse mixing zone, and its transverse width and density will be controlled by transverse dispersion of the electron donor and U(VI) in the absence of iron. In the presence of iron, the biomass / U(IV) phase will form wherever iron and U(VI) are present; (5) Biomass components will retard the re-oxidation of U(IV), because they will limit transverse mixing of alternative electron acceptors; (6) A continuous, low concentration electron donor amendment will promote simultaneous Fe(III) and U(VI) reduction and foster an Fe(III)-reducing microbial community; whereas a high surge of electron donor will stimulate a sulfate-reducing community and effectively prevent U(VI) reduction; and (7) A continuous, high concentration electron-donor amendment will more effectively promote and maintain U(VI) reduction than a single, high surge of electron donor, because this will maintain precipitated U(IV) along the transverse mixing zone.

The experimental tasks will utilize etched silicon microfluidic pore networks (micromodels) to simulate micro-scale 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 will use micromodels that contain Fe(III) oxides versus micromodels devoid of all iron, to assess the critical role that total iron plays in uranium bioremediation. We will introduce electron donors, U(VI), and Fe(III)-reducing cells 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 will also use strictly abiotic micromodels to determine the influence of abiotic reductants, such as sulfides and hydroquinones, on U(VI) reduction in the presence and absence of Fe(III). Finally, we will use uranium-contaminated groundwater to establish a community of native microorganisms (Fe(III) versus sulfate reducers) and characterize the effects of electron-donor concentration and alternate electron acceptors (sulfate) on U(VI) reduction using 16S rRNA-FISH.

The experimental procedure outlined above will not only identify the mechanisms for reduction of U(VI) and oxidation of U(IV) in flowing pore scale models, but it will also determine if the mechanisms are relevant in the presence and absence of Fe(III), by changing one variable at a time. The data regarding electron-donor concentration and mixing will be critical to future remediation efforts; this study will allow practitioners to optimize the type and concentration of electron donor based on specific geochemical conditions. Finally, there is still debate over the relative contribution of biological versus abiotic U(VI) reduction under stimulated conditions. Although all involved can agree that it is likely mixed biotic-abiotic interactions promoting U(VI) reduction, it is important to understand the mechanisms and kinetics of each process.

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

*Grantee-Led Research*

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

Recently, two large undisturbed soil cores (50 cm diameter, 60 cm height), taken out of the Hanford formation sediments from the 200 Area at a depth of 20 m, have been instrumented with TDR and tensiometer sensors and are being irrigated under unsaturated flow. One core has been irrigated with a steady-state flow rate of 20 mm/yr, and column outflow has been collected to analyze for colloid concentrations. Outflow volume, pH, ionic strength and colloid concentrations are being analyzed in the column outflow. No colloids have been detected in the column outflow so far. We will now increase the flow rate to 40, 80, and finally 160 mm/year. A second core has been set up to study unsaturated, transient flow, i.e., we simulate large rainfall events typical for winter storms at the Hanford site. No outflow has been collected out of this core so far, since the water infiltration front has not reached the 60 cm depth yet. A third column will be used to study the effects of water table fluctuations on colloid mobilization.

We have instrumented the field lysimeters at the Hanford Vadose Zone Field Lysimeter Facility (Hanford 300 Area), and we applied Eu-hydroxycarbonate and polystyrene colloids to the lysimeters in January 2009. The lysimeters have been irrigated with different flow rates (two lysimeters are exposed to natural rainfall, the others are being irrigated regularly with multiples of the natural rainfall and are covered to prevent evaporation). Irrigation water matches the chemistry of natural rainfall at Hanford. Fiberglass wicks were installed at 1, 2, 4, and 7 ft below surface to collect vadose zone water. The tension pulled at the wicks is 50 cm-H<sub>2</sub>O, matching the water potential in the soil. Wick outflow is being analyzed for pH, electrical conductivity, colloid concentrations, electrophoretic mobility, and particle size. Electron microscopy is being used to visualize colloidal material in the outflow. Colloids have been detected in the outflow, and we are currently working on exact quantification of the concentrations. We are planning to instrument one additional lysimeter in the Hanford 200 Area in late February 2010. Undisturbed soil cores that have been taken are being prepared for geocentrifuge studies.

To obtain mechanistic understanding of colloid mobilization, we have studied colloid mobilization by a moving air-water interface. Fluorescent polystyrene colloids were deposited in a glass channel, and air-water interfaces were moved over the deposited colloids at different velocities. Colloid removal was quantified by confocal microscopy. Results showed that infiltration (advancing air-water interface) could remove more colloids than drainage (receding air-water interface); but nonetheless, a receding interface still could remove colloids. These results have important implications for colloid mobilization in the vadose zone. We have also experimentally quantified capillary and Laplace pressure forces at particles of different shape and contact angles, and are using natural Hanford particles to quantify interaction forces.

A mechanistic description of surface complexation reactions is being added to the massively parallel code PFLOTTRAN. Allowance is made for sorption onto mobile and immobile colloids, in addition to competitive sorption with host rock minerals. The surface complexation reactions may obey conditions of local equilibrium, or be described by reversible or irreversible kinetic reaction rates. Also included in the model is colloid filtration.

## Uranium(VI) Interaction with Aqueous/Solid Interfaces Studied by Nonlinear Optics

Grantee-Led Research

F.M. Geiger, *Northwestern U.*

We discuss an exploratory laboratory study aimed at understanding the molecular-level interactions that govern contaminant mobility and fate when uranyl species interact with aqueous/solid interfaces. Our experiments yield information that is critical for the BER long-term performance measure 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.” Specifically, working with CO<sub>2</sub>-equilibrated and CO<sub>2</sub>-limited aqueous solutions with pH values where precipitation can be avoided and electrolyte concentrations between 1 and 100 mM, we use nonlinear optics to

- 1) Detect uranyl species at the fluid/solid interface directly, *in situ*, under flow conditions, and in real time by using resonant second harmonic generation at 306 nm
- 2) Determine the adsorption free energies and binding constants for uranyl species
- 3) Determine the absolute uranyl surface coverage via interfacial charge density measurements by using the Eisenthal chi(3) method
- 4) Elucidate the dominant speciation state of the adsorbed uranyl species from the change in charge state of the adsorption site from free adsorption energy versus interfacial potential plots determined via chi(3) charge screening studies
- 5) Image the temporal and spatial evolution of interfacial charge density over 100 x 100 micron areas in real time during U(VI) adsorption and desorption via nonlinear optical microscopy
- 6) Correlate the imaging data maps with results from conventional scanning probes; and
- 7) Work with the Sandia geochemical modeling group to benchmark their computer simulations with our experimentally determined thermodynamic and spectroscopic data.

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

Grantee-Led Research

Y. A. Gorby (PI), J. Craig Venter Institute; M. Fields, Montana State U.

Electrically conductive extracellular appendages known as *bacterial nanowires* have been confirmed in organisms ranging from oxygenic, photosynthetic cyanobacteria to thermophilic, methanogenic co-cultures. Nanowires produced by metal-reducing bacteria *Geobacter sulfurreducens* PCA and *Shewanella oneidensis* MR-1 coordinate extracellular electron transfer to solid phase electron acceptors such as iron and manganese oxides and electrodes in microbial fuel cells, at distances reaching 10s of microns away from cell surfaces. Reduction of U(VI) and precipitation of nanocrystalline uraninite on bacterial nanowires has been observed in suspensions of metal-reducing and sulfate-reducing bacteria. 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 recently funded project 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*. The project targets environmentally relevant bacteria isolated from uranium-reducing sediments at the Oak Ridge Field Research Center (ORFRC). *Geobacter* FRC32<sup>T</sup> is an iron(III)- and uranium(VI)-reducing bacterium whose abundance and activity at the ORFRC has been confirmed using structural and functional gene analysis. FRC32<sup>T</sup> produces branched extracellular appendages in response to electron acceptor limitation, and these "putative nanowires" are morphologically similar to those produced by *Geobacter sulfurreducens* strain PCA. "Putative nanowires" have also been detected in the iron(III)- and uranium(VI)-reducing isolate *Anaeromyxobacter* fw109-5 and the sulfate-reducing organism *Desulfovibrio* FW1012B under electron acceptor-limited conditions that promote nanowire production in other organisms.

This project is predicated on four experimentally tractable hypotheses.

1. *Branched extracellular appendages produced by Geobacter FRC<sup>T</sup>, Anaeromyxobacter fw109-5 and Desulfovibrio FW1012B under conditions of electron-acceptor limitation are electrically conductive.*
2. *Bacterial nanowires from ORFRC isolates are protein assemblages containing a combination of structural proteins (pilin) and electron transport proteins (multi-heme cytochromes).*
3. *Nanowires produced by ORFRC isolates can reduce and transform heavy metals and radionuclides over distances that exceed 10 microns.*
4. *Bacterial nanowires rapidly transform uranium into insoluble precipitates and mitigate its transport in porous subsurface media.*

We have developed a suite of approaches for completing the proposed research. Controlled cultivation methods are used to produce high-quality samples for conductivity and compositional analyses. Novel nanofabrication and nanoelectronic methods developed by the PI and his collaborators are being applied to characterize the conductive properties of nanowires from each of the target organisms. Mass spectrometry-based proteomics will be used to tentatively identify nanowire proteins, and targeted mutagenesis will be used to confirm these protein assignments. The contribution of nanowires to the fate and transport of uranium in saturated porous media will be evaluated using sediment columns. Research targeting bacterial nanowires produced by metal-reducing organisms from uranium-contaminated subsurface environments will directly contribute to an improved understanding of biogeochemical processes controlling uranium migration and advance the goals of the DOE Office of Basic Energy Sciences.

## Electrode-Induced Recovery of Uranium(VI) from Acidic Subsurfaces

Grantee-Led Research

K. B. Gregory (PI), J. Peng—Carnegie Mellon U.

Poised electrodes emplaced in the subsurface have emerged as a strategy for *in situ* bioremediation for removal and recovery of heavy-metal contaminants from groundwater. However, little is known about the biological or geochemical conditions that may impact the removal and recovery processes arising as a result of site-specific conditions. Area 3 of the Oak Ridge FRC exhibits not only high concentrations of U(VI), but also of  $\text{Al}^{3+}$ ,  $\text{NO}_3^{2-}$  and low pH, among other unique site-specific conditions. These studies were initiated to better understand the underlying principles of electrode-based remediation and the aqueous geochemical conditions that will impact remedial design and implementation.

Previous results demonstrated that low-pH decreases removal rates of U(VI), but also that this detrimental impact may be overcome by increasing the applied potential, albeit with diminishing returns. In addition, at low applied potentials (2.5 V between anode and cathode), the presence of potentially competing trivalent and divalent cations did not impair U(VI) removal rates. Recent results show similar findings for monovalent cations. However, at high potential (5 V), the presence of  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  decreased removal rates of U(VI), whereas  $\text{Na}^+$  increased rates of removal of U(VI). AQDS as a humic acid analog did not impact U(VI) removal.

Semi-continuous removal of U(VI) at graphite electrodes was examined by repeated spikes of U. The kinetics of removal were similar for all additions of U(VI). However, the capacity for removal decreased after each addition. This decreased capacity for removal was partially overcome by increasing the applied potential. The data suggest that a Langmuir isotherm model is appropriate, and that adsorption-desorption equilibrium rather than electro-reduction is likely to be the dominant removal process in the absence of microbial reduction processes.

Long-term, bench-scale studies with electrodes deployed in site water and sediment have been initiated to more closely model *in situ* conditions and evaluate the impacts of electrodes on the microbial community at low pH. U(VI) and total radioactivity decrease rapidly at an applied potential of 2.5 V between the anode and cathode. Moreover, results show that U(VI) and total radioactivity removal occur before nitrate removal despite a much higher concentration of nitrate present in Area 3 groundwater. This finding suggests that electrode-based bioremediation is a promising alternative for traditional bioremediation, since the necessity of removing nitrate as both an alternate electron acceptor and oxidant of U(IV) may be avoided.

Ongoing results from this project demonstrate not only the feasibility but also the distinct advantages of an electrode-based remedial process for U(VI) in acidic environments such as those observed in Area 3 of the Oak Ridge FRC. Future work will examine microbial processes stimulated by poised electrodes and the microbial community that develops on electrodes deployed in site water and sediment.

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

Grantee-Led Research

K.F. Hayes (PI), S.P. Hyun, Y.S. Han, K. Sun, Y. Bi, J. Carpenter, T. Clancy, G. Upadhyaya, L. Raskin—*U. of Michigan*; B.E. Rittmann, R. Vannela, C. Zhou—*Arizona State U.*; J.A. Davis, *USGS*; J.R. Bargar, *Stanford U.*; R.K. Kukkadapu, *PNNL*

This research seeks to identify the role of reduced iron sulfide minerals in the long-term sequestration of uranium (U) at DOE contaminated sites treated with sulfate-reducing bacteria (SRB). SRB utilize sulfate as a terminal electron acceptor and produce sulfide, which may subsequently serve as a reducing agent to produce insoluble reduced metal solids or as an anion to produce insoluble metal sulfides. When using SRB, it is recognized that uranium concentrations can be effectively lowered via reduction of soluble U(VI) uranyl complexes by sulfide to produce more reduced solid forms of U(IV), such as the insoluble uraninite ( $\text{UO}_2(\text{s})$ ). One of the possible limitations for the long-term sequestration of U by SRB is the potential for its remobilization when oxidants such as oxygen, nitrate (or nitrite), or Fe(III) are formed or flow into the treated subsurface environment. *As such, one of the overarching goals of this research is to assess the potential for mobilization of U solids formed under sulfate-reducing conditions when subjected to re-oxidizing conditions.*

Under sulfate-reducing conditions, a variety of iron sulfide minerals may form, such as mackinawite ( $\text{Fe}_{1+x}\text{S}$ ), greigite ( $\text{Fe}_3\text{S}_4$ ), or pyrite ( $\text{FeS}_2$ ), depending on the solution-phase composition, the type of background solids in the subsurface, and the type of sulfate-reducing bacteria present. *In this context, this research is investigating the impact of iron sulfides formed by various SRB on long-term sequestration of U when subjected to re-oxidizing conditions.*

In the first phase of this project, biogenic and synthetic versions of the iron sulfide, mackinawite, and the reduced uranium solid, uraninite, are being produced and characterized. X-ray diffraction (XRD), X-ray Absorption spectroscopy (XAS), Mossbauer spectroscopy (MS), and Transmission Electron Microscopy (TEM) are being used to confirm the identity of the reduced iron sulfide and uranium solid phases produced, and will also be used to characterize the oxidation products when these solids and their mixtures are subjected to the chosen oxidants.

In the case of the biogenic production of solids, two organisms have been under investigation, the gram-positive bacterium *Desulfotomaculum nigrificans* (*Firmicutes*) and the gram-negative bacterium *Desulfovibrio vulgaris* (*Deltaproteobacteria*). So far, using standard media for SRB with lactate and acetate as electron donors and with dissolved Fe(II) as the iron source, both *D. nigrificans* and *D. vulgaris* have been found to produce mackinawite as confirmed by XRD. The recipe for *D. vulgaris* is currently being scaled-up to produce enough mackinawite to be used in batch oxidation experiments. Experiments are also planned to produce biogenic uraninite with *D. vulgaris* for subsequent rate studies.

Batch reactor studies are planned, in which synthetic  $\text{FeS}(\text{s})$  (mackinawite) and  $\text{UO}_2(\text{s})$  (uraninite) will be introduced into the reactors to identify factors controlling the rate of oxidation as a function of groundwater conditions, including pH, and carbonate, calcium, FeS, and oxidant concentrations. These experiments will be used to identify the mechanism of oxidation and conditions under which mackinawite is an effective scavenger of oxygen compared to uraninite.

Column experiments are also planned in which a ten-fold excess of mackinawite relative to uraninite will be packed with natural sand. In these experiments, the rate of oxidation of uranium by oxygen will be compared to a control column packed with only  $\text{UO}_2(\text{s})$  and no  $\text{FeS}(\text{s})$  to verify the protective nature of the presence of iron sulfide. Initial results from the biogenic studies and solid-phase characterizations of the synthetically and biogenically produced solids and their oxidation reaction products will be discussed in the poster presentation.

## Biochemical Basis for Survival and Growth Advantage in Stationary Phase Adaptation of *Geobacter sulfurreducens* under “Famine” Conditions

Grantee-Led Research

R. A. Helmus (PI), A. Stanley, B. Stanley, S. Brantley, M. Tien—Penn State U.

Since microorganisms typically exist in the environment in a state with limited access to electron donors and/or electron acceptors, gaining an understanding of how organisms involved in bioremediation persist under such conditions is relevant for effective planning of bioremediation efforts. This work aims to identify survival mechanisms of *Geobacter sulfurreducens* under growth conditions in which electron donor and electron acceptor are scarce. Microorganisms display five typical states of growth: lag, log, stationary, death, and survival phases. In some species, individuals with a growth advantage adaptation begin to emerge from a community starting in stationary phase; this adaptation is termed “growth advantage in stationary phase,” or GASP.

The first objective was to determine if *G. sulfurreducens* displays a GASP response, which would enhance the selection of specific strains of *G. sulfurreducens* under starvation conditions through an increased ability to compete for limited resources. A GASP response is defined by the outgrowth of an older strain of bacteria when co-cultured with a younger strain. By co-culturing antibiotic-resistant strains of *G. sulfurreducens* originating from batch cultures of different ages, we determined that organisms of equal age rarely displayed a GASP response. However, in 86% of co-cultures with unequal-aged strains, a GASP response was observed, with the older strain persisting by a cell density of at least one log higher than the younger strain.

Next, we investigated possible genetic causes of GASP in *G. sulfurreducens*. Multiple genes were selected for sequencing from GASP strains of *G. sulfurreducens* based on their ability in *E. coli* to induce a GASP response via nucleotide insertion or deletion mutations. The *rpoS* gene (GSU1525) and a homologue to *E. coli lrp* (GSU3370) were sequenced from at least five GASP strains each. No mutations were identified in the screening of these two genes, indicating that other genes may be more likely to be responsible for GASP response formation in *G. sulfurreducens*.

Proteomic features of survival phase *G. sulfurreducens* organisms were then explored, with the objective of identifying proteins that are differentially expressed in survival phase cultures compared with mid-log phase cultures. Sixty proteins were identified using iTRAQ analysis as being significantly down-regulated in survival phase organisms. These included 27 proteins involved with translation and protein synthesis, five associated with electron transfer, and three involved in nucleotide biosynthesis. Two proteins that play a role in heme biosynthesis, the gene products of GSU3285 and GSU3458, were down-regulated. However, preliminary results did not indicate that total heme levels were lower in survival phase cultures compared with mid-log phase cultures. There were 74 proteins that were significantly up-regulated in survival phase organisms. These included components of the ATP synthase and NADH dehydrogenase enzymes, as well as Fe (III) reductase subunits and all three components of the heterotrimeric fumarate reductase FrdCAB. In addition, several outer membrane proteins and putative lipoproteins were up-regulated. These results indicate that the composition of the inner and outer membranes of survival phase *G. sulfurreducens* had drastically changed from that of mid-log phase *G. sulfurreducens*.

The current study discovered a growth advantage in stationary phase response in *G. sulfurreducens* and identified protein-level differences in survival-phase strains that may explain how *G. sulfurreducens* can survive in environments with limited electron donor and/or acceptor. The genetic bases of the GASP phenomenon and survival capacity will continue to be investigated by genomic sequencing.

# Time-Lapse Tomographic Approaches for Monitoring Remediation Processes

*Grantee-Led Research*

Z. Hou (PI), N. Terry, X. Xu, A. Flaxman—*U. at Buffalo, SUNY*

Recent studies suggest that time-lapse crosshole geophysical methods may be effective in monitoring subsurface hydrological and biochemical processes. These methods provide minimally invasive, cost-effective, high-resolution information previously limited to wellbore data.

Our study area is located at the 100H area of the Department of Energy (DOE) Hanford Site, with biostimulation treatments performed to reduce and immobilize Cr(VI). Time-lapse crosshole seismic and radar data sets have been collected in order to monitor spatio-temporal responses to remediation processes. Before using these field data for parameter estimation and monitoring hydrobiogeochemical processes, we are making efforts to (1) identify the critical (field/flow/transport) parameters that affect these hydrobiogeochemical processes; (2) determine the sensitivity of geophysical (seismic/radar) responses to these parameters; and (3) develop and evaluate different geophysical forward models and inverse strategies, through synthetic analyses.

Meanwhile, two other concerns are addressed: (1) to reduce the dimension of parameter space to a reasonable level, when estimating flow/transport parameters, or spatial distribution of dielectric permittivity/electrical conductivity; (2) to take advantage of time-lapse data by treating the updated "posterior" pdf as a memory function, which stores all the information up to date about the state variables, then considering the memory function as a new prior and generating samples from it for further updating when more geophysical data is available.

Preliminary results include comparison of the impacts of field/hydraulic parameters on geophysical signals, estimation of hydraulic parameters using tomographic radar data, estimation of spatio-temporal distribution of dielectric permittivity, and using combined approaches, including minimum-relative-entropy, quasi-Monte Carlo, Markov-Chain Monte-Carlo, and Bayesian updating.



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

*Grantee-Led Research*

Q. (Max) Hu (PI), *U. of Texas-Arlington*; R. Ewing, *Iowa State U.*

At the Hanford site, an estimated 202,703 kg of uranium (U) was released to the vadose zone over the course of site operations between 1940 and 1990. In the 300 Area, while groundwater U concentrations range from 5 to 150  $\mu\text{g/L}$ , solid-phase U concentrations in aquifer fines may be as high as 250 mg/L. The fines ( $<53\ \mu\text{m}$ ) comprise only 1.78% of the total sediment mass, but hold a disproportionately large fraction of the total U. We hypothesize that the mass-transfer limitation of U release at the Hanford site is partly due to low connectivity of intragranular pores in the Hanford sediments. Low pore connectivity results in porosity decreasing, and tortuosity increasing, as functions of distance from a grain's surface. A low-connectivity pore-space would produce both concentration profiles with edge effects and an anomalous pattern of contaminant release over time. This hypothesis offers a testable, physically based explanation for the prolonged U release and groundwater contamination observed at Hanford.

The objectives of this new SBR project are to (1) reduce uncertainty in assessing remediation options in the Hanford 300 Area by measuring U(VI) concentrations at greatly increased precision across and within sediment size classes; (2) determine whether, and to what extent, low pore connectivity affects solute diffusion in Hanford 300 Area sediments of various size classes; (3) evaluate the suitability of polyphosphate as a U-immobilizer by examining the diffusion of orthophosphate ( $\text{PO}_4^{3-}$ ) into Hanford 300 Area sediments; and (4) test grain-scale and core-scale scaling relationships relating to diffusion in Hanford 300 Area sediments.

To achieve these objectives, we will use state-of-the-art ICP-MS instrumentation with dynamic reaction cell technique, coupled with laser ablation (LA), to measure U concentration as a function of distance from the grain surface across the range of sediment particle size classes. With the same instruments, we will conduct several diffusion experiments to examine scale-dependent accessible porosity and diffusivity of both U and conservative tracers. A final set of experiments will examine scale-dependence of porosity and diffusivity at the core scale. Experiments will also be simulated at the pore network scale, aiding analysis.

This work will examine a key scientific issue, mass-transfer limitations at the pore scale, using both new instruments with high spatial resolution, and new conceptual and modeling paradigms. The complementary laboratory and numerical approaches connect pore-scale physics to macroscopic measurements, providing a previously elusive scale integration. This work will provide sufficient scientific understanding such that DOE sites will be able to incorporate coupled physical and chemical processes into decision making for environmental remediation and long-term stewardship.

# Mercury Stable Isotopes as Indicators of the Biogeochemical Cycling and Transport of Mercury

*Grantee-Led Research*

T.M. Johnson (PI), Gideon Bartov—*U. of Illinois at Urbana-Champaign*

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 methyl mercury and Hg reduction, and to reveal mechanisms of those reactions. Hg has seven stable isotopes, ranging in mass from 196 to 204. Through two distinct mechanisms, the relative abundance of the isotopes are altered by certain processes. 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  $^{202}\text{Hg}/^{198}\text{Hg}$  ratio. The second mechanism is the magnetic isotope effect, whereby the reaction rates of odd isotopes depart from those of the even isotopes because of coupling of electron spins with nuclear spins, which exist in odd nuclei only. This effect causes anomalies in  $^{201}\text{Hg}/^{198}\text{Hg}$  and  $^{199}\text{Hg}/^{198}\text{Hg}$  ratios, and occurs in photochemical reactions involving radical pairs.

This project is a one-year exploratory effort that will begin to apply Hg isotope data at Oak Ridge. The work is organized around three objectives:

- 1) Determine if different potential sources in the system or different generations of Hg contamination have distinct Hg isotope ratio “signatures” that can be traced through the system. Preliminary data suggest there is a strong  $^{202}\text{Hg}/^{198}\text{Hg}$  ratio contrast between contaminant Hg and background Hg found in nominally uncontaminated soils: Background ratios appear to be a few per mil less (measurement precision  $\sim \pm 0.1$  per mil). There also appear to be differences in  $^{202}\text{Hg}/^{198}\text{Hg}$  between different generations of Hg in the EFPC system.
- 2) Develop preconcentration methods that will be needed to obtain precise Hg isotope ratio measurements on water samples, whose concentrations are usually extremely low, obtain pilot data from EFPC waters, and look for evidence of Hg(II) reduction. Possible methods to preconcentrate and measure methyl-Hg will also be explored, though this is an extreme challenge because of the extremely low methyl-Hg concentrations.
- 3) Collect pilot data on biota sampled from EFPC and assess their ability to be used as proxies for direct measurements of methyl mercury or indicators of biogeochemical processes in the system.

Our group at Illinois is developing ties with ORNL-SFA team members and obtaining samples in conjunction with existing sampling plans. In the long run, a collaborative effort using Hg stable isotope data should improve understanding of Hg transport from various sources and improve determination of rates of key processes, such as demethylation and Hg reduction, that affect the bioavailability and environmental impact of contaminant Hg at DOE sites.

## Determination of Hexavalent Chromium Reduction Using Cr Stable Isotopes: Isotopic Fractionation Factors for *In Situ* Redox Manipulation Zones

Grantee-Led Research

A. Basu, T.M. Johnson (PI)—*U. of Illinois at Urbana-Champaign*

Cr stable isotope measurements in groundwater can provide improved estimates of the extent of Cr(VI) reduction, which immobilizes the Cr and renders it less toxic. Cr isotope fractionation occurs during Cr(VI) reduction; lighter isotopes react more readily than heavier ones. As reduction proceeds, the remaining Cr(VI) becomes enriched in heavier isotopes. We quantify this enrichment by measuring the  $^{53}\text{Cr}/^{52}\text{Cr}$  ratio. The intensity of isotopic fractionation varies between reduction mechanisms, depends on biogeochemical conditions, and is expressed as a fractionation factor,  $\alpha$ :

$$\alpha = R_{\text{reactant}}/R_{\text{product}}$$

where  $R_{\text{reactant}}$  and  $R_{\text{product}}$  are the  $^{53}\text{Cr}/^{52}\text{Cr}$  ratios of the reactant and the reaction product flux. For convenience,  $\alpha$  is often expressed in per mil units using the symbol  $\epsilon$  where  $\epsilon = (\alpha - 1) \times 1000\text{‰}$ . In order to know the relationship between the extent of Cr(VI) reduction and observed  $^{53}\text{Cr}/^{52}\text{Cr}$  ratio shifts at a particular site, we must know  $\epsilon$  for the reactions occurring at the site.

The Cr isotope method should prove useful in studying the performance of In Situ Redox Manipulation (ISRM) zones—for example, to determine if Cr breaking through an ISRM barrier passes through zones of substantial but incomplete reduction or merely bypasses the barrier completely. Here, we determine the site-specific calibration factor,  $\epsilon$ , for the ISRM, created by injecting sodium dithionite into the subsurface at the Hanford 100-D area, from laboratory microcosm studies and experiments with pure mineral phases. These results will be used to interpret Cr isotope data being collected at the ISRM barrier, will be useful for other Cr(VI) ISRM projects, and more generally improve our knowledge of Cr isotope fractionation.

Sodium dithionite injection creates reduced Fe(II) phases (sorbed Fe(II),  $\text{FeCO}_3$ , FeS, and perhaps other phases such as green rust) in the sediment, among which sorbed Fe(II) is thought to be dominant. Isotopic fractionation factors were measured for Cr(VI) reduction in two batch incubations of sediments from the Hanford 100-D ISRM collected at two different depths from a borehole down-gradient from a dithionite injection well. Results indicate  $\epsilon$  was 3.5‰. This coincides with a preliminary  $\epsilon$  value of 3.5 ‰ for Cr(VI) reduction by synthesized  $\text{FeCO}_3$ . The value of  $\epsilon$  for Cr(VI) reduction by synthesized green rust is 2.7 ‰, significantly lower than the ISRM sediments, suggesting that green rust is probably not a dominant reducing phase. Further work will determine fractionation factors for reduction by FeS and Fe(II) sorbed onto/into hematite particles.

So far, it appears fractionation factors for Cr(VI) reduction by ISRM sediment and likely ISRM phases fall within a narrow range similar to that observed in the few previously published studies. This aids in interpretation of Cr isotope data from the ISRM's, by providing a better calibration of the relationship between  $\delta^{53}\text{Cr}$  and the extent of Cr(VI) reduction.

## Limited Immobilization of Hexavalent Chromium via Reduction at Idaho National Laboratory: Chromium Stable Isotope Results

*Grantee-Led Research*

A.L. Raddatz, T.M. Johnson (PI)—*U. of Illinois at Urbana-Champaign*; T. McLing, *INL*

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

We obtained high-precision  $^{53}\text{Cr}/^{52}\text{Cr}$  measurements on 60 groundwater samples from Idaho National Laboratory (INL) using a multicollector ICP-MS method. Results are expressed as  $\delta^{53}\text{Cr}$  values (per mil deviations from NIST SRM-979) and carry uncertainty of  $\pm 0.15\%$ . Analysis was challenging for some samples with Cr(VI) concentrations as low as 0.001 mg/L.

The original contaminant Cr(VI) had  $\delta^{53}\text{Cr}$  values close to zero: Two archived samples of highly contaminated groundwater collected in 1967, when contaminant releases were occurring, give near-zero values. This is expected; Cr ores, industrial supplies, and reagent Cr also have near-zero  $\delta^{53}\text{Cr}$ . Several groundwater samples from 2008 or 2009 have similar low  $\delta^{53}\text{Cr}$  values and elevated Cr(VI) relative to background. For example, well USGS-65, located immediately downgradient from a contaminant source has the greatest recent Cr(VI) concentration (0.098 mg/L), and its measured  $\delta^{53}\text{Cr}$  is  $+0.30\%$ . This is nearly identical to the  $\delta^{53}\text{Cr}$  of the original contaminant, and we conclude that little or no Cr(VI) reduction has occurred. Two other contaminated samples have similar  $\delta^{53}\text{Cr}$  values. Clearly, there are some paths through the aquifer along which reduction is insignificant.

Elevated  $\delta^{53}\text{Cr}$  values observed in several other locations strongly suggest the occurrence of Cr(VI) reduction. 12% of the sampling locations have values greater than 1.5‰. Some of these elevated values may result from mixing of low- $\delta^{53}\text{Cr}$  contaminant Cr with natural background, which exhibits a range of  $\delta^{53}\text{Cr}$  values (0.79‰–2.45‰), but is present only at low concentration ( $0.005 \pm 0.002$  mg/L). However, four contaminated wells ( $> 0.015$  mg/L) have  $\delta^{53}\text{Cr}$  values much greater than any reasonable mixture of contaminated water with natural background water. These elevated  $\delta^{53}\text{Cr}$  values indicate Cr(VI) reduction has occurred. Quantifying reduction is more difficult, but a semi-quantitative estimate can be made using a Rayleigh distillation model and a rough estimate of the magnitude of isotopic fractionation induced by reduction in this setting. Using a fractionation factor of 1.0035, we estimate at least 25% of the original contaminant Cr(VI) has been removed in some places. This is probably an underestimate, because of known problems applying the Rayleigh model to real aquifers. Results from several multi-level wells strongly suggest Cr(VI) reduction occurs only in a thin layer of the aquifer immediately below the water table, perhaps because the Fe(II)-bearing minerals there have been exposed to little saturated zone weathering and are more reactive.

Overall, the results suggest that Cr(VI) reduction occurs in certain zones of the Snake River Plain aquifer, despite the general presence of oxic conditions, and that contaminant Cr(VI) is slowly converted to the less dangerous Cr(III) form.

## Processes Controlling Enhanced Transport of Plutonium in Unsaturated Conditions

Grantee-Led Research

D. Kaplan (PI), C. Bagwell—SRNL; R. Kukkadapu, PNNL; F. Molz, Clemson U.; H. Nitsche, LBNL; B. Powell, Clemson U.

The objective of this study is to understand the processes responsible for enhanced transport of Pu in the vadose zone. Previously, we demonstrated in a 11-year field study conducted in 51-L lysimeters at the Savannah River Site that Pu is extremely immobile, where <97% of the Pu remained within 5 cm of the source. However, both upward and downward migration of Pu away from the source was observed in all lysimeters. This observed mobility was greater than the steady-state ( $K_d$ ) geochemical models would predict using steady-state or transient transport models. Through laboratory and spectroscopic studies, it was possible to attribute some of the enhanced mobility to Pu oxidation state transformations not accounted for in the steady-state model. However, this accounted for only the downward enhanced transport. Simulations indicated that key processes were not accounted for in our conceptual model. This year's studies have focused on identifying some of these key yet-unidentified processes, specifically the role of biological influences (microbial and plant) and  $\text{Fe}^{2+}$  mineralogy on Pu redox chemistry, and incorporating them into a numerical model to permit hypothesis testing for Pu long-term stewardship in the vadose zone.

The role that microbial organic acids may play in enhanced Pu mobility was evaluated. Twenty soil strains were isolated from the lysimeter sediments, of which eight strains were down selected by starving and isolated for organic acid production. Five relatively small organic acid compounds produced by these eight strains were identified and quantified by ion chromatography. The tendency of Pu(IV) to complex with these compounds were determined. In a related set of studies, Np(V), Pu(V), and U(VI) adsorbed strongly onto cell surfaces of *Pseudomonas putida* F1, a lysimeter sediment isolate; distribution coefficients ranged from  $10^3$  mL/g for Np(V) to  $10^5$  mL/g for U(VI) and Pu. Differences between the Np and Pu distribution coefficients were attributed to reduction of Pu(V) to Pu(IV) by the cell surface or exudates. Filtration tests at 4 nm and 200 nm indicated that a fraction of the aqueous Pu and U were associated with organic exudates, which could represent a potentially mobile actinide organic-complex species.

Under oxidizing conditions, surface-mediated reduction of Pu(V) to Pu(IV) occurred in neutral pH lysimeter sediment suspensions, a sediment that contains <0.05 ppm organic carbon and <0.5% Fe(II) (by Mössbauer spectroscopy). This result was reported by XANES (> $\mu\text{M}$  Pu concentrations) and wet chemistry measurements (nM Pu concentrations). This sediment was treated to reduce and solubilize iron oxide and phyllosilicate/clay Fe(III). Sorption and subsequent reduction of Pu(V) correlated with the concentration of Fe(II) remaining within each sediment. The correlation between Fe(II) concentrations and Pu(V) reduction demonstrates the potential impact of changing Fe mineralogy on Pu subsurface transport if subsurface environments are cycled between oxidizing and reducing conditions.

The initial conceptual model of the transport process was based on steady-state and then fully transient soil water movement coupled to redox reactions between reduced and oxidized Pu species. Simulations from this model yielded reasonable below-source transport, but little above-source transport. The conceptual model was then modified to include Pu sorption and uptake by plant roots and upward movement in the transpiration stream. Resulting simulations in the lysimeters were consistent with Pu activity concentrations in the top 20 cm of soil and suggested Pu transport into the shoots of the annual grasses growing in the lysimeters and an accumulation on the soil surface. Such a residue has now been verified by thermo-ionization mass spectroscopy (TIMS) isotope ratio analysis. At this point, it became clear that plants were an important pathway for Pu transport. This motivated laboratory experiments with corn, which demonstrated that DFOB-complexed Pu moved a million times faster in corn than reduced Pu moved in lysimeter sediment. It suggests the probable involvement of Pu complexing agents in the absorption and transport process in plants. Recent data imply that plants may co-metabolize Pu with Fe, elements with nearly identical charge to ionic radii ratios and similar extraordinarily large complexation complexes with multidentate organic acids, such as siderophores.

Throughout this study, mathematical models were used to analyze data. This analytical process can be conceived as a type of computer-aided thinking that when combined with additional experiments can result in new insights. Such an approach has been used to derive future research questions, test hypotheses and to visualize complex, nonlinear, property relationships. Computer-aided thinking differs from classical prediction in that results must be highly constrained by experimental data.

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

*Grantee-Led Research*

T.C. Kenna (PI), M.M. Herron—*Columbia U.*; A. Ward, *PNNL*

The goal of our exploratory project, recently funded under the new category of “Enabling Scientific Tools,” is to determine the parameters necessary to translate existing neutron and gamma ray borehole log data into reservoir properties of clay content, porosity, permeability, and vadose zone water saturation, following scientifically sound petrophysical relationships. These techniques have been successfully employed in the petroleum industry, but the approach is new when applied to subsurface remediation. The optimization of well log interpretation could lead to a breakthrough, enabling the characterization of reservoir properties that control subsurface flow and transport at significant spatial resolution.

The specific research objectives of our project are to:

- 1) Analyze core and outcrop samples from representative facies for a variety of mineralogical, chemical, and physical properties (in collaboration with Dr. Andy Ward-PNNL).
- 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 useful reservoir properties, such as lithology, matrix density, porosity, water saturation, and permeability.

Our work will lead to an improved interpretation of existing well log data. The algorithms we develop can utilize available existing spectral gamma and neutron logs collected over the past several decades, and the approach is applicable to recent wells with more complete suites of logs. Our work can provide guidance in selecting the most appropriate logs to run in future logging programs depending on the desired properties and the uncertainty of the estimate.

Initial work performed on eleven samples from Hanford well 399-3-18 (C4999), located near the 300 A IFRC, indicates a significant correlation between core gamma ray data and total clay as determined by Dual Range Fourier Transform Infrared spectroscopy (DRFT-IR). The core gamma ray data permit calibration of the existing gamma ray log into standard API units and then into total clay as a function of depth. This is a simple example of the type of translation of existing log data into meaningful reservoir property that we will accomplish in this project. Hundreds of these gamma ray logs already exist at Hanford.

This project will enable us to improve our ability to remotely define subsurface petrophysical and flow properties, which is listed as a research priority in the ERSP Strategic plan (DOE, 2006). Without calibration of existing borehole log data, their application to the ERSP goal of understanding subsurface processes is limited. Significant added return on DOE investment comes from calibration of the core data and derivation of reservoir properties and loggable parameters that improve the interpretation of well log data already acquired. This exploratory project focuses on a single site; however, our approach may serve as a template for application at other sites where DOE has remediation responsibilities and stewardship challenges.

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

Grantee-Led Research

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Major advances in DNA sequencing capability have begun to provide an unprecedented view of the community composition and genetic potential of the subsurface microbial world. However, the metabolic potential gleaned from community sequencing must be coupled to the cultivation and physiological characterization of model microorganisms in order to effectively interrogate microbially mediated mechanisms of uranium (U(VI)) transformation. Nitrate is a critical co-contaminant that intimately impacts the fate and transport of U(VI) in groundwaters. An improved understanding of denitrifying microbial communities is required to predict and control nitrate attenuation mechanisms for the remediation of the contaminated terrestrial subsurface at U.S. 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.

A diverse set of metal- and nitrate-reducing bacteria were isolated, and environmental sequence data indicate that these species are abundant in the contaminated subsurface of DOE sites. Although denitrifying *Betaproteobacteria* have been frequently detected and previously cultivated from the contaminated subsurface, we isolated denitrifying bacteria from the taxa *Alphaproteobacteria*, *Gammaproteobacteria*, *Actinobacteria*, and *Firmicutes*, and revealed a greater diversity than had been previously characterized. Isolates from the phylum *Proteobacteria* were complete denitrifiers, whereas the Gram positive isolates reduced nitrate to nitrous oxide. Isolates from the genus *Rhodanobacter* were shown to be members of a large guild of denitrifying bacteria in the uranium-contaminated subsurface. Analyses of genomic data and denitrification gene sequences from the isolates revealed that commonly employed primer sets have a narrow target range and can greatly underestimate the diversity of denitrifiers. Furthermore, they cannot be used to amplify nitrite reductase genes (*nir*) from members of the key genus *Rhodanobacter*. Similarly, commonly used primer sets targeting nitrous oxide reductase genes (*nosZ*) were found to exclusively target nitrous oxide genes from *Proteobacteria*, though unable to amplify *nosZ* genes from the abundant *Rhodanobacter spp.* in the uranium-contaminated subsurface. In addition to denitrifying bacteria, a Fe(III)- and U(VI)-reducing bacterium was isolated. Based on phylogenetic and phenotypic differences, *Geobacter daltonii sp. nov.* strain FRC-32T was described as a novel species within the subsurface clade of genus *Geobacter*. Comparison of *G. daltonii* to closely related *Geobacter* species indicates the remarkable genotypic and phenotypic plasticity of bacteria within the genus. A genome-genome comparison of *G. daltonii* to *G. uraniireducens* revealed not only a significant divergence in total genome size, but also that the organisms share few genes with greater than 90% DNA sequence similarity. Analyses of *Geobacteraceae*-specific citrate synthase (*gltA*) mRNA gene sequences retrieved from U.S. DOE sites at Oak Ridge, TN, and Hanford, WA, have elucidated the active *Geobacter* species, including *G. daltonii*, in subsurface sediments undergoing U(VI) bioremediation.

## Design and Application of Microbial-Uranium-Reduction Monitoring Tools

Grantee-Led Research

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Diverse groups of microorganisms affect the oxidation state and complexation of metals and, therefore, influence or control the mobility of toxic radionuclides in subsurface environments. Relevant to uranium (U) speciation are bacteria that reduce predominantly water-soluble and mobile hexavalent U, U(VI), to U(IV), which has reduced solubility and typically forms the uraninite mineral  $\text{UO}_2$ . This research project aims to enhance understanding of the microbiology controlling radionuclide mobility by developing enhanced and novel molecular and biogeochemical tools with which microbial activity can be better monitored and predicted. The new tools will be used to study the underpinning mechanisms contributing to metal and radionuclide reduction, and will benefit the broad scientific community exploring the distribution, activity and ecology of metal-reducing bacteria. Application of the new tools to DOE IFRC sites will demonstrate the value of the tools to assess, monitor, and predict *in situ* reductive processes--and their power to guide decision making for environmental restoration and provide long-term stewardship at radionuclide-contaminated DOE sites.

For novel molecular tool development, the research focus is on bacteria that use U(VI) as growth-supporting electron acceptor, because processes linked to the energy metabolism of the microbial catalysts are most relevant for contaminant turnover in oligotrophic subsurface environments. *Anaeromyxobacter dehalogenans* are common subsurface bacteria and gain energy for growth from U(VI) to U(IV) reduction; however, the growth yields measured with U(VI) as electron acceptor were about 10-fold lower than predicted from thermodynamic calculations. Knowledge of the amount of catalyst in the contaminated subsurface is relevant for developing predictive models, and the reasons for the lower-than-predicted growth yields are under investigation. Reduced U is susceptible to reoxidation by oxidants (e.g., oxygen) and controlling oxic/anoxic interface processes to ensure the long-term stability of the precipitated material is critical for lasting immobilization. *Anaeromyxobacter* spp. grow using oxygen as an electron acceptor at partial pressures at and below 0.18 atm. Thus, *Anaeromyxobacter* are uniquely adapted to life at the oxic-anoxic interface, where they consume oxygen and take advantage of oxidized metal species, including U(IV) as electron acceptors. Monitoring the *Anaeromyxobacter* community at the Oak Ridge IFRC site using 16S rRNA gene-targeted qPCR approaches revealed unexpected strain diversity and responses to environmental conditions (i.e., ethanol biostimulation, oxygen intrusion). While *Anaeromyxobacter* 16S rRNA gene sequences increased, the 16S rRNA gene copies of *Geobacter lovleyi*, a population present at this site, remained unchanged or declined following oxygen intrusion. The combined applications of genus- and strain-level qPCR approaches, along with 16S rRNA gene clone libraries, provided novel information on patterns of microbial variability within the *Anaeromyxobacter* group and among metal-reducing bacteria relevant to U bioremediation. Importantly, the quantitative assessment suggested that members of an *Anaeromyxobacter* clade with no cultured representatives dominated the U reduction treatment zone, emphasizing the need for continued isolation efforts.

The main biogeochemical tool focus is on quantifying U isotopic fractionation with high precision mass spectrometry. This tool identifies specific isotope enrichments associated with metabolic or abiotic reduction or reoxidation reactions that occur in groundwater. Our analysis of samples from the Rifle IFRC site indicate that U(VI) becomes isotopically lighter as it becomes depleted due to reduction. The nature of this isotopic fractionation will be evaluated under different controlled experimental settings, so that the influence of different microorganisms, including *Anaeromyxobacter* spp., the specific nature of abiotic reduction reactions, and the reactions rates can be determined.



## Diagnosis of *In Situ* Metabolic State and Rates of Microbial Metabolism During *In Situ* Uranium Bioremediation with Molecular Techniques

Grantee-Led Research

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The overall objective of these studies is to further develop molecular tools for analyzing the activity of the microorganisms that are important in *in situ* bioremediation of uranium-contaminated groundwater and to use these tools to better understand the factors influencing the effectiveness of *in situ* uranium bioremediation at Department of Energy sites. The short-term objectives in the next three years are: (1) to determine whether quantifying the abundance of key proteins in subsurface *Geobacter* communities can diagnose their metabolic status and rates of metabolism; and (2) to begin extending the molecular diagnostic approach to the study of subsurface sulfate-reducing microorganisms that may also contribute to *in situ* uranium bioremediation.

To determine whether there was a correspondence between the abundance of key proteins and metabolic activity of *Geobacter* species, antibodies were produced against polypeptides that were designed to be specific for the citrate synthase, the A subunit of RNA polymerase (RpoA), or a subunit of nitrogenase (NifD) of *Geobacter* species in the subsurface clade 1, because this is the predominant *Geobacter* clade in a diversity of subsurface environments. Antibody-based quantification of these proteins in the subsurface clade 1 isolate, *Geobacter bemidjiensis*, demonstrated that there was a direct correlation between the abundance of citrate synthase and the rate of metabolism in chemostat cultures, whereas RpoA abundance remained constant. NifD was abundant during growth in nitrogen-free medium, but not when ammonium was available. Analysis of protein samples collected during *in situ* uranium bioremediation studies at the Rifle, CO, study site revealed a strong correspondence between the abundance of citrate synthase and acetate availability and removal of U(VI) from the groundwater. NifD increased in abundance when ammonium concentrations declined, consistent with previous detection of an increase in nifD expression in the same samples. These studies suggest that antibody-based quantification of key metabolic proteins, which should be readily adaptable to standardized kits, can be diagnostic of metabolic status of subsurface microorganisms. Investigations into the abundance of other important *Geobacter* proteins that have been difficult to detect with mass spectrometer-based methods, such as c-type cytochromes and pili, are under way.

Sulfate reduction is an important process following acetate addition at the Rifle site. Previous studies have demonstrated that there is a diversity of sulfate-reducing microorganisms present, but there has been little information on which sulfate reducers are active. Our previous pure culture studies demonstrated that the expression of genes for dissimilatory sulfite reductase were diagnostic of the specific per-cell sulfate reduction rate. PCR primer sets were designed to specifically quantify the transcripts of the dsrAB genes of each of the three major groups of sulfate reducers at the Rifle site. Quantitative PCR assays revealed that the *Desulfobacteraceae* cluster was the most active, as the number of dsrAB transcripts rapidly increased and remained at high levels ( $1.0 \times 10^4$  transcripts/cell) during the sulfate reduction phase. Although *Desulfobulbaceae* and *Firmicutes* were present at the site, their levels of dsrAB expression were significantly lower ( $1.0 \times 10^1$ – $1.0 \times 10^2$  transcripts/cell) than that of the *Desulfobacteraceae*. These results demonstrate that molecular analysis of the *in situ* physiological status of subsurface communities can provide important insights into subsurface bioremediation.

## BUGS Modeling: Integrating Dynamic Genome-Based Metabolic Models with Reactive Transport Models to Predict the Fate of Contaminants in the Subsurface

Grantee-Led Research

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One of the stated goals of the DOE Environmental Remediation Sciences Program is to develop tools that will make it possible to predict the fate of subsurface contaminants under either natural attenuation or engineered bioremediation conditions, to optimize bioremediation strategies. The overall objective of our research is to develop integrated microbial-reactive transport models that can predict the fate of contaminants in the subsurface under a wide diversity of conditions, based on first principles rather than empirical parameter fitting. This will be accomplished by the BUGS (Bottom Up Genome Scale) Modeling approach, in which genome-scale metabolic models of the relevant microorganisms are coupled with appropriate geochemical and hydrological models. Our previous studies demonstrated the utility of the BUGS Modeling approach to predict the fate of uranium following the addition of acetate to groundwater at the Rifle, CO, study site and to predict the outcome of competition between *Geobacter* and *Rhodoferrax* species under a diversity of geochemical conditions at the site. We have since expanded this modeling framework to include a central metabolic model of *Desulfovibrio vulgaris* and a kinetic model of the acetate-oxidizing sulfate reducer, *Desulfobacter*, based on literature data. Simulations of the response of this microbial community to lactate additions suggest that it may be possible to obtain prolonged uranium reduction by *Desulfovibrio* even after the depletion of the microbially reducible Fe(III). However, laboratory incubations of sediments demonstrated that fermentative *Clostridia* are also likely to compete for the lactate, which has led to an ongoing expansion of the community modeling framework to include these organisms. Additional experimental evaluation revealed that, in contrast to *Geobacter* species, which are primarily planktonic during active Fe(III) reduction, sulfate-reducing microorganisms are primarily attached to sediment surfaces, demonstrating the importance of accounting for attached versus planktonic cells in future modeling efforts. The planktonic nature of *Geobacter* species and their associated need to continue electron transfer while in the planktonic state was captured in a model of the capacitance capability of the c-type cytochromes. This novel mechanistic model of uranium reduction was successfully coupled with a column-scale reactive transport model. An extensive sensitivity analysis of biogeochemical model parameters was conducted to evaluate the effect of hydrological, geochemical, and microbiological phenomena on the extent of uranium removal. These results suggest that biological parameters are highly critical close to the acetate injection site, and that hydrological parameters are more important farther away from the injection site. To better integrate the genome-scale metabolic model and the reactive transport models, we developed a novel computationally efficient approach that enables the integration of large-scale models with minimal computing effort. These results demonstrate that the strategy of integrated, iterative modeling and experimental studies is providing increasingly sophisticated tools for predictively modeling subsurface bioremediation.

## Development and Fundamental Understanding of Catalytic DNA Biosensors for On-site and Real-time Detection and Quantification of Radionuclides and Metal Ions

*Grantee-Led Research*

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We are developing novel catalytic DNA biosensors for simultaneous detection and quantification of bioavailable radionuclides such as uranium, and metal contaminants such as mercury and lead. The sensors are highly sensitive and selective, not only for different metal ions, but also for different oxidation states of the same metal ion (such as U(IV) vs. U(VI)). Such sensors allow for on-site and real-time monitoring of concentration, speciation, and stability of radionuclides and metal contaminants at DOE sites. The richer information will help researchers to better understand subsurface physical, chemical, and biological processes, and to carry out more accurate modeling to explain and predict remediation effects. It will not only help assess the effectiveness of science-based solutions for cleanup by researchers and engineers who perform the cleanup, but also contribute significantly to the long-term monitoring of DOE contaminated sites by DOE staff members, state and local regulation agents, and concerned citizens around the sites.

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

A major obstacle to using this class of sensors for practical applications is the wide temperature variations of sensor performance. We have overcome this obstacle by demonstrating the use of DNA mismatches in tuning the thermal stability, so that the DNAzyme Pb sensor performance can be constant over a wide temperature range. Furthermore, most of the sensor research focuses on labeled sensors with fluorophores or chromophores, not only with a high cost of operation, but also with potentially weaker activity compared with unmodified analogue. We have overcome this limitation by introducing an abasic site of dSpacer into DNAzymes for label-free fluorescent detection of  $\text{Pb}^{2+}$ , with an even lower detection limit (4 nM) than the labeled sensor while maintaining the same selectivity. Finally, we have reported a novel liposome-based system that can detect and respond to the presence of as low as 10 nM of mercury, the Hg MCL defined by the US EPA. In particular, we can modulate the release profile of Hg chelators by the local concentration of Hg, so that more chelators are released in regions of high concentration, and no chelators are released in regions of low concentration. This “budgeted” release profile will be particularly useful in situations where the local levels of Hg contamination vary, or if such contamination is time dependent.

In addition to sensor development for field applications, we are also using biochemical and biophysical techniques, including luminescence and EXAFS spectroscopy, to elucidate the structural elements responsible for high selectivity for radionuclides and metal ions,<sup>7</sup> and the knowledge gained can be very useful for designing more selective sensors as well as chelators for radionuclides and metal ions in remediation.

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

*Grantee-Led Research*

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This project addresses the interrelationships between pore-, core-, and field-scale reactive transport. Our focus is on the geochemical reactions driven by release of caustic Hanford radioactive wastes, their impact on pore structure, and the evaluation of *in situ*, up-scaled rates for these reactions. We target four issues central to the reactive transport scaling problem.

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

Our approach couples (1) reactive column experiments; (2) synchrotron X-ray computed microtomography (CMT), synchrotron micro X-ray fluorescence (XRF), backscattered electron (BSE) and scanning electron (SEM) microscopy, and energy dispersive X-ray (EDX) spectroscopy; and (3) lattice-Boltzmann, network flow, and continuum-based flow and reactive transport models.

During the past year, we have focused on the following goals.

- 1) Experimental investigation of where, within a network of pores and within a single pore, zeolite precipitation will occur. SEM studies of polished sections cut from the flow column experiments (after CMT scanning) will provide this information.
- 2) A new series of flow column experiments with: (1) more careful coordination in the timing between the column experiment and CMT scanning to reduce the closed “batch reactor” time period; and (2) introduction of caustic solution with and without Al to assist in differentiation of dissolution-only effects from those of combined dissolution and precipitation.
- 3) Development of a preliminary reactive network flow model which will be used, ultimately, to compute pore-to-core up-scaled reaction rates.
- 4) Extensive analysis of core up-scaled reaction rates in a reactive network flow model applicable to CO<sub>2</sub> sequestration, to obtain preliminary experience with the computational technique as well as to benchmark general findings that may be applicable to this project.

$^{238}\text{U}/^{235}\text{U}$  Isotope Ratios as Tracers of Chemical Reduction:  
Integrating Observations from the Rifle Field Site and U Ore Deposits

Grantee-Led Research

C.J. Bopp IV, C. Lundstrom (PI), T. Johnson, R. Sanford—*U. of Illinois at Urbana-Champaign*; K.H. Williams, *LBNL*; P.E. Long, *PNNL*

Redox reactions can greatly influence the toxicity and mobility of certain groundwater contaminants. Assessing the extent of reduction using concentration measurements alone can be difficult, especially when concentrations are influenced by sorption, mixing, and complex advection patterns. Isotopic ratio measurements of redox active metals (such as Cr) provide an alternative approach to quantifying reduction and extent of immobilization. We have developed techniques to measure  $^{238}\text{U}/^{235}\text{U}$  to high precision by MC-ICP-MS/double spike methods; this provides a new tool for assessing U(VI) reduction and immobilization. Here, we integrate new  $^{238}\text{U}/^{235}\text{U}$  data for groundwater samples from the Rifle Integrated Field Research Challenge Site with observations of isotopic shifts occurring in different types of U ore deposits, to develop a consistent view of uranium isotope fractionation during subsurface reduction. The Rifle project focuses on a near-surface aquifer contaminated by earlier uranium milling operations. This alluvial aquifer provides an excellent natural laboratory for studying in situ bioremediation of U(VI) (e.g., Anderson et al., 2003). The Winchester Experiment investigated U(VI) reduction accompanying the injection of acetate-amended groundwater. The carbon-rich groundwater stimulates the growth of native iron-reducing bacteria, such as *Geobacter* sp., which reduce mobile U(VI) to U(IV) and sequester it as immobile solids. Our analyses of groundwater samples from this experiment show that  $^{238}\text{U}/^{235}\text{U}$  in dissolved U(VI) decreases steadily with time as U(VI) reduction proceeds. As dissolved U(VI) concentrations decrease from  $\approx 200\mu\text{g/L}$  to  $\approx 20\mu\text{g/L}$ , we observe a systematic change to lower  $^{238}\text{U}/^{235}\text{U}$  of  $\approx 1.00\%$ . In contrast, analysis of a suite of natural background samples shows no systematic change in  $^{238}\text{U}/^{235}\text{U}$  with time or concentration. The shift to lower  $^{238}\text{U}/^{235}\text{U}$  in residual waters observed at Rifle is complementary to the isotopic signature observed in solids produced by reduction of U, such as roll front ore deposits. For instance, systematic difference in  $^{238}\text{U}/^{235}\text{U}$  exists between high temperature hydrothermal uranium ore deposits and those formed by low-temperature U(VI) reduction in aquifers (Bopp, et al, 2009; *Geology*); these latter deposits have higher  $^{238}\text{U}/^{235}\text{U}$ , consistent with preferential incorporation of  $^{238}\text{U}$  into the solid  $\text{UO}_2$  product. Finally, new measurements of  $^{238}\text{U}/^{235}\text{U}$  across the toe of a small roll front in the Colorado front range show this same sense of fractionation. Thus,  $^{238}\text{U}/^{235}\text{U}$  provides a tool for identifying chemical reduction of U, free of the problems that hamper concentration measurements.

## Uranium Biomineralization by Natural Microbial Phosphatase Activities in the Subsurface

*Grantee-Led Research*

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The goal of this project is to examine the role of microbial phosphohydrolases in naturally occurring subsurface microorganisms for the purpose of promoting the immobilization uranium through the production of insoluble uranium phosphate minerals. The results of our prior NABIR-ERSP (SBR) project demonstrate that subsurface microorganisms isolated from radionuclide- and metal-contaminated soils at the DOE Oak Ridge Field Research Center (ORFRC) are acid-tolerant and resistant to numerous toxic heavy metals, including lead. In addition, many of these lead-resistant isolates exhibit phosphatase phenotypes (i.e., in particular those surmised to be phosphate-irrepressible) capable of ameliorating metal toxicity by the liberation of inorganic phosphate during growth on organophosphorus compounds, with the concomitant production of a metal-phosphate precipitate. Liberated phosphate from glycerol-3-phosphate was sufficient to precipitate as much as 95% of U(VI) as low-solubility uranium-phosphate minerals in synthetic groundwater containing either dissolved oxygen or nitrate as terminal electron acceptor in the pH range 5 to 7. In this current project, we have developed an experimental approach to determine whether the activities of naturally occurring microbial phosphatases in subsurface microbial communities result in the immobilization of uranium via the formation of phosphate minerals in contaminated soils.

In collaboration with DOE LBNL investigators (G. Anderson, C. Wu, and T. Hazen), we have been characterizing the subsurface microbial community responses of U(VI) and  $\text{NO}_3^-$  contaminated ORFRC Area 2 and Area 3 soils, as well as the microbial population responses to exogenous organophosphate additions under oxic and anoxic growth conditions, soil slurry, and flow-through reactor experiments conducted at pH 5.5 and 7.0. Soil slurry and flow-through reactor experiments were conducted for 36 days and 80 days at 25°C with 10 mM G2P and 15 mM  $\text{NO}_3^-$  as the sole C, P, and N sources, respectively. Under oxic growth conditions, greater than 4 mM soluble  $\text{PO}_4^{3-}$  was measured at the end of the slurry incubations, and  $\text{NO}_2^-$  was not detected. Preliminary data obtained for anoxic soil slurry incubations indicated an accumulation of greater than 1mM  $\text{PO}_4^{3-}$ , as well as the accumulation and subsequent removal of  $\text{NO}_2^-$ . Following triplicate incubations, 16S rDNA diversity of slurries were analyzed via high-density 16S oligonucleotide microarrays (PhyloChip). Preliminary results suggest that under oxic conditions, the microbial community structure is enriched in proteobacterial taxa at low pH as compared to the diversity of unamended soils. Analyses of slurries incubated under anoxic conditions are under way to identify bacterial taxa capable of organophosphate hydrolysis under both oxic and anoxic environments. Flow-through reactor studies of soils with an initial pH of 3.7 demonstrated robust microbial activities once a pore water pH of 5.5 was achieved. Both denitrification and organophosphate hydrolysis were measured within 2 days of pH adjustment. Our soil slurry and column studies demonstrate the potential efficacy of organophosphate-mediated sequestration of U(VI) by the microbial community residing in ORFRC contaminated subsurface soils.

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

Grantee-Led Research

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From 1957 to 1992, the U.S. DOE conducted 828 underground nuclear tests at the Nevada Test Site (NTS). Today, the resulting cavities (sterilized by temperatures and pressures sufficient to melt rock) represent a unique natural laboratory for studies of legacy waste transport and microbial recolonization of materials altered by nuclear detonations. During the 2008 and 2009 field seasons, in coordination with ongoing “hotwell” monitoring by the DOE Underground Test Area Program (UGTA), fluids from five cavities (GASCON, CHANCELLOR, NASH, ALEMAN, and ALMENDRO) and one tunnel (U12N) were sampled by means of bailers or pumps. Microbial diversity was assessed by a combined molecular (16S rRNA gene libraries) and cultivation-based approach. Our ongoing characterizations represent the first targeted investigation of microbial ecosystems associated with radionuclide-contaminated groundwater at the NTS.

The presence of microorganisms was confirmed for all locations except CHANCELLOR; where very high temperatures (~95°C), 27 years after the detonation that created it, indicate hydrological isolation. Phylogenetic analysis revealed substantially differing microbial communities at individual sites; however, several inferred physiologies and phylogenetic groupings were common between sites. Of particular note were predicted iron-oxidizing and -reducing bacteria (most similar to *Siderooxidans ghiorisii* and *Peelobacter venetianus*, respectively) and a cluster of sequences related (at up to >98% identity) to Firmicute clones from South African gold mines (*Candidatus Desulforudis audaxviator*). As in the deep subsurface, *D. audaxviator* is surmised to subsist on hydrogen and sulfate produced via radiolytic cleavage of water, its appearance at the NTS suggests a role for this novel microbial lifestyle at DOE sites. Additionally, 16S rRNA gene clones most closely related to microorganisms capable of degrading organic (*Delftia acidovorans*) and phenolic compounds (*Phenylobacterium* spp.) were identified, as well as those putatively involved in sulfur cycling (*Desulfovibrio* sp. zt31 and *Thioverga sulfuroxydans*), selenate/selenite reduction (*Azospira oryzae*) and arsenite oxidation (*Ralstonia* sp. 22). Selective NTS-specific media were used to cultivate and quantify selected phenotypes including nitrate-, sulfate-, and iron-reducers, fermentative anaerobes, and methanogens. Several isolates thus obtained are being utilized in related studies.

## Resolving Biological-IP Mechanisms Using Micron-Scale Surface Conductivity Measurements and Column IP Data

*Grantee-Led Research*

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Spectral induced polarization (SIP) measurements appear to be sensitive to microbial activity. If the biogeochemical mechanisms that lead to charge polarization are known, then SIP measurements can be used as a diagnostic and monitoring tool for subsurface microbial activity in soils. Our approach bridges polarization and conductive force microscopy (PCFM) observations made at the nano-to-micron scale with column-scale IP data. Initial PCFM point measurements made on a simple, under-saturated system containing 200 micron silica beads at 1 Hz show an exponential increase in apparent surface conductivity, from a baseline of  $\sim 7.2 \times 10^{-13}$  S to  $7.7 \times 10^{-13}$  S ( $\pm 2 \times 10^{-14}$ ) at 70-75% relative humidity (RH) and  $1.7 \times 10^{-11}$  ( $\pm 1 \times 10^{-13}$ ) S at 90% RH. In the presence of NaCl, values exponentially rise from to  $1.5 \times 10^{-12}$  S ( $\pm 1 \times 10^{-14}$ ) at 50% RH and  $1.65 \times 10^{-8}$  ( $\pm 1 \times 10^{-10}$ ) S at 90% RH. Current maps of the bead surface show approximate registration between  $dI = 1-5$  picoAmps changes in current and nanometer-scale surface features, including scratches and asperities. Parallel column SIP measurements between 0.1-1000 Hz show the expected decrease in phase with increasing salt concentrations. For example, at 1 Hz phase shifts decrease from 0.25 to 0.03 mrad as NaCl concentration is increased from 50 to 300 mg/L. A good level of reproducibility was observed in the IP measurements as the average coefficient of variation across all frequencies was 9% and at 1Hz was 16%. Future, near-term experiments will explicitly connect the effect of surface roughness on the column IP and PCFM measurements in preparation for probing the increased complexity of the microbe-mineral interface.



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

*Grantee-Led Research*

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This project is testing the hypothesis that sedimentary lithofacies determine the geochemical and physical hydrologic properties that control reactive solute transport. The representative geochemical and physical aquifer properties selected for quantification in the proposed project are the properties that control carbon tetrachloride (CT) transport at the Hanford Site (Ringold Formation): hydraulic conductivity and reactivity (sorption distribution coefficient and transformation rate constant). We are combining borehole grain-size data (to identify lithofacies and estimate hydraulic conductivity and porosity) with measurements from archived and fresh core samples (for geochemical experiments and to provide additional constraints on the reactive transport properties) to estimate the spatial distributions of properties that control reactive solute transport in the subsurface.

We have used a surrogate compound approach to determine that (1) the CT sorption distribution coefficient ( $K_d$ ) is low in the Ringold formation and (2) reasonable  $K_d$  estimates can be obtained from the measured organic carbon content ( $f_{oc}$ ) of the sediments using the literature or reference value for  $K_{oc}$  independent of CT concentration. We combined  $f_{oc}$  measurements from fresh and archive samples performed for this project with existing data from the Ringold Fm. at the Hanford Site. In all cases,  $f_{oc}$  data were restricted to samples where the grain-size distribution was known, so that the lithofacies could be determined. We also measured  $K_{oc}$  for duplicate Ringold Formation samples. The  $f_{oc}$  and  $K_{oc}$  sample data were used to estimate the average  $K_d$  for the three different lithofacies.

Through analysis of the boreholes and outcrop, we determined that (1) the Ringold E unit (the primary saturated zone unit contaminated by the CT plume) consists primarily of gravel to cobble material deposited by large multichannel streams, and (2) this unit is lithologically relatively homogeneous with minor sandbody inclusions that appear to be bar-top facies. We used transition probability geostatistical methods to simulate the three-dimensional (3D) distribution of lithofacies that had been identified using grain-size data from boreholes. The 3D distribution of hydraulic conductivity and porosity were generated conditional on borehole data and the previously generated realizations of lithofacies. Because there were not enough data to map the  $K_d$  within the Ringold Fm. aquifer, realizations of the  $K_d$  distribution were generated by substituting the mean  $K_d$  for each lithofacies into the previously generated lithofacies realizations.

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

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

*Grantee-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. Macroscopic metal sorption studies and recent molecular 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  $\text{Hg}^{2+}$  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.

Our preliminary studies indicate that Hg adsorbs strongly on all examined bacterial surfaces, with nearly complete removal of Hg from aqueous solutions. 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 micromolar, with minimal changes below this concentration. The Hg-EXAFS spectra indicate that Hg complexes entirely with cysteine groups at nanomolar and low-micromolar concentrations, and with carboxyl sites at high micromolar concentrations. In addition, the structure of the cysteine complex changes from primarily  $\text{HgS}_3$  to  $\text{HgS}_2$  and  $\text{HgS}$  ( $\text{S}$  = cysteine), with increasing Hg concentrations in the submicromolar range.

The future research plan is to estimate the concentration of these sulfhydryl sites relative to carboxyl and phosphoryl sites on the cell wall, the thermodynamic stability of the important Hg-bacterial surface complexes, and the conditions under which these sites affect Hg speciation. We will explore how these surface sites on bacteria bind to Hg in the presence of different inorganic and organic ligands common in the environment. The surface structures of Hg-sulfhydryl complexes will be analyzed using XAS, and we will make correlations between the structure of the Hg-cell surface complex and Hg methylation rate by the organism. Using this information, we plan to expand this study further to evaluate the release mechanisms of Hg from these sites during bacterial cell lysis. This is important in evaluating whether the biologically reduced or adsorbed metals are released back into the aquatic systems. Release mechanisms and rates are important for modeling the ultimate fate of metals in these bacteria-bearing aquatic systems. 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

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The focus of this research is to determine mechanisms that can release divalent mercury [Hg(II)] from natural organic matter, thus making it available for transformation to methylmercury, the dominant mercury species taken up in the food chain. Contamination by mercury, a neurotoxin, occurs globally from coal-burning, incineration, and volcanism and locally from industrial point sources, including the U.S. Department of Energy's Oak Ridge Y-12 Site where 212,000 kg of Hg were released during the Cold War and dispersed into the floodplain of the East Fork Poplar Creek. The forms of mercury in the flood plain now include Hg(II) bound to natural organic matter and mercury sulfide minerals. When strongly bound to reduced sulfur in soil or dissolved natural organic matter, organic matter coatings on mineral surfaces, or sulfide minerals mercury(II), may become immobilized and/or unavailable for the methylation reaction, depending on particle size. We are learning more about how Hg is bound to sulfur and other sites in organic matter, but the rates and mechanisms of potential Hg release from such sites are largely unknown. For example, how irreversible is Hg sorption to organic matter and organic-coated minerals, and are there natural mechanisms that can remove strongly-bound Hg(II) at fast rates? The goals of this project are to characterize the kinetics of Hg release from soil organic matter, minerals, and minerals coated with organic matter. Understanding these processes is critical in evaluating the potential dispersal of Hg(II) in soil-sediment-water systems and remediation approaches.

We will determine Hg release from organic matter, mineral surfaces, and organic matter-coated mineral surfaces as a function of likely reactants expected in oxidative and reductive natural environments. Testable hypotheses include (1) release of Hg(II) bound to reduced sulfur groups in soil organic matter, and 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.

Experiments and bulk- to molecular-scale analyses, including x-ray absorption spectroscopy (XAS) of natural and experimental materials, will provide quantitative data on immobilization mechanisms for mercury associated with soil organic matter and minerals. Soil organic matter (including humic and fulvic acids) isolated from ORNL's Integrated Field Research Challenge (IFRC) site and redox-sensitive reactants such as ascorbic acid or quinones will be tested for their ability to release bound Hg(II) under controlled oxidation states from mercuric sulfides, phyllosilicate clays, and OM-coated clays characteristic of the Oak Ridge site. Results will be formatted for use in models of mercury transport and bioavailability.

Quantitative data describing the mechanisms and rates of mercury release from soil organic matter, minerals, and OM-coated minerals characteristic of the subsurface environment at the Oak Ridge Site is essential to assess the extent of natural remediation and success of applied remediation technologies. Soluble mercury(II) generally is considered to be bioavailable, and the reversibility of the exchange of Hg(II) between aqueous species and immobilized forms is of critical importance to predicting the distribution of mercury in the environment over short and long time scales.

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

### Grantee-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 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 second year, laboratory studies focused on additional sorbent testing, a redesign of the sensor to include multiple sorbents, sensor validation in laboratory-scale box aquifers, advanced geochemical modeling, and initial field testing in the La Quinta Gallery at the Rifle IFRC site. The new sensor design used an anion exchange resin to measure uranium fluxes and activated carbon to measure water fluxes. Several anion-exchange resins, including Dowex 21K and 21K XLT, Purolite A500, and Lewatit S6328, were tested as sorbents for capturing uranium on the sensor, and Lewatit S6328 was determined to be the most effective over the widest pH range. Four branched alcohols proved useful as tracers for measuring groundwater flows using activated carbon under laboratory and field conditions. The flux sensor was redesigned to prevent the discharge of tracers to the environment, and the new design was tested in laboratory box aquifers and the field. Geochemical modeling of equilibrium speciation using an up-to-date thermodynamic data base suggested Ca-triscarbonato-uranyl complexes predominate under field conditions, while calculated uranyl ion activities were sensitive to changes in pH, dissolved inorganic carbon (DIC) and alkaline earth metals. Results suggested geochemical modeling of the Old Rifle site could probably use any of the standard ionic strength corrections, but should (1) be sure to use accurate pH measurements as a constraint and (2) be sure that the thermodynamic database is up to date. In addition, it would be useful to measure DIC directly rather than to rely upon alkalinity titrations. Initial field tests in the La Quinta gallery at the Rifle IFRC site were conducted to assess ambient groundwater and uranium fluxes, monitor microbial growth on the sensor during field deployment, and further resolve any unforeseen problems evolving from field deployment. Ten flux sensors were deployed in five wells (2 five-foot flux sensors in each well) for three weeks, from mid-November to early December 2009. Observed water fluxes varied from 1.2 to 5.3 cm/d, while uranium fluxes ranged from 0.01 to 2.2 ug/cm<sup>2</sup>d. Uranium and water flux variations corresponded closely with changes in lithology. Uranium fluxes were typically observed to increase with depth and in zones where natural bioreduction is suspected. Stochastic simulations were conducted to estimate the magnitude of uranium discharge over a 10.5 m<sup>2</sup> transect. The mean discharge was ~52 mg/d with a narrow 90% confidence interval of  $\pm 11\%$ . Preliminary microbial biomass and community composition measured on flux meter sorbents varied between well location and depth; however, data are still under review.

Future field studies in the La Quinta and other galleries will be closely coordinated with existing SBR projects to complement data collection. For these studies, the uranium flux sensor will be used in conjunction with molecular-biological tools to characterize microbial community and active biomass at synonymous wells. These field efforts will be designed to quantify spatial changes in uranium flux and field-scale rates of uranium attenuation (ambient and stimulated), 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 generated 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.

## Microbial Community of U-Polluted Subsoil: Search for New Bioremediation Agents Based on *In Situ* Domestication and Kinetic Studies

*Grantee-Led Research*

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The ultimate goal of this project is to achieve a full mechanistic understanding of biotic interactions within microbial community of polluted subsoil, and subsequent use of this knowledge to optimize bioremediation process. We focus on U-contaminated groundwater at several FRC sites, and on building a library of isolates and their consortia from FRC sites— including previously uncultivated ones—which could be used in bioremediation practice as bioactive reagents. Since the majority of subsoil microorganisms resist cultivation on artificial lab media, we use a novel cultivation approach based on exposure of microorganisms to a natural environment in diffusion chambers.

Samples of groundwater (GW) and sediments were taken from the Area2 SRS stimulation plot and used for two kinds of lab experiments: (1) GW was amended with various organic compounds (glucose, ethanol, acetate, glycerol, cysteine, yeast extract, starch) and incubated at 20°C under oxic or anoxic (N<sub>2</sub>) conditions with continuous recording of CO<sub>2</sub> formation rate (IR-gas analyzer), substrate-induced microbial growth (increase in the pellet proteins, plating and direct count of cells with UV-microscope) and U(6)-U(4) transition with scintillation count of radioactivity in solution and solid phase; (2) isolation of bacteria from intact and amended GW after its serial dilution and plating on nonspecific nutrient media in standard Petri dishes and in multi-well diffusion chambers (ichips) as well as in microtiter plates. Degree of cultivability was estimated by (a) comparison of number of colony-forming units (CFU) observed in regular plates and ichips with direct microscopic count and (b) assessment of phylogenetic diversity (16S RNA) of culture collection versus community.

Diffusion chambers and ichips proved to be efficient in cultivating numerous previously uncultivable bacteria in GW. They displayed high resistance to heavy metals, nitrate and acidity. Unexpected result was that anoxic plating on the non-specific complex media (YE, cosamine agar, etc.) always produced 2-3 orders lower CFU counts than oxic plating. Uptake and metabolic conversion of added organic substrates was also less intensive in anoxic incubations with longer lag-phase as compared with oxic conditions. We conclude that GW may not be fully anoxic, and may contain aerobic microsites. Therefore the most promising bioremediation agents should be found among facultative anaerobes able to switch from aerobic respiration to fermentation and anaerobic respiration. At the same time, we cannot completely exclude the assumption that strictly aerobic species can preserve activity in GW if they have high affinity to dissolved oxygen. Reductive immobilization of U was found to occur either in synchrony with microbial growth or precede accumulation of cell mass. The highest stimulatory effect on U-reduction was observed when adding cysteine, followed by glucose and ethanol.

We accumulated more than 1000 isolates from GW and presently are testing their purity and taxonomic position based on 16S RNA sequencing and conventional taxonomic tests. An express high-throughput technique has been developed to screen isolates for their ability to reduce U(6). Serial dilutions in microtiter plates turned out to be convenient alternatives to conventional plating in the primary isolation and screening of active strains. Some isolates were represented by stable consortia rather than pure cultures of bacteria. With the most active pure cultures and consortia, we plan to document their population dynamics in sterile and nonsterile GW enriched with C-substrates, to see whether they are able to survive *in situ* and compete for common substrates with endogenous populations. The most robust strains may be considered as candidates for introduction to contaminated GW for U and nitrate removal.

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

Grantee-Led Research

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Subsurface bacteria, including sulfate-reducing bacteria (SRB), can reduce soluble U(VI) to insoluble U(IV) with subsequent precipitation of  $\text{UO}_2$ . However, it has been shown that SRB-mediated uraninite nanocrystals can be reoxidized (and thus remobilized) by Fe(III)-(hydr)oxides, common constituents of soils and sediments, once the electron donor is exhausted. This reoxidation has also been shown to occur under sulfate-reducing conditions; however, the mechanism of this process remains unresolved. Under abiotic conditions, we have investigated the influence of siderophore compound desferrioxamine B (DFB) on reoxidation of biogenic  $\text{UO}_2$  by ferrihydrite, goethite, and hematite-coated quartz sand in batch systems. Preliminary results indicate that 0.2 mM DFB increases U(VI) formation in solution. DFB promoted solubilization of  $\text{UO}_2$  and Fe(III) minerals show an order of magnitude more Fe(III) than U(IV) solubilized by DFB, suggesting DFB likely promotes reoxidation through dissolution of Fe(III) minerals.

Experiments were performed with *Desulfovibrio desulfuricans* G20 to investigate biotic influences on the system. Following growth of G20 in lactate-limited medium containing 0 or 180 mM U(VI) and sulfate, previously produced and cleaned biogenic  $\text{UO}_2$  was added to systems without U(VI) to compare rates of U(IV) reoxidation in the presence of hematite. Following 4 d of incubation, soluble U(VI) was detected only in systems that began with U(VI) as opposed to previously produced  $\text{UO}_2$ . Fe or S secondary minerals formed during U reoxidation were analyzed using Mössbauer, XRD, HR-TEM, EDS, and SEAD. Results showed that no goethite peaks were present in the samples, and a fraction (~20-25%) of the hematite was transformed. HR-TEM and EDS showed various forms of iron sulfides (predominately amorphous), and intracellular and extracellular coexistence of uraninite and iron sulfides. Mössbauer data suggested the presence of greigite.

Exploratory multicomponent biogeochemical simulations were executed to evaluate thermodynamic and kinetic constraints affecting uranium reduction and the subsequent onset of reoxidation. We have expanded our previous modeling work to include the effect of Fe(II) mineral precipitation, notably FeS, siderite, and magnetite. Reoxidation by hematite and goethite thermodynamically does not produce sufficiently high enough levels of Fe(II) for siderite and/or magnetite to form. However, both of these minerals become thermodynamically stable when ferrihydrite is the oxidant. At pH ~7 and 3 mM  $\text{HCO}_3^-$ , U(VI) concentrations upon reoxidation by hematite or goethite are calculated to remain in the low to sub  $\mu\text{M}$  range, compared to values in the 10–100  $\mu\text{M}$  range when ferrihydrite is the oxidant. However, based on the literature, the hydration of hematite and goethite could yield higher  $\text{UO}_2$  reoxidation than predicted on strict thermodynamic grounds. A system containing only magnetite and  $\text{UO}_2$  was investigated for comparison. When sulfide was added to the system, it was shown that Fe(III) (hydr)oxides oxidize sulfide preferentially to  $\text{UO}_2$ . Therefore, the relative rates of sulfide and  $\text{UO}_2$  oxidation determine the final U(VI) concentrations upon reoxidation. Interactions of  $\text{UO}_2$  with hematite in the presence of the siderophore DFB were also simulated.

## **Metaproteomics Identifies the Protein Machinery Involved in Metal and Radionuclide Reduction in Subsurface Microbes and Elucidates Mechanisms and U(VI) Reduction Biomarkers**

*Grantee-Led Research*

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The overall goal for this newly funded project is to develop and exploit environmental metaproteomics tools to identify biomarkers for monitoring microbial activity affecting U speciation at U-contaminated sites, correlate metaproteomics profiles with geochemical parameters and U(VI) reduction activity (or lack thereof), elucidate mechanisms contributing to U(VI) reduction, and provide remediation project managers with additional information to make science-based site management decisions to achieve cleanup goals more efficiently.

Although significant progress has been made elucidating the microbiology contributing to metal and radionuclide reduction, the cellular components, pathway(s), and mechanisms contributing to U transformation remain poorly understood. Recent advances in (meta)proteomics technology enable detailed studies of complex samples, including environmental samples, which differ between sites and even show considerable variability within the same site (e.g., the Oak Ridge IFC site). Additionally, site-specific geochemical conditions affect microbial activity and function, suggesting generalized assessment and interpretations may not suffice. This research effort will integrate current understanding of the microbiology and biochemistry of U(VI) reduction and capitalize on advances in proteomics technology made over the past 2 years. Initial studies will use Area 2 Oak Ridge IFC sediment for continuous flow sediment column studies, followed by analyses of Oak Ridge IFC field samples from locations where slow-release substrate biostimulation has been implemented to accelerate *in situ* U(VI) reduction rates.

Our overarching hypothesis is that the metabolic signature in environmental samples, as deciphered by the metaproteome measurements, will correlate with U(VI) reduction activity. The detailed analysis of proteins linked with U(VI) reduction activity will (1) test if c-type cytochrome isoforms, previously associated with radionuclide reduction activity, are suitable biomarkers, (2) identify new biomarker targets for site assessment and bioremediation monitoring, and (3) provide new information about specific proteins and mechanisms involved in U(VI) reduction and immobilization.

Since metaproteomic and metagenomic characterizations are computationally challenging and time-consuming, we will use a tiered approach that combines database mining, controlled laboratory studies, U(VI) reduction activity measurements, phylogenetic analyses, and gene expression studies to support the metaproteomics characterizations. Optimization of the proteomic tools will include in-depth comprehensive metagenomic and metaproteomic analyses on a limited number of samples. Optimized metaproteomic analyses will be applied to Oak Ridge IFC field samples to demonstrate the utility of the new tools for site assessment, efficient implementation of bioremediation and long-term monitoring. The expanded toolbox will contribute to implementing science-driven site management with broad benefits to the DOE mission.

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

*Grantee-Led Research*

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This project was selected for funding through the Environmental Remediation Sciences Program Solicitation 09-07 and work will begin in the spring of 2010. 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. The novelty of the proposed work lies largely in the manner the information from these measurements and calculations will be combined into a model that will be used to evaluate the thermodynamics of plutonium sorption reactions, as well as predict sorption of plutonium to sediments from DOE sites, using a component additivity approach. Additional studies will incorporate effects of surface-mediated redox interactions, as well as complexation with natural organic matter (NOM), into the model.

This research will test the hypotheses that (1) strong interactions of plutonium with mineral surfaces are due to formation of inner-sphere Pu(IV) complexes, which are mechanistically driven by displacement of solvating water molecules from the actinide and mineral surface during sorption; and (2) under environmental conditions, desorption of these inner sphere complexes can only be accomplished by mineral surface-mediated oxidation to more soluble Pu(V) or Pu(VI) or through ligand-enhanced dissolution with NOM.

Testing these hypotheses and meeting the objective of developing a mechanistically based sorption model will require coupling studies of macroscale plutonium sorption with an understanding of the chemical speciation and bonding environment of Pu at the mineral surface. These studies will follow an experimental program with three tasks. The first task will measure sorption values and enthalpy of Pu(IV) and Pu(V) sorption to pure mineral phases, as well as sediments from the Savannah River Site and the Hanford Site. The batch sorption data will be coupled with the results of XAS and electron microscopy. Task 2 will consist solely of quantum-mechanical calculations to determine sorption energies and activated states involved in adsorption, and observations of the electronic interactions between the adsorbate, co-adsorbate, and substrate, and possible reduction of actinide species. Both the computational studies and experimental studies will be used to build a surface complexation model of plutonium speciation consistent with experiments and atomistic simulations, and based on actual reaction mechanisms. This model will evaluate the strength of plutonium surface interactions, as noted in Hypothesis 1 above. The third task of this project is to test the second hypothesis by performing plutonium desorption tests under variable redox conditions and in the presence and absence of organic ligands intended to simulate NOM and microbial exudates.



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

*Grantee-Led Research*

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This exploratory 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 (DS) 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 mineral 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, bio-mineralization rate).

We have started testing H1 by performing DS measurements (alpha and beta range) on iron (Fe) particles of dimensions similar to microbial cells, dispersed within agar gels over a range of Fe concentrations. We have tested the ability of the physics-based modeling to predict volume concentrations of the Fe particles by assuming that the Fe particles are polarizable inclusions within an otherwise nonpolarizable medium. We are currently evaluating the smallest volume concentration that can be detected with the DS method. Similar experiments and modeling have been performed on the sulfate-reducing bacteria *D. vulgaris*. Synchrotron x-ray absorption measurements have been conducted to determine the local structure of biominerals coatings on *D. vulgaris* which were grown in the presence of different Fe concentrations. Future plans include quantifying the thickness of the mineral growth on cell wall using DS in the beta region. Future work will also involve total scattering measurements with hard x-ray beams (high-q) to determine the structure characteristics of atomic sites for a range significantly beyond that determined by x-ray absorption.

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

*Grantee-Led Research*

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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 the “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 rock-mass characteristics, including network structure and matrix properties. There is significant recent evidence for an apparent scale-dependence in “effective matrix diffusion”—estimated values appear to increase with scale and sometimes far exceed typical molecular diffusivities. 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. 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 3-D 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 on (1) the relationship between effective fracture-matrix interaction parameters, network structure, and matrix properties and (2) their scale dependence in different types of fractured rock environments.

We will show results obtained using “very-high-resolution” particle tracking algorithms at the single fracture scale and at fracture intersections. We are developing upscaled particle-tracking algorithms for the field-scale three-dimensional code, which will allow use of much larger time steps. The next step in the research is to verify the accuracy of the upscaled algorithms using the “very-high-resolution” simulation results as a benchmark, and refine them as needed. We will then pursue simulations on large field-scale fracture networks.

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 initial 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

Grantee-Led Research

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Surface-attached communities or 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 biofilms are often assumed in the subsurface, particularly at the matrix-well screen interface, evidence for their development in the bulk aquifer matrix is scarce, and their potential contribution to contaminant transformations remains largely unknown. To gain insights into how biofilms contribute to uranium transformations in the subsurface, we used *Geobacter sulfurreducens* as a model representative of the *Geobacter* species that predominate during active *in situ* bioremediation of uranium and other soluble metal contaminants with acetate as an electron donor. We investigated the potential of *G. sulfurreducens* biofilms in uranium bioremediation by studying the role of biofilm developmental stages (monolayer, immature microcolonies and mature microcolonies) in uranium transformations using X-ray absorption (XAS) spectroscopy. Soluble U(VI) was the predominant form of uranium in monolayered and immature biofilms, while U(IV) was most abundant in mature biofilms. These results indicate that biofilms play a role in U(VI) transformations via first a sorption and then a reductive mechanism. The reductive precipitation of U(VI) increased as the biofilms developed and proportionally to the expression of the pilus nanowires, which electronically connect the biofilm cells and also maintain the biofilm structure. In order to investigate a potential role of the biofilm nanowires in the reduction of U(VI) to U(IV) during biofilm development, we studied uranium transformations by biofilms of a pilin-deficient mutant, which cannot assemble the pilus nanowires and is interrupted at the immature microcolony stage. Although it adsorbed soluble U(VI) at rates comparable to the wild type biofilms at this stage in development, it could not reduce U(VI). Microscopy, physiological and XAS analyses confirmed the role of *G. sulfurreducens* conductive pili in U(VI) reduction as a U(IV) precipitate. Furthermore, expression of the pilus nanowires also protected the cells from the toxic effects of soluble U(VI) and preserved cell viability after prolonged exposure to U(VI). These results 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.

## **Advanced Self-Potential and Induced Polarization Inversion: Development and Use for Investigating Natural Recharge Processes**

*Grantee-Led Research*

A. Revil (PI), *Colorado School of Mines*; S.S. Hubbard, *LBL*

The goal of our project is to develop a new approach to imaging preferential groundwater flow paths and to be able to understand the biochemistry taking place in these areas. We have developed a general theory of two geophysical methods that are both sensitive to groundwater flow and the presence of contaminants. The self-potential method is a passive geophysical method in which the electrical potential is recorded at the surface of the earth with non-polarizing electrodes. It is very similar in essence to electroencephalography used to localize nonintrusively brain activity. The self-potential signals are sensitive to the Darcy velocity, the TDS of the pore water, and the presence of contaminants. This method can also be used to get information on the distribution of the redox potential in unconfined aquifers in the presence bacterial biofilm in the capillary fringe, pointing out the role of bacterial pili in the generation of these signals. A general theory of self-potential signals related to reactive transport modelling has been developed recently, and an application to the time-lapse monitoring of a salt plume moving by buoyancy in a tank has been performed by Martínez-Pagán et al. (2010). We have developed both deterministic and stochastic inverse modelling to solve the self-potential inverse problem.

A complementary geophysical method concerns complex resistivity tomography (also called spectral-induced polarization). We have developed a new theory in which induced polarization can be used to image nonintrusively the distribution of permeability and the presence of heavy metals. We are presently developing a 3-D tomography technique (combining both deterministic and stochastic approaches) to image preferential fluid flow pathways (Jardani et al., in preparation), using this approach and joint inversion algorithm of self-potential with induced polarization.

We are presently coupling these modelling efforts to the reactive transport code TOUGHREACT, to be able to perform both forward and stochastic inverse modelling of reactive transport in porous media through the use of these geophysical techniques. This modelling effort will be tested at Oak Ridge in April 2010 downstream of Pond S3.

## Physiological Models of Microbial Redox Metabolism in Biostimulated Uranium(VI)-Contaminated Subsurface Sediments

Grantee-Led Research

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R. Findlay, *U. of Alabama*; Q. Jin, *U. of Oregon*

The goal of this project is to obtain explicit information on the linkage between microbial redox transformations associated with U(VI) reduction and the physiology and phylogeny of microorganisms that proliferate in subsurface sediments undergoing biostimulation through ethanol or acetate amendment. The central hypothesis is that patterns of U(VI) reduction in sediments experiencing shifts in the predominant terminal electron accepting pathway (TEAP) will be directly linked to changes in the relative abundance and activity of different types of respiratory microorganisms, which in turn are controlled by the abundance and availability of electron donors and acceptors. A key goal of the work is to analyze and interpret the results of experimental sediment reactor studies using a microbial population dynamics-based model of TEAPs in biostimulated subsurface sediments. Development and refinement of this model will ultimately produce a reaction module that can be incorporated into field-scale reactive transport simulations for sites (e.g., the ORFRC) where a complex assemblage of TEAPs is likely to take place over space and time during in situ biostimulation.

Initial project activities focused on analysis and simulation of two long-term (3-4 months) flow-through (semicontinuous culture) reactor experiments with different types of U(VI)-contaminated sediment from Area 2 at the ORFRC, where ongoing field-scale U(VI) remediation research involving biostimulation is taking place. The sediments examined included (1) Fe(III) oxide-rich saprolite from a depth of ca. 5 m, just below the gravel layer at Area 2; and (2) Fe(III) oxide-depleted material from a depth of ca. 4 m within the gravel layer. Continual addition of small amounts of ethanol (equivalent to an average input of ca.  $0.1 \text{ mM d}^{-1}$ ) led to complete consumption of all incoming nitrate (ca.  $0.05 \text{ mM d}^{-1}$ ), and to sequential (and in some cases partially overlapping) reduction of Fe(III) and sulfate. Changes in the chemical composition of the reactors were consistent with the results of culture-based (roll tube enumerations) and molecular (T-RFLP and gel-element microarray analysis of PCR-amplified 16S rRNA genes) microbial community analyses, both of which revealed proliferation of *Betaproteobacterial* nitrate-reducing and *Deltaproteobacterial* Fe(III)- and sulfate-reducing taxa. The results of these experiments were simulated using a microbial physiology-based reaction model that accounts for both kinetic and equilibrium chemical reactions and potential thermodynamic controls on TEAPs (Jin and Roden, submitted). The model was initially developed based on a batch reactor experiment with Fe(III) oxide-rich Area 2 sediment (Mohanty et al., *Environ. Sci. Technol.*, 2008), and calibrated for simulation of semicontinuous culture reactor (SCR) experiments based on an initial SCR experiment with Fe(III) oxide-rich Area 2 sediment. The simulations effectively reproduced the results of the SCR experiments with the two different types of Area 2 sediment, in which the relative importance of Fe(III) versus sulfate reduction differed significantly as a result of differences in the abundance of solid-phase Fe(III).

As yet unexplained complexities in the aqueous/solid-phase partitioning and redox behavior of endogenous U(VI) in highly U(VI)-contaminated Area 2 sediments made it difficult to understand and simulate the behavior of uranium in the SCR experiments. A preliminary “fed batch” (with ethanol as the electron donor) experiment was conducted with a slurry of deeper (7-9 m depth), less U(VI)-contaminated saprolite from Area 2 sediment, to which ca.  $0.15 \text{ mmol L}^{-1}$  of exogenous U(VI) (uranyl-acetate) was added from a stock solution. Progressive reduction of the added U(VI) was observed in connection with Fe(III) and sulfate reduction following nitrate depletion. The sediment slurry is being used to test our implementation molecular-microbial-community analytical techniques (qPCR of functional and 16S rRNA genes, stable isotope probing of 16S rRNA genes and PLFAs), and to evaluate the efficacy of ethanol versus acetate as an electron donor for U(VI) reduction under sulfate-reducing conditions.

## Effect of Physical Heterogeneity and Flow Transients on the Mobilization of Colloids and Colloid-Associated Radioactive Cations in Partially Saturated Porous and Fractured Media

*Grantee-Led Research*

J. Ryan (PI), *U. of Colorado*; J. Saiers, *Yale U.*; P. Jardine, *ORNL/U. of Tennessee*

The objective of this study has been to elucidate the influences of physical heterogeneity, flow transients, solution chemistry, moisture content, and cation desorption kinetics on the mobilization and transport of colloids and colloid-associated radioactive cations in water-unsaturated porous and fractured media. To address these objectives, we have conducted laboratory experiments examining (1) the transport of mineral colloids,  $^{137}\text{Cs}$ , and  $^{90}\text{Sr}$  in saturated and water-unsaturated quartz sand columns at varying moisture contents and with physical heterogeneity simulating macropore flow; (2) the transport of mixtures of mineral colloids and  $^{137}\text{Cs}$  in water-unsaturated sediments under steady-flow conditions; (3) the mobilization of *in situ* colloids and  $^{137}\text{Cs}$  induced by drainage and imbibition of  $^{137}\text{Cs}$ -contaminated sediments, and (4) the mobilization of colloids and organic matter from saprolitic soil collected at the Oak Ridge National Laboratory under simulated rainfall conditions. Results of the first experiments demonstrate that lower moisture content enhances the importance of cation desorption kinetics in determining the extent of colloid-facilitated transport in unsaturated porous media, and that physical heterogeneity in the form of a simulated macropore accelerates facilitated transport, such that desorption kinetics do not significantly affect the extent of colloid-facilitated transport of  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$ . Results of the second experiment show that substantial quantities of *in situ* colloids and colloid-associated  $^{137}\text{Cs}$  are mobilized during porous-medium drainage and imbibition, and that the effect of colloids on  $^{137}\text{Cs}$  transport is sensitive to changes in pore-water ionic strength and dissolved-organic-matter concentration during both steady and transient pore water flow. Also, the mass of colloids and  $^{137}\text{Cs}$  transported during transient flow is greater than what would be inferred on the basis of steady-flow experiments, at high ionic strengths in particular. Results of the fourth experiment show that colloid mobilization in the water-unsaturated Oak Ridge saprolite is controlled by fracture flow and greatest during transients in flow.

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

*Grantee-Led Research*

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One of the key risk drivers at radioactive waste disposal facilities is radioiodine, especially  $^{129}\text{I}$ . Little research has been carried out at these facilities beyond simple monitoring of groundwater plumes. The objective of this work is twofold: (1) to determine the chemical speciation (iodide, iodate, organo-iodine) of  $^{129}\text{I}$  and  $^{127}\text{I}$  in groundwater samples from two  $^{129}\text{I}$ -contaminated DOE sites and (2) to investigate the extent that organic matter content, microbial cells, enzymatic activity, and exopolymeric substances affect the speciation and mobility of iodine in sediments at these sites. Three hypotheses will be tested through detailed environmental characterization paired with controlled laboratory experiments: (H1) The chemical form of iodine in groundwater is dependent not only on abiotic, but also biotic processes. Importantly, these processes must be investigated using environmentally relevant iodine and ligand(s) concentrations; (H2) Iodine mobility through sediment is highly dependent on its speciation; organo-iodine mobility can be enhanced or retarded depending on the organic moiety, whereas inorganic iodine mobility is largely dependent on its oxidation state; (H3) Naturally occurring microorganisms present in the Hanford Site and Savannah River Site subsurface environment can alter the chemical form of iodine. Our experimental approach determines how microbial activity, concentrations and chemical speciation (iodide, iodate, and organo-iodine) of  $^{129}\text{I}$  and  $^{127}\text{I}$ , as well as organic carbon, affects iodine mobility in contaminated groundwater at the Hanford and Savannah River Sites. Methods will include: 16S rRNA gene sequencing, FISH, MICRO-FISH, and species-specific DNA probes for microbiology, EM, HPLC, CHN, TOC, GC-MS, NMR and AMS for iodine and organic iodine characterization, and use of  $^{125}\text{I}$  radiotracers. Results using tracer  $^{125}\text{I}$ -and stable  $^{127}\text{I}$  additions to Savannah River Site sediment slurries demonstrate that iodine mobility varies greatly with iodine concentration, and contact time with the sediment and organic carbon (OC) content, mostly due to covalent binding of iodine to OC moieties in soils and aquifer sediments (Schwehr et al., 2009). Column experiments using  $^{125}\text{I}$  and  $^3\text{H}$  as well as aquifer sediments confirmed significant retardation of iodine at ambient concentrations, i.e.,  $10^{-8}$  to  $10^{-7}$  M, but not at artificially high concentrations of iodide (i.e. concentrations used in most laboratory analysis,  $\geq 0.1$  mM/L). Over 30 mg of organic and inorganic matter within the size fraction  $> 0.5$  microns and 20 mg of organic and inorganic matter within the size fraction  $> 3$  kDa was isolated from water equilibrated with SRS aquifer sediments for molecular-level chemical characterization. The aromatic fraction, which is expected to contain most of the iodine, was quantified by using ATR-FTIR and spectrometric methods. Enzymatic analysis of the sediments revealed significant, though low,  $\text{I}_2$  oxidizing activity. Iodide accumulation by F-area bacterial isolates revealed that accumulate iodine when  $\text{H}_2\text{O}_2$  was added. Finally, we developed a new and convenient method for determining the speciation (i.e., iodide, iodate, organo-iodine) of  $^{129}\text{I}$  and  $^{127}\text{I}$  in water from SRS aquifers at ambient conditions, using GC-MS methods after sediment combustion and derivatization of the leachate solution, giving a low detection limit of 10 ng/L.

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

Grantee-Led Research

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

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

Our research this past year has been focused primarily in two areas: (1) preparation and immobilization of appropriate redox active catalysts (e.g., metalloporphyrins) and (2) preparation and immobilization of effective solid phase electron donors (e.g., micro- and nano-sized zero valent iron). Efficacy of these prepared materials for use in the reduction of contaminants at DOE facilities were screened using Cr(VI), so that promising systems could be identified for subsequent testing with U(VI). Natural porphyrins selected for testing in this project were protoporphyrin (Proto) and uroporphyrin (Uro). These two porphyrins were then complexed with Co(II) or Fe(II) as to form their corresponding metalloporphyrins. A mixed solvent reflux procedure was used to rapidly promote metal complexation by the porphyrins, and synthesis completion was verified by absorption spectroscopy as well as ICP-MS measurements of the remaining (uncomplexed) Co and Fe in solution. Yields of Proto-Co, Proto-Fe, Uro-Co, and Uro-Fe were found to be  $98.5 \pm 2.5\%$ ,  $100.6 \pm 5.1\%$ ,  $98.9 \pm 4.0\%$ , and  $101.2 \pm 3.4\%$ , respectively. Vitamin B12 (VB12), a cobalt-containing natural corrin (i.e., porphyrin analog), was also included for testing.

Previous reports have suggested that immobilizing redox catalysts can extend their lifetimes without adversely affecting their catalytic capabilities. Such systems, however, have never been fully examined for use in the reduction of metals. The four metalloporphyrins synthesized above and VB12 were immobilized in sol-gels using a variety of procedures previously reported in the literature. The sol-gels containing the catalysts were then ground to a fine size and characterized using a variety of electron microscopy and surface area techniques. Nano-sized zero valent iron (nZVI) was immobilized in Ca-alginate beads. First, nZVI particles were synthesized following our previously reported procedure and then characterized by electron microscopy, ICP-MS, and surface area analysis. The nZVI particles ranged in size from 30-50 nm, consisted of  $99.3 \pm 0.4\%$  Fe, and had a surface area of  $26.9 \pm 5.9 \text{ m}^2/\text{g}$ . The nZVI particles were then added to a sodium alginate solution and mixed with sonication for 30 min. The nZVI-containing alginate solution was then drawn into a syringe and added dropwise to a  $\text{CaCl}_2$  solution in an anaerobic chamber. The Ca-alginate beads formed immediately and were hardened in solution for 6 hr before filtering and drying in the anaerobic chamber for 48 hr. While in solution the beads were elastic, with a diameter of about 4 mm, but upon drying and hardening they shrank to a diameter of about 1 mm. Scanning electron microscopy revealed that the surface of the dry beads were rough and had visible cracks.

Electron donor-catalyst systems are now being screened under a variety of experimental conditions using Cr(VI) reduction first. Promising systems will then be tested subsequently for reduction of U(VI). Both micro-sized ZVI (mZVI) and nZVI are being tested in the presence and absence of free and immobilized metalloporphyrin catalysts. Because metal-reduction reactions often lead to precipitation of the reduced metal species, reuse experiments are also being conducted to determine whether the immobilized and/or free catalysts and electron donors will be adversely affected over longer reduction reaction times.



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

*Grantee-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 to date has explored the use of continuous waterborne electrical imaging (CWEI), in conjunction with fiber-optic distributed temperature sensor (FO-DTS) monitoring, to improve the conceptual model for how groundwater/surface water exchange regulates uranium transport.

Inversion of the CWEI datasets (a data rich survey containing ~60,000 measurements) provided predictions of the distributions of electrical resistivity and polarizability, from which the spatial complexity of the primary hydrogeologic units along the river corridor has been reconstructed. Variation in the depth to the interface between the overlying coarse-grained, high-permeability Hanford Formation and the underlying finer-grained, less permeable Ringold Formation, an important contact that limits vertical migration of contaminants, has been resolved along ~3 km of the river corridor centered on the IFRC site in the Hanford 300 Area. Polarizability images were translated into lithologic images using established relations between polarizability and surface area normalized to pore volume ( $S_{por}$ ). Spatial variability in the thickness of the Hanford Formation captured in the CWEI datasets indicates that previous studies based on borehole projections and drive-point and multi-level sampling overestimate the contributing area for uranium exchange within the Columbia River at the Hanford 300 Area. The FO-DTS data recorded along a 1.5 km of cable with a ~1-m spatial resolution and 5-minute sampling interval revealed subreaches showing (1) temperature anomalies (relatively warm in winter and cool in summer) and (2) a strong correlation between temperature and river stage (negative in winter and positive in summer), both indicating reaches of enhanced, surface-water/groundwater exchange. The FO-DTS datasets confirm the hydrologic significance of the variability identified in the CWEI and reveal a pattern of highly focused exchange, concentrated at springs where the Hanford Formation is thick, and coinciding with a paleochannel identified in ground penetrating radar surveys at one location. No evidence for focused exchange is observed in the FO-DTS data where the Ringold Formation is in contact with the streambed or the Hanford Formation is thin. Our work performed to date illustrates how the combination of CWEI and FO-DTS technologies can characterize surface-water/groundwater exchange in this complex, coupled river-aquifer system.

Vertical temperature arrays and pressure transducers were installed at locations determined by the FO-DTS anomalies in August 2009. Datasets recorded with these arrays will (1) be modeled for groundwater fluxes through the river bed and hydraulic properties, and (2) be used to support time-series analysis of the FO-DTS to determine forcing mechanisms driving the nonstationary temperature signals captured with the FO-DTS. Data from a three month time-lapse 2D resistivity survey along a 755 m line parallel to shore will be processed to further constrain groundwater/surface-water exchange at spatial scales not captured with the FO-DTS. Monitoring results from this survey also will guide a focused 3D resistivity monitoring effort in Year 3 of the project. Electrical measurements on cores obtained from the Hanford IFC database will be used to help constrain the hydrogeological interpretation (in term of solute and/or uranium exchange) of the 2D and 3D resistivity datasets. A revised stratigraphic model accounting for spatial variations in Hanford/Ringold contact is being developed for predicting contaminant fluxes to the river, using the STOMP code.

## Molecular Mechanisms of Bacterial Mercury Transformation

*Grantee-Led Research*

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Mercury is a key contaminant found at DOE sites. Understanding the many biotic and abiotic factors contributing to mercury mobility and transformation is critical for devising new remediation strategies. Among these factors, detoxifying bacteria have a particularly strong influence on toxic methylmercury levels. The research here will characterize the physical pathways and chemical transformations that mercury undergoes in these bacteria, by combining long-standing expertise in the biochemistry and structural biology of bacterial mercury detoxification in the UGA and UCSF teams with the expertise and world-class facilities in neutron scattering and computational science at UT and ORNL. A fundamental understanding will be obtained of two basic intracellular bacterial mercuric biotransformation processes: regulation and reduction. These processes are mediated by the proteins of the mer operon. The regulatory protein, MerR, senses intracellular levels of Hg(II) and activates expression of all other mer genes. By combining low-resolution molecular envelope measurements from neutron scattering with high-resolution computer simulation, we will test specific hypotheses for the mechanism of MerR action at atomic-detail. The flavin oxidoreductase MerA performs the reduction of Hg(II) to inert, non-toxic Hg(0) which readily diffuses from the cell. High-performance mixed quantum mechanical/molecular mechanical (QM/MM) computer simulations will be performed to elucidate the catalytic mechanism of MerA, i.e., the physical principles with which MerA accelerates the reduction process. Elucidation of the molecular principles of mercury regulation and reduction in bacteria is critical to understanding bacterial function in Hg-contaminated areas, and to the conception of a design strategy for biotransformation enhancement. The totality of the information obtained will enable us to assemble a quantitative physicochemical picture of the transformation processes undergone by mercury in the bacterial cell, which the UGA and UCSF teams and other ORNL investigators will be able to relate quantitatively to extracellular mercury and organomercury mobilization processes.

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

Grantee-Led Research

R.W. Smith (PI), Y. Fujita—*INL*; T. R. Ginn, *U.C. Davis*; S.S. Hubbard, *LBNL*

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  $^{90}\text{Sr}$ , is co-precipitation in calcite. We have previously found that calcite precipitation and co-precipitation of Sr can be accelerated by the activity of urea hydrolyzing microorganisms, that higher calcite precipitation rates can result in increased Sr partitioning, and that nutrient additions 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, and investigating the coupling between flow/flux manipulations and precipitate distribution and metal uptake.

Our first field campaign was conducted in 2008 at the Vadose Zone Research Park (VZRP), an uncontaminated aerobic site with subsurface characteristics similar to much of the nearby  $^{90}\text{Sr}$  contaminated Idaho Nuclear Technology and Engineering Center (INTEC). Well-to-well experiments using conservative tracers, molasses, and urea were conducted to estimate *in situ* first-order urea hydrolysis rate constant of  $0.034\text{ d}^{-1}$ . This was more than an order of magnitude higher than rate constants estimated above-ground using water samples, suggesting that attached microbial communities may play a significant role for *in situ* urea hydrolysis. In 2009, *in situ* static experiments were conducted and revealed that the activity of the attached microbial community ( $17,000\text{ pmol kg}^{-1}\text{ hr}^{-1}$ ) was more than 3 orders of magnitude higher than the unattached communities ( $3.6\text{ pmol kg}^{-1}\text{ hr}^{-1}$ ). Preliminary biomass estimates based on phospholipid fatty acid analyses for the attached and unattached communities differ by approximately the same order of magnitude.

Planning for a field campaign at the Rifle Integrated Field Research Challenge site has been initiated. Using qPCR, *ureC* genes have been detected in water and sediment samples from across the site, and laboratory estimations of ureolysis rates in Rifle groundwater are an order of magnitude greater than those measured in VZRP groundwater. These data, together with results from mining of metagenomic surveys and knowledge of the geochemistry at the site, suggest that stimulation of ureolytically driven calcite precipitation should also be effective at Rifle. We are evaluating the ability of organic carbon substrates to increase ureolytic activity in Rifle microcosms, to support the design of field experiments planned for the summer of 2010.

Combining TOUGHREACT with the inverse modeling tool UCODE, we have calibrated ureolysis and precipitation kinetics models from batch data and successfully applied the result to simulate precipitate formation in columns under both stop-flow and continuous injection (of reagent solutes) scenarios. We find that the strategy of stop-flow injection, in a direction opposite to that used to emplace the attached (filtered) ureolytic bacterial cells, most effectively resulted in a uniform spatial distribution of precipitate. This reaction model is being upscaled via incorporation in streamtube-ensemble representations of *in situ* flows. The developed reactive transport models will be used for the design and interpretation of our field experiments.

## Mineral Solubility and Free Energy Controls on Microbial Reaction Kinetics: Application to Contaminant Transport in the Subsurface

*Grantee-Led Research*

P. Van Cappellen, *Georgia Tech*; C.I. Steefel (PI), *LBNL*; M. Taillefert, *Georgia Tech*; T. Behrends, *Utrecht U. (The Netherlands)*; S. Webb, *Stanford U.*

Recent developments in the theoretical treatment of geomicrobial reaction processes have resulted in the formulation of kinetic models that directly link the rates of microbial respiration and growth to the corresponding thermodynamic driving forces. In this new project, we propose to verify and calibrate these kinetic models for the microbial reduction of uranium(VI). The approach will combine laboratory experiments on uranium bioreduction and field-scale reactive transport modeling.

Rates of enzymatic reduction of U(VI) will be measured under variable, but controlled, geochemical conditions. Experiments will be carried out in mixed flow-through retentostat reactors, by supplying known uranium reducing bacteria (*Geobacter metallireducens*, *Shewanella putrefaciens*, *Desulfovibrio desulfuricans*) with inflow solutions containing soluble U(VI) and an electron donor. Gibbs energy yields will be manipulated by varying the concentrations of U(VI) and electron donor, as well as the carbonate alkalinity. Once we have made headway with the uranium-only experiments, we will supply the bacteria with both U(VI) and Fe(III), using different iron(III) oxyhydroxides. The aim is to test whether the competition between microbial U(VI) and Fe(III) reduction can be rationalized in terms of the relative Gibbs energy yields of the two reduction pathways. Rates of U(VI) reduction, Fe(III) reduction and biomass growth will be monitored as a function of time; mineral precipitates will be characterized by XRD, electron microscopy, EXAFS and micro-XRD; solubility products of the mineral reactants and biogenic precipitates will be measured independently.

The experiments will yield a data set of co-varying rates of U(VI) and Fe(III) reduction, bacterial growth rates, as well as mineral solubilities and Gibbs energies of reaction. The data will be used to derive the functional dependencies of enzymatic U(VI) and Fe(III) reduction kinetics on the corresponding Gibbs energies of reaction. The new kinetic descriptions of microbial U(VI) and Fe(III) reduction will replace those currently used in reactive transport models that couple catabolic energy generation and growth of microbial populations to the rates of biogeochemical redox processes. Reactive transport calculations incorporating the new, bioenergetically-dependent, rate expressions for microbial reactions will be used to simulate the fate and transport of DOE relevant subsurface contaminants in aquifers, with an emphasis on uranium. Sensitivity analyses will determine how alternative formulations of microbial and chemical reaction kinetics affect the model predictions. Model applications will focus on fate and transport issues at the Old Rifle site. Of particular interest is whether our current process understanding is sufficient to explain the switch from iron reduction to sulfate reduction and the concurrent decrease in uranium bioreduction observed at this DOE site.

## Characterization of Outer Membrane Proteins Involved in Iron Reduction and Biofilm Formation in *Geobacter Sulfurreducens*

Grantee-Led Research

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*Geobacter sulfurreducens*, an iron-reducing bacteria commonly found in subsurface environments, is capable of utilizing iron and other metals in the environment for respiration. It has been proposed that *Geobacter* reduces iron oxides via direct contact, and that biofilm formation facilitates direct interaction with solid electron acceptors. Great progress is being made to understand the iron-reduction pathway in *G. sulfurreducens* with respect to the electron acceptor being utilized. However, it is not well understood how protein expression changes in the context of a biofilm.

Biofilm research has shown that cells undergo genetic changes as they switch from a free-living lifestyle to a sessile lifestyle. This renders the biofilm cells phenotypically different from their planktonic counterparts. The literature has shown that there are several outer membrane c-type cytochromes (Omc) and a pilin protein that are important for iron oxide reduction in *G. sulfurreducens*. These proteins are OmcE, OmcS, and PilA. The loss of these proteins does not affect the reduction of iron citrate, a soluble electron acceptor. The loss of OmcB, on the other hand, affects both soluble and insoluble iron reduction, and it has been proposed that OmcB plays a central role in the iron-reduction pathway. Although it is clear that there are certain proteins in *Geobacter* that are geared towards the reduction of solid electron acceptors, how the expression of these proteins change throughout a multilayered biofilm based on where the cells are in the biofilm relative to the electron acceptor remains to be discovered. As a result, our research will focus on differential protein expression in multilayer *Geobacter* biofilms.

Based on the known roles of outer membrane c-type cytochrome (Omc) proteins OmcB, OmcE, and OmcS in iron reduction and of pili proteins PilA and PilT in biofilm formation, we will examine their expression and localization in *Geobacter* biofilms. The genes encoding these proteins were amplified from the genomic DNA, subcloned into a pET21a (+) expression vector, and expressed as a His-tag fusion protein in BL21 (DE3) cells. Plasmid DNA containing the *omc* gene insert was transformed into BL21 (DE3) competent cells harboring the pEC86 plasmid, which incorporates the heme into the heterologously expressed protein to produce a functional heme protein. Once expressed, these proteins were purified on a Ni-NTA column, and antibodies were generated against purified OmcB, OmcE, OmcS, and PilT. Subsequently, each antiserum was affinity purified, and the antibodies against OmcB, OmcE, OmcS and PilT were tested for their specificity against their respective protein in *Geobacter* whole cells and total membrane samples via Western blotting, which demonstrated specificity for detection. The OmcB antibodies were used to immunolocalize OmcB in a multilayered *Geobacter* biofilm grown in a microbial electrolytic cell (MEC) on a graphite electrode. Antibodies against OmcE, OmcS and PilT will also be used to immunolocalize these protein in the biofilm. Immunogold labeling and transmission electron microscopy were used to visualize expression in the biofilm. Preliminary results show that OmcB may be differentially expressed in the biofilm. In addition to understanding the expression of these cytochrome and pili proteins in *Geobacter* biofilms, we are also interested in determining whether any of the outer membrane cytochrome proteins are present in protein complexes. Blue native-polyacrylamide gel electrophoresis was used to separate protein complexes in *Geobacter* membrane fractions. Preliminary results show that there are at least seven heme proteins present in complexes as determined by chemiluminescent heme staining. Immunoprecipitation will also be used to determine whether OmcB, OmcE OmcS, and PilT are present in complexes, and liquid chromatography tandem mass spectrometry will be used to identify the proteins in the complexes.

## Proteomic Changes Induced by Chromate under Nitrate-Reducing Conditions in *Geobacter metallireducens* and *Desulfovibrio desulfuricans*

Grantee-Led Research

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A major challenge for the bioremediation of radionuclides and metals is the co-occurrence of nitrate, as it can inhibit metal transformation. We have been investigating the physiology of three metal-reducing bacteria, *Geobacter metallireducens*, *Desulfovibrio desulfuricans* 27774, and *Sulfurospirillum barnesii*. Our research objectives were to (1) investigate the role of Cr(VI) concentration on the kinetics of both growth and reduction of nitrate and Cr(VI) in these three organisms, (2) develop a profile of bacterial enzymes involved in nitrate transformation (e.g., oxidoreductases) of each organism using a proteomic approach, (3) investigate the function of periplasmic nitrite reductase (Nrf) as a chromate reductase from the three different organisms, and (4) use the information gathered in Objectives 1-3 to develop a strategy to maximize microbial chromium reduction in the presence of nitrate.

We report here the results of our proteomic investigation of *G. metallireducens* and *D. desulfovibrio* to identify the specific mechanisms by which these metal-reducing bacteria respond to Cr(VI) exposure (e.g., oxidative stress, redox active proteins) in the presence of nitrate. Cells from *G. metallireducens* were grown for 3 days anaerobically with 20 mM sodium nitrate (control sample) and exposed to 100 uM chromate for 12 hours before harvesting (treated sample). Cells from *D. desulfuricans* were grown anaerobically (3 days) with 20 mM sodium nitrate (control sample) amended with 100 uM chromate (treated sample 1) or exposed to 100 uM chromate for 2 hours before harvesting (treated sample 2). An equal volume of TFE was added to 25 µg of cell lysate. Samples were reduced, alkylated, completely dried in a SpeedVac, and resuspended in 50 µL of 100 mM ammonium bicarbonate buffer. Samples were acetone precipitated, trypsin digested and reconstituted in 20 µL of 0.1% formic acid for LC-MS/MS analysis. Over 240 (*G. metallireducens*) and 313 proteins (*D. desulfuricans*) were unequivocally identified and semi-quantitative analysis based on spectral counts yielded several up- and down-regulated proteins for which the levels were changed >2 fold compared to control samples. There are unique proteins found in the Cr(VI) exposed samples of *G. metallireducens*, including redox active proteins, a flagellin-like protein (Gmet\_0442) and chemotaxis sensory proteins (Gmet\_2478 and Gmet\_1641). Identification of outer membrane efflux proteins suggests that certain bacterial (heavy metal) resistance mechanisms are involved. Moreover, the presence of cytochromes highlights the flexibility of *G. metallireducens* in the electron-transfer networks that are of importance to reduce chromate ions. HPLC-Chip-MS/MS technology was used for high throughput analysis of bacterial metabolism of chromate reduction to find associated biomarkers. Proteins involved in chemotaxis, redox active proteins, and cytochromes were identified among others, suggesting their role in microbial enhanced reduction of chromium. In the case of *D. desulfuricans*, a significant number of proteins were seen to be down-regulated. This approach is providing a greater understanding of how these organisms respond to chromium under nitrate-reducing conditions and insight into potential strategies to enhance Cr(VI) reduction.

## Abiotic Nitrate Reduction by Redox-Modified Fe-Bearing Smectite

*Grantee-Led Research*

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Reduction of nitrate and nitrite by bacteria is well documented, occurring in a vast array of soil types via several distinct and well described biotic pathways. However, the inorganic portion of soils may play a larger role in these redox reactions than previously thought, particularly Fe-containing clay minerals. In a previous study, assays of biological activity in soils found low levels of bacterial denitrifiers, yet nitrate was completely removed at the oxic/anoxic interfacial zone in the soil profile. This suggests that structural Fe(II) within the clay minerals is likely a key player, but attempts to replicate the field results in the laboratory were unsuccessful in spite of the reaction appearing to be theoretically possible. The objective of the present study was to investigate the capability of chemically reduced clays—as an analog for bacteria reduced clays—to reduce nitrate. Results revealed for the first time in the laboratory that significant levels of nitrate reduction were coupled to oxidation of Fe(II) in the clay structure. Among the reduction products observed were nitrite and ammonium. Nitrate reduction in natural soils may, therefore, occur via abiotic processes at reduced clay mineral surfaces.

## Defining the Molecular-Cellular-Field Continuum of Mercury Detoxification

### Grantee-Led Research

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Hg is of special interest to DOE due to past use at the Oak Ridge Reservation (ORR). Its facile redox [ $\text{Hg}^{2+/0}$ ] chemistry, bonding to carbon [e.g.  $\text{MeHg}^+$ ] and unique physical properties [e.g.,  $\text{Hg}^0$  volatility] underlie a complex global Hg cycle involving biotic and abiotic chemical and physical transport and transformations in soils, sediments, waterways, and the atmosphere. Management to minimize negative impacts in diverse settings requires rich knowledge of *all* contributing processes. Bacteria are critical in the Hg cycle. Facultative and anaerobic bacteria make  $\text{MeHg}^+$ , which is neurotoxic to wildlife and humans. Sustainable stewardship requires eliminating both  $\text{MeHg}^+$  and even more toxic  $\text{Hg}^{2+}$ , which is also the substrate for methylation. Using proteins encoded by *mer* locus mercury resistant (HgR) aerobic and facultative bacteria can convert soil or waterborne  $\text{Hg}^{2+}$  or  $\text{MeHg}^+$  to less toxic, relatively inert gaseous  $\text{Hg}^0$ . HgR microbes are present in highly contaminated sites, and field data show they depress  $\text{MeHg}^+$  >500-fold in such zones. So, enhancing the capacity of natural HgR microbes to remove  $\text{Hg}^{2+}$  and  $\text{MeHg}^+$  from soils, sediments, and waterways is a logical component of a plan for stewardship of contaminated sites. To do so requires knowing how each part of the HgR pathway works and what it demands from the global metabolic capacity of the cell where it resides—the entire cell is the relevant catalytic unit. Since HgR loci are found in metabolically diverse bacteria, unique *mer*-host co-evolution is expected.

Here, we propose to test the “cell-as-catalyst” hypothesis in model HgR bacteria representative of 3 bacterial genera prevalent in Hg-contaminated sites at ORR:  $\alpha$ -proteobacteria,  $\gamma$ -proteobacteria, and actinobacteria. At the molecular and subcellular levels, we will test the hypothesis that *mer* proteins interact with each other and with specific host proteins to rapidly and completely convert  $\text{Hg}^{2+}$  or  $\text{RHg}^+$  to  $\text{Hg}^0$ . We will dissect the metabolic support of HgR in the well-studied *Tn501 mer* operon in  $\gamma$ -proteobacterium *E. coli* using the EZ-Tn5 transposome to knock out nonessential genes, and biochemically and genetically test those unable to express Hg resistance for the exact basis of their inability in this regard. We will also do kinetic analyses, NMR, crystallography, and crosslinking to quantify and map transient and stable interactions of *mer* proteins with each other and host proteins.

This effort will be leveraged by our ongoing collaboration with the ORNL SFA team (L. Liang, J. Smith, H. Guo), who will use neutron scattering and computational chemistry to define complexes of *mer* proteins interacting in solution with each other and with host proteins (e.g., RNA polymerase). We'll also use these biochemical and biophysical tools to characterize the naturally occurring MerB-MerA fusion enzyme of  $\alpha$ -proteobacterium *Xanthobacter autotrophicus* and the *mer* enzymes and regulatory protein from actinobacterium *Streptomyces lividans*, and use the EZ-Tn5 transposome method to compare what cellular genes underpin expression of Hg resistance in *Streptomyces*.

Lastly, in consultation with the SFA metagenomics group, we'll generalize these observations by interrogating their datasets for the presence/absence of such HgR-support systems in contaminated sites at ORR. To use HgR microbes in stewardship requires measuring their contribution to their environment. To connect these cellular catalysts to the larger environment, we will devise reaction-based models for Hg moving from the external milieu to the interior of a bacterial cell in terrestrial, aerobic surface, and subsurface water systems, taking into account available ligands for  $\text{RHg}$  and  $\text{Hg(II)}$  in the environment (with data on ORR Hg species from B. Gu) and the thermodynamic and kinetic parameters of the *mer* proteins. The outcome will be predictive models for the rates at which substrate mercurials in different environmental contexts can reach the cytosolic enzymes for biotransformation and how rapidly the end product  $\text{Hg(0)}$  leaves the cell. Furthermore, in collaboration with K. Kritee and J. Blum, we will conduct a pilot study to determine Hg stable isotope fractionation during  $\text{Hg(II)}$  reduction by MerA. This will be a first step towards connecting Hg isotope fractionation signatures at the molecular, cellular, and ecosystems levels.



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

### Grantee-Led Research

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We have made good progress on three fronts: cell biology of mercury exposure, proteomic identification of Hg-adducts, and completion of the first global proteome for *E.coli* with or without R/Hg exposure. **(1) Biology: bio-chemical & biophysical correlates of Hg damage in *E.coli*.** In late-log phase *E.coli* cells treated briefly, bulk thiol (RSH) content was  $\sim 4 \times 10^6$  molecules of RSH per cell ( $\sim 10$  mM). At growth-arresting concentrations of merthiolate (MT, 160  $\mu$ M), phenylmercuric acetate (PMA, 40  $\mu$ M) or 16  $\mu$ M HgCl<sub>2</sub> [Hg(II)] dropped this to  $\sim 0.5$  mM (MT) or obliterated bulk RSH entirely (PMA and Hg). Similar effects were seen on cysteines of proteins tagged with BODIPY-iodoacetamide. The hierarchy of RSH blockage (Hg(II)>PMA>MT) corresponded to cell uptake of each compound measured by ICP-MS. Only 16  $\mu$ M Hg(II) saturated all cellular RSH, but 10-fold more MT (160  $\mu$ M) was needed to achieve a 1:1 ratio with cellular thiols. PMA had an intermediate absorption efficiency. Interestingly, both PMA and Hg(II) were absorbed at higher ratios than total cell thiols could account for, suggesting that these mercurials take other ligands in addition to sulfur. Indeed, XAFS analysis of treated cells showed that coordination of PMA and MT was dominated by S1 and C1 ligands, with a little N or O. In contrast, XAFS of HgCl<sub>2</sub>-treated cells had a small, broad S2 peak, indicating a very heterogeneous ligand mix, very likely including N and/or O. Additional XAFS work is ongoing to identify these other ligands. In pursuing the hypothesis that mercurials amplify their damage by provoking an ROS cascade, we found that the standard OxyBlot assay for protein carbonylation is inhibited by Hg(II), so we're exploring tests of oxidative damage to lipids and nucleic acids. However, in a direct biophysical test of the ROS-cascade hypothesis using EPR quantification of redox active free Fe(II)/Fe(III) in intact cells (with UGA colleague, Mike Johnson), we found that 16  $\mu$ M Hg(II) increased free Fe(II)/(III) two-fold, and 80  $\mu$ M Hg(II) provoked a 3-fold increase, equivalent to  $\sim 80\%$  of total cellular iron based on ICP-MS. Such high levels of free Fe were not achievable with standard ROS-stress compounds 4 mM H<sub>2</sub>O<sub>2</sub> or 8 mM t-butylperoxide. Thus, Hg(II), and to a lesser extent, PMA, strongly blocked formation of and/or directly destabilized Fe-S metalloproteins. The free Fe was not lost from the cell, nor was there any change in cellular content of seven essential metals (Mg, Mn, Fe, Co, Ni, Cu, and Zn). However, Hg(II) significantly altered the electrolyte balance, causing a  $\sim 3$ -fold drop in K and a corresponding increase in Na. When remaining XAFS data are collected and analyzed, this study phase will be completed, but we will continue to use these tests to benchmark proteomics samples. **(2) Methods: Iodoacetamide optimizes peptide and cys-peptide recovery and an improved computational filter optimizes detection of Hg-adduct peptides.** We have devised a best practice global proteomics protocol using iodoacetamide (IAM) to minimize Hg/RHg reassociation during processing and urea for protein denaturation prior to trypsinolysis. Our modified LC-MS preparation recovered peptides and proteins at least as effectively as current standard methods used by others. IAM at 40, 20, or 10 mM had no impact on total peptides recovered. However, recovery of Cys peptides at their genomic frequencies required at least 10 mM IAM. PMA also enhanced observation of Cys peptides, perhaps by blocking post-lysis S-S crosslinks, but PMA was only  $\sim 30\%$  as effective as IAM alone. We also improved our stable isotope-based computational filter for Hg-containing adducts by including multiple spectral scans of a given peptide in each run. With these summed spectra, we can distinguish bona fide RHg- and Hg-modified peptides with a specificity of 99.4% and sensitivity above 95%. **(3) Global Proteomics for Hg Biomarkers: The first high resolution global proteome of *E.coli* treated (or not) with PMA or MT and carrying (or not) a plasmid-borne *mer* locus and identification of key biomarker proteins.** Overall, proteome coverage was very good (91%) for both plasmid-free and plasmid-carrying cells; observation of chromosomal genes in both cell types was 85-86% and of plasmid genes in that cell line was 76%. Considering all 188 Cys-adduct events that have at least 5 RHg modification events (hits) across all IAM levels, the hit frequencies at any given Cys site ranged from 12% to 100%; the hit frequency is sharply skewed, with 170 of these Cys-adducts being observed at least 50% of the time. We have cataloged the homologs and structures for half the 188 proteins in which these Cys-RHg adducts occur; 31 of them have human, mouse, zebrafish, nematode, yeast, and bacterial homologs, which retain one or more of the modified cysteines observed in the *E.coli* version. These proteins are variously involved in the TCA cycle, glycolysis, the pentose phosphate pathway, the translational apparatus, nucleotide metabolism, membrane transport, Fe-S cluster assembly, and redox homeostasis. Examples of proteins with one or more cysteines modified at 100% include the F1F0 ATPase, RecA, citrate synthase, succinate dehydrogenase, and the RpoA and RpoC subunits of RNA polymerase. We are cataloging the rest of the RHg-targeted proteins and comparing the RHg-modified and unmodified cysteines in each to understand what makes a given cysteine more or less vulnerable to RHg- modification.

## Manganese Redox Mediation of $\text{UO}_2$ Stability and Uranium Fate in the Subsurface: Molecular and Meter-Scale Dynamics

*Grantee-Led Research*

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Bioreduction of U(VI) to U(IV) has been proposed as a strategy for the *in situ* immobilization of U contamination in the subsurface. For this to be a viable long-term solution, the U(IV) products must remain reduced and immobile after the remediation effort has ceased. We have observed that Mn oxides--known as potent environmental oxidants--readily oxidize U(IV) and are effective sorbents for the resulting U(VI). The presence of either preformed biogenic  $\text{MnO}_2$  or conditions leading to active biological Mn(II) oxidation resulted in the rapid oxidation of biogenic  $\text{UO}_2$ . Another observation underscores the complex interplay of Mn and U redox cycling: the presence of Mn(II) during microbial U(VI) reduction resulted in the formation of a biogenic  $\text{UO}_2$  phase for which Mn(II) was incorporated in the lattice, and onto which Mn(II) was extensively sorbed. The reactivity of this material with respect to oxidation was considerably lower than that of biogenic  $\text{UO}_2$  produced in the absence of Mn(II). The slower oxidation of  $\text{UO}_2$  was observed for oxidants that included  $\text{O}_2$  and biogenic  $\text{MnO}_2$ . The incorporation of Mn(II) into the crystal lattice as well as the sorption of Mn(II) onto the surface of  $\text{UO}_2$  appear to contribute to the increased stability of the material.

Based on these results, we will investigate the dynamics and mechanisms of redox coupling between Mn and U. It is likely that in the bioremediated subsurface, biological Mn(II) oxidation would take place once micro-aerobic conditions return at the periphery of the plume of reduced U. The production of  $\text{MnO}_2$  could potentially lead to a catalytic redox cycle that re-oxidizes U(IV) and creates a redox front moving farther afield. The specific objectives of our project are to (1) characterize the physical, chemical and biological factors that govern the enhanced rates of  $\text{UO}_2$  oxidation by  $\text{MnO}_2$ , (2) determine the structure and composition of the reaction products of the coupled oxidation/reduction of Mn oxides and  $\text{UO}_2$ , and (3) evaluate the transferability of microscale processes to larger scales in heterogeneous porous media.

To achieve these objectives, we will study the redox- and nonredox-mediated reactions between U and Mn in batch and flow-through reactors, as well as in column and 2D reactors. The latter setups are designed to capture the potential importance of a peculiarity of this system, which is that the two main reactants ( $\text{MnO}_2$  and  $\text{UO}_2$ ) are solid phase. Limitations in physical contact between the reactants may be a major impediment to their interaction and may lead to lower than expected reactivity in the porous media reactors.

This work is designed to consider the effect of Mn on the feasibility of *in situ* U(VI) reductive immobilization. The pragmatic approach bridges spatial scales in a way that can allow molecular- and microscale information to have an impact on the design of field-scale remediation strategies.

## Resource-Induced Shifts in Metal Reducing Microcosms Revealed Communities Dominated by *Geobacter* and *Pelosinus*

Grantee-Led Research

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Studies performed on *in situ* bioremediation of uranium sediments have revealed numerous bacterial populations potentially involved in metal reduction. To assist in the identification of unknown metal reducing populations, we have employed an anaerobic serial enrichment strategy coupled with 454 amplicon pyrosequencing to identify putative uranium-reducing populations.

Three uranium-contaminated sediment samples (FW107, FW102-2 and FW102-3) were obtained from the FRC (Field Research Center) S3 area and used as initial inocula. Bacterial communities enriched in freshwater medium (FWM) with sodium lactate as electron donor and nitrate, Fe(III) and U(VI) as terminal electron acceptors were sequentially transferred every 35 days for three cycles and sampled at each cycle for community analysis. The 454 amplicon pyrosequencing revealed that FW107 is different from FW102-2 and FW102-3. The latter two samples are from the same sampling well but different depths. Iron- and uranium-reducing communities had similar abundant bacterial populations (*Pelosinus* and *Sulfurospirillum* for FW107, *Geobacter* and *Pelosinus* for FW102-2 and FW102-3), but were highly skewed communities. *Pelosinus* sequences were 79% and 84% of the total in Fe(III) and U(VI) reducing communities respectively in FW107. In FW102-2, *Geobacter* dominated with 42% and 84% of the total sequences in Fe(III) and U(VI) reducing communities respectively. Nitrate-enriched communities were substantially different and more diverse than the metal-reducing enrichments, dominated by *Pseudomonas* and *Simplicispira* in FW107 and FW102-2 respectively. Although different populations emerged as dominant from FW107 and FW102, both enrichments had large amounts of U(VI) precipitants due to the complexation of uranium and medium components. In order to better evaluate uranium reduction, we have developed new conditions to eliminate uranium precipitation. A previously reported PIPES-buffered artificial groundwater (PBAGW) with modifications was used as the minimal media, and sodium pyruvate and uranyl acetate were supplemented as electron donor and acceptor. Sample FW107 and FW102-2 were first enriched in 1/2X R2B broth anaerobically prior to performing the uranium-reduction experiments to obtain greater biomass for inoculation. During the first ten days of uranium reduction, cell protein of both communities increased from 0.1 µg/mL to 8µg/mL, while increasing amounts of cell-associated U particles were also detected. In addition, depletion of pyruvic acid and accumulation of acetic acid were observed in both FW107 and FW102-2 uranium microcosms. The enriched community derived from FW102-2 appeared to be the more effective uranium-reducing community. Within 20 days, uranium in solution decreased from approximately 250 µM to 196 µM and 86 µM in FW107 and FW102-2, respectively. We also observed that the abundance of *Pelosinus* in both uranium-enriched communities increased as a function of time. Transcriptomics will be used to better understand the functioning of these uranium-reducing communities.

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

Grantee-Led Research

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

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

The overall objective of this work will be to provide the fundamental understanding necessary to evaluate the feasibility of reductive immobilization of  $\text{TcO}_4^-$  in the vadose zone or groundwater by controlled application of  $\text{Fe}^0$ ,  $\text{Fe}^0/\text{S}^0$  (and sulfate or organic carbon, if necessary) to stimulate microbial sulfate and sulfur reduction.

Nonsulfur-containing nanoscale zero-valent iron particles (nZVI) were synthesized using a refined procedure based on reduction of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  with  $\text{NaBH}_4$ . To assess biotic and abiotic sulfate reduction in the presence of this nZVI, we have developed a method based on ion chromatography with electrochemical detection that should give simultaneous speciation of sulfate ( $\text{SO}_4^{2-}$ ), sulfide ( $\text{S}^{2-}$ ) and other possible sulfur species. Preliminary data obtained with this method show no detectable transformation of sulfate to sulfide due to direct abiotic reduction by nZVI.

For biotic experiments, we have cultured a pure SRB isolate (*Desulfotomaculum reducens*) in both the presence and absence of nZVI to assess the effect of the latter on growth. Also, both SRB and possibly  $\text{S}^0$  reducers have been enriched from Hanford sediments using synthetic groundwater media HS300 containing a cocktail of C sources and electron donors (including  $\text{H}_2$ ). The enriched microbes, which display a range of rod morphologies, will be used in batch and column nZVI experiments.

## Applications of GeoChip for Analysis of Different Microbial Communities

### Grantee-Led Research

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Microarray technology provides the opportunity to identify thousands of microbial genes or populations simultaneously. The objective of this study was to further develop and apply a comprehensive functional gene array (GeoChip) to detect and monitor microbial communities. GeoChip 2.0 is a functional gene microarray which allows for the simultaneous detection of >10,000 genes involved in the geochemical cycling of C, N, and S, metal reduction and resistance, and organic contaminant degradation. Based on GeoChip 2.0, a new generation, GeoChip 3.0 has been developed, which has several new features. First, GeoChip 3.0 covers more gene groups, including antibiotic resistance, energy processing, and additional functional genes involved in C, N, P, and S cycling. Second, the homology of automatically retrieved sequences by key words is verified by HMMER using seed sequences, so that unrelated sequences are removed. Third, a common oligo reference standard (CORS) has been implemented, so that data normalization and comparison of different microbial communities can be conducted. Fourth, a genomic standard is used to quantitatively analyze gene abundance. In addition, GeoChip 3.0 includes phylogenetic markers, such as *gyrB*. Finally, a software package has been developed to facilitate management of probe design, data analysis, and future updates. The OU GeoChip (based on GeoChip 3.0) was a recipient of a 2009 R&D100 Award from *R&D Magazine*, which recognizes the 100 most innovative scientific and technical breakthroughs of the year. Additional expansion is currently under way and will include genes related to stress response, virulence factors, human-microbiome, and phage genes.

The GeoChip has been used to examine dynamic functional and structural changes in microbial communities from many different environments. Here, examples of studies utilizing the GeoChip to examine microbial communities at contaminated sites are presented. These studies illustrate the ability of the GeoChip to provide direct linkages between microbial genes/populations and ecosystem processes and functions. These three studies examined areas within the U.S. DOE's Field Research Center (FRC) in Oak Ridge, TN. (1) Microbial communities within a pilot-scale test system established for the biostimulation of U(VI) reduction in the subsurface by injection of ethanol were examined using GeoChip 3.0. Functional community dynamics were examined during a period of nitrate exposure. After exposure to nitrate the diversity and richness increased several fold, but quickly returned to pre-nitrate levels. Detrended correspondence analysis (DCA) indicated a shift in the overall community structure after nitrate exposure, but the community began to return to pre-exposure structure once nitrate was removed. The relative abundance of several nitrogen cycling genes showed an increase immediately after nitrate exposure, including ammonification, denitrification, and nitrogen fixation genes indicating a stimulation of these communities. (2) In the second study from the FRC, analysis of groundwater monitoring wells along a contamination gradient using GeoChip 2.0 revealed less overlap between wells with different levels of U and  $\text{NO}_3^-$  contamination. While diversity of nitrate-fixation genes decreased in  $\text{NO}_3^-$  contaminated wells, the diversity of metal reduction and resistance genes did not correlate with metal concentrations. Signal intensity did, however, increase in heavily contaminated wells, indicating a larger percentage of organisms with metal-related genes. Sulfate-reduction genes had greater diversity and greater signal intensity in more contaminated wells. Individual principle component analyses (PCA) of the gene diversity and geochemistry of the wells separated them in similar ways. CCA indicated that pH was an important variable that correlated with gene diversity in the lowest-contamination well, while  $\text{NO}_3^-$  and U correlated with the most highly contaminated well. Overall, contaminant level appears to have significant effects on the functional gene diversity along the contaminant plume at the FRC.

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

*Grantee-Led Research*

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Organic acids, organic bases, and detergent-like chemicals change the properties of organic chemical mixtures and surface wettability. The wastewater and NAPL mixtures discharged at the Hanford site contain such chemicals, and their proportions likely change over time due to reaction-facilitated aging. The specific objectives of this work are to determine (1) the effect of organic chemical mixtures on surface wettability, (2) the effect of organic chemical mixtures on carbon tetrachloride (CT) mass-transfer rates from NAPL, and (3) the migration and redistribution of organic chemical mixtures.

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

The homogeneous micromodels were fabricated and then used to perform NAPL entrapment and dissolution experiments for pure CT and the MIX1 NAPL. After NAPL entrapment, initial perimeter length of MIX1 was higher than that of pure CT. As a result, initial NAPL dissolution was higher for MIX1 than for pure CT. Since MIX1 has more small NAPL blobs than CT, overall NAPL dissolution rate was faster in MIX1 until a large fraction of high-solubility components (DBBP and CT) in MIX1 dissolved. At late time, the NAPL dissolution rate in MIX1 decreased dramatically, probably due to a high fraction of TBP and LO remaining because of their lower solubility relative to DBBP and CT, while the dissolution rate in pure CT decreased steadily. A set of micromodel experimental results will be analyzed using a multi-component NAPL dissolution code with an upscaled mass-transfer model.

Three flowcell experiments were conducted in a 100 cm long, 80 cm high, and 5.5 cm wide 2-D flowcell to account for the impact of the organic mixtures on DNAPL distribution and migration in a layered vadose zone, which contained a fine lens surrounded by coarser sand. After packing, either the organic mixture or pure CT was released into the flow cell. After no apparent movement of NAPL, soil vapor extraction (SVE) was applied. Pure CT penetrated the bottom of the fine sand lens by fingering, while the organic mixture did not penetrate. STOMP simulation results qualitatively predicted both experimental results well; however, simulations significantly underpredicted the amount of CT penetrating through the fine lens and failed to predict the finger formation. For the SVE experiment with pure CT, numerical modeling with no fitting parameters matched experimental results well. This showed that local equilibrium assumption can be used to simulate CT volatilization in the layered system, provided that permeability and initial NAPL distributions are known.

The impact of chemical properties on DNAPL migration and redistribution at the Hanford site was evaluated using STOMP. Pure CT penetrated the low-permeability layer easily, due to a higher density difference between CT and pure water than between MIX1 and wastewater, and lower water saturation in the low-permeability layer in the case of pure CT and water. As a result, NAPL volume across the groundwater table at 38 yrs was significantly higher for pure CT-water than for MIX1-wastewater. Simulation results show that multiphase flow and transport models need to account for the properties and behavior of chemical mixtures in order to provide accurate predictions of NAPL distribution.

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

Grantee-Led Research

N. A. Wall (PI), *Washington State U.*; B. Gu, *ORNL*

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 Tc(IV) and acetate at pH 4.5 and varying ionic strengths (NaCl). Such data allowed us to calculate the stability constant at zero ionic strength, using the Specific Ion Interaction Theory (SIT) equations. The logarithm of the stability constant of the formation of the complex  $\text{TcO}(\text{OH})\text{Ac}$  ( $\log\beta_1, 1,1$ ) at zero ionic strength was determined to be  $2.8 \pm 0.3$ . These results were presented at the Migration '09 conference (Kennewick, September 2009), and a journal article has been accepted for publication in *Radiochimica Acta*. We have also successfully determined the binding constants and dissolution kinetics of Tc(IV) with the natural humic (HA) and fulvic (FA) acids, including those isolated from the Oak Ridge Integrated Field Research Challenge (IFRC) sites. The pK<sub>a</sub> and carboxylate capacity values of the IFRC HA and IFRC FA were determined via potentiometric titrations, and their binding constants with Tc(IV) were quantified at pH 4.5 and two different ionic strengths. The logarithm of the apparent binding constants at the ionic strengths 0.5 M and 1.0 M (NaCl) are  $5.9 \pm 0.4$  and  $5.8 \pm 0.2$ , respectively, for the Tc(IV) complexes with IFRC HA; and  $6.4 \pm 0.3$  and  $5.9 \pm 0.3$ , respectively, for the Tc(IV) complexes with IFRC FA. These results will be presented at the Spring ACS meeting in San Francisco, CA. In agreement with the binding constant presented above, both synthetic and natural organic ligands were found to greatly enhance the dissolution of reduced Tc(IV) under anaerobic conditions. The IHSS soil HA is among the most effective in causing the solubilization of Tc(IV), followed by the synthetic ligands, EDTA, and the IFRC-HA and IFRC-FA. In comparison with the ligand-promoted dissolution, Tc(IV) solids were also found to rapidly dissolve under oxidizing conditions, but the presence of EDTA decreased the oxidative dissolution, owing to its strong complexation with Tc(IV). In contrast, the presence of IHSS soil HA significantly increased the oxidative dissolution of Tc(IV), which was attributed to the presence of both redox-active and complexing functional groups in the HA. These observations indicate that the presence of oxygen and complexing organic ligands may greatly alter the dissolution and mobility of reduced Tc(IV). We will continue determining the stability constants of Tc(IV) with other organic ligands (e.g., IHSS soil HA, EDTA, citrate, isosaccharinic acid) and perform the dissolution and mobilization studies of reduced Tc(IV) under conditions that are relevant to DOE contaminated sites. All data obtained from these studies can then be integrated in geochemical speciation models. The research is anticipated to provide new thermodynamic data on the behavior of reduced Tc(IV) with organic ligands and ultimately to improve predictive capabilities for the long-term stability and mobility of these contaminants at DOE contaminated sites.

## Viral Infection of Subsurface Microorganisms and Metal/Radionuclide Transport

*Grantee-Led Research*

K.A. Weber (PI), *U. of Nebraska*; K.S. Bender, *Southern Illinois U.*; Y. Li, *U. of Nebraska*

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 isolates from DOE sites, *G. uraniumreducens* Rf4, *Geobacter* sp. FRC-32, and *Anaeromyxobacter* sp. Fw109-5, revealed phage genes integrated into the chromosome. Presence of these gene sequences indicates that 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 soils and sedimentary environments remains poorly understood. 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) among viruses, subsurface microbial communities, and contaminant metals/radionuclides. 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: (1) subsurface microorganisms are susceptible to viral infections by the indigenous subsurface viral community, and (2) viral surfaces will adsorb heavy metals and radionuclides. Since very little is known about viral and microbial community interactions in these contaminated subsurface environments, the hypotheses outlined will address basic research needed to accomplish the BER Long Term Measure, 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. This will be done by establishing viral-microbial relationships and the subsequent fate and transport of heavy metals and radionuclides.



## Three-Dimensional Imaging and Quantification of Biomass and Biofilms in Porous Media

*Grantee-Led Research*

G. Iltis, R. Armstrong, D. Jansik, B. Wood, and D. Wildenschild (PI)—*Oregon State U.*

The overall objective of this exploratory project is to develop pore-scale imaging techniques based on x-ray computed microtomography (CMT) suitable for quantifying microbial structure, and eventually growth patterns, in 3-D porous media. Synchrotron-based x-ray microtomography will be used to visualize biofilm/biomass structure and distribution in porous media by attaching a suitable x-ray contrast agent to the biophase.

In the first year we have developed a new methodology using CMT to produce high-resolution measurements of the spatial distribution of biofilms in porous media. This methodology has the advantages that (i) a variety of solid substrates (where biofilms occur naturally) can be used; (ii) the method is non-destructive; and (iii) the method is relatively rapid, so that the progression of the spatial evolution of a biofilm can, in principle, be carried out. One caveat is that CMT is nondestructive to the porous matrix, but x-rays are destructive to the majority of microorganisms. However, some bacteria have elaborate DNA repair mechanisms that confer radiation resistance; we propose that such a bacterium could be used as a model microbe for temporal CMT imaging. Synchrotron-based tomographic imaging of biofilms within porous media at the pore scale has yet to be accomplished, primarily due to the fact that obtaining x-ray contrast between the biomass and water has posed a significant challenge. To address the contrast issue, we have developed a fluid-phase agent that provides x-ray absorption contrast between the fluid and biofilm phases by adsorbing to the surface of the biofilm, thereby delineating the biofilm/aqueous phase interface. By outlining the biofilm surface with a contrast agent, we propose that unique biofilm geometries/features can be imaged using CMT.

A preliminary three-dimensional proof-of-concept experiment was conducted at beamline 13-BMD, GSECARS at the Advanced Photon Source (APS/ GSECARS), Argonne National Laboratory, in a porous medium within which biofilm had been grown. This initial experiment provided compelling evidence that our choice of contrast agent and CMT imaging methods provide three-dimensional representations of biofilm present in the experimental packed-bead column. However, we were not able to directly validate (using a separate imaging technique) that the structures we observed corresponded to regions known, at the microscale, to be biofilm. To provide the necessary validation, we developed an experimental system with which the distribution of biofilm within a porous flow cell could be visualized using both digital microscopy and CMT. This validation of the CMT imaging technique for visualizing biofilm *in situ* in porous media has been the focus of this work carried out in the first year of the project.

For the validation study, silver-coated microspheres were used for contrast; however, alternative surface coatings that do not exhibit antimicrobial characteristics will be pursued in future applications. Visual comparison of the light microscopy images to the CMT composite images indicate very good agreement between the data collection methods and suggests that the silver particles detected using CMT imaging do, in fact, allow for the delineation of biofilm within the pore space. The correlation between the two imaging techniques was quantified via surface area using two different surface-generating algorithms. This exercise resulted in a quantitative comparison of the light microscopy and CMT images with percent differences, normalized to the light microscopy images, of between +1 and -5 percent. Thus, the differences between light microscopy and CMT images are sufficiently small to consider this a successful proof-of-principle validation for the CMT approach.

Additional work in year one includes recent experiments at the APS that investigate the effectiveness of the technique for evaluating biofilms formed by various bacteria, as well as for different flow rates to assess variation in biofilm characteristics, including density and distribution within the pore space. Work in the second year of the project will focus on further evaluation of the physical straining technique, including the use of an iodine-based suspension instead of discrete particles, and using the anti-body approach outlined in the original proposal.

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

Grantee-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 that 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. To test this hypothesis, we are proceeding with the following tasks: (1) synthesizing and characterizing nanoporous alumina and goethite as analog materials; (2) investigating U(VI) sorption and desorption on nanopore surfaces in controlled carbonate-bearing model systems; (3) investigating U(VI) sorption and desorption of natural sediments from the Oak Ridge National Laboratory FRC (ORFRC) site; (4) investigating effects of nanopore size 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) carrying out EXAFS studies of U(VI) sorbed on normal surfaces, on nanopore surfaces, and in post-reduction samples to understand chemical environments of the sorbed U(VI) and its redox reactivity at the molecular level.

To isolate the effect of nanopore surfaces, we conducted parallel experiments on porous and nonporous alumina and goethite. Our results show that nanopore surfaces can preferentially enhance carbonate sorption from ambient aqueous solutions, and therefore promote formation of uranyl tricarbonate complexes in the nanopores. According to extraction experiments using different bicarbonate concentrations, sorbed U(VI) can be divided into three categories: (1) extractable by low concentration (< 50 mM) of bicarbonate (i.e., U(VI) on normal outer surfaces), (2) extractable by high concentration ( $\geq$  50 mM) of bicarbonate (i.e., U(VI) on large nanopore surfaces), and (3) nonextractable by very high bicarbonate ( $\geq$  500 mM) (i.e., U(VI) on small nanopore surfaces, < 2 nm). Ongoing experiments will correlate the three types of sorbed U(VI) complexes to (a) bicarbonate extractable and microbe/hydroquinone reducible, (b) bicarbonate extractable but microbe/hydroquinone nonreducible, and (c) bicarbonate unextractable and microbe/hydroquinone nonreducible U(VI) complexes in ORFRC sediments. Nanopore surfaces have higher charge density than those of non-nanopore surfaces. We propose that both size of the uranyl tricarbonate complex and high charge density result in the formation of stable, nonextractable uranyl tricarbonate complexes in the small nanopores.

We also carried out adsorption and sequential desorption experiments for three nearly pristine (< 0.01  $\mu\text{M/g}$  U) saprolite samples from ~7 m depth at the ORFRC. Dithionite-citrate-bicarbonate (DCB) extractable Fe contents in the three samples range from 7.1 to 11.12 and 22.6 g Fe/kg, respectively. Adsorption experiments were carried out in the presence of  $\text{HCO}_3^-$  (0.1, 1, 5 mM) and  $\text{Ca}^{2+}$  (0, 5, 10 mM) at pH ~6.5. Bicarbonate nonextractable U(VI) was observed in these ORFRC materials. A surface complexation model (based on the hydrous ferric oxide surface in the PHREEQC database) that included three sorption sites with weak, strong, and very strong (i.e., nonextractable) U(VI) binding affinity adequately reproduced the U(VI) sorption/desorption data. It is suggested that the influence of nanopore surfaces on U(VI) sorption/desorption and reduction processes is likely to be significant in virtually all subsurface environments.

## Reduction of Hg(II) to Hg(0) by Denitrifying and Iron-Reducing Bacteria

Grantee-Led Research

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We conducted laboratory studies to investigate the mechanisms of Hg(II) reduction by denitrifying and iron-reducing enrichments, derived from subsurface sediments taken from Oak Ridge, TN. Based on previous observations, we hypothesized that reduction by Hg resistant bacteria would dominate under denitrifying conditions, while under iron-reducing conditions, reduction would be carried out by metal reducers. An oligotrophic medium was constructed with principal chemical components constrained by on-site monitoring wells to simulate *in situ* groundwater chemistry. In the nitrate reducing enrichments, Hg inhibited denitrifying activities at concentrations higher than 0.5 microM. Under this threshold, the enrichments detoxified Hg by reducing Hg(II) to Hg(0), as indicated by the formation of Hg(0), which was trapped in oxidizing trap solution. In addition, two strains isolated from the enrichments with 5 microM Hg(II)-spike and identified as *Ralstonia* sp. (betaproteobacterium) and *Bradyrhizobium* sp. (alphaproteobacterium; 99 percent identity, based on 16S rRNA gene sequencing) were able to reduce Hg(II). Using degenerate primers, merA, the gene encoding for mercuric reductase, was detected in both cultures and was found to be most similar to that of gammaproteobacteria (98 percent identity) and beta- gamma-proteobacteria (83 percent identity) in *Ralstonia* sp. and *Bradyrhizobium* sp., respectively. This Hg resistance phenotype has never been described in the Alphaproteobacteria.

Enrichment cultures established with FRC subsurface sediments and ferrihydrite as electron acceptor resulted in the isolation of a pure culture of a spore-forming bacterium designated as FRC-RU4. Ribosomal RNA sequence analysis suggested that FRC-RU4 may represent a new genus among the phylum *Firmicutes*, because its 16S rRNA gene was only 94 percent similar to sequences in databases. FRC-RU4 is an obligate anaerobe that grows in the artificial groundwater medium using ferrihydrite, nitrate, or fumarate (30 mM) as terminal electron acceptors and acetate (20 mM) as electron donor. Hg reduction experiments showed that reduction of Hg(II) to Hg(0) by FRC-RU4 was enhanced under iron-reducing conditions as compared to fumarate-reducing conditions, similar to our previous observations with other iron-reducing bacteria. Electron microscopy, Mossbauer spectroscopy and X-ray diffraction analysis revealed that FRC-RU4 induced the formation of nanoparticulate goethite and magnetite when grown on ferrihydrite. Abiotic experiments showed that magnetite can rapidly reduce Hg, whereas ferrous iron sorbed to goethite cannot. Taken together, these results suggest that Hg reduction processes, likely resulting from the activity of Hg-resistant denitrifiers and biotic/abiotic interactions of iron-reducers, can affect Hg speciation and hence mobilize Hg as Hg (0) in anoxic groundwater environments.

## Geochip-Based Analysis of Metabolic Diversity of Microbial Communities During *In Situ* Biostimulation at a Uranium-Contaminated Aquifer

Grantee-Led Research

P. Zhang (PI), *U. of Oklahoma*; W. Wu, *Stanford U.*; J.D. Van Nostrand, Y. Deng—*U. of Oklahoma*

The potential for removing U(VI) from groundwater by stimulating *in situ* microbial activity using a slow-released substrate (SRS), consisting primarily of vegetable oil, was evaluated in a highly contaminated aquifer (5–6  $\mu\text{M}$  of U(VI) at the Oak Ridge Integrated Field Research Challenge (ORIFRC) site in Tennessee. SRS was injected within 2 hrs into the subsurface, and the metabolic capabilities of the microbial communities in one upgradient control- and seven downgradient monitoring-wells were monitored over a period of 140 days, using GeoChip3.0, an array capable of detecting and monitoring ~28,000 functional genes responsible for C, N, S, and P cycling (e.g., sulfate and nitrate reduction), electron transfer (e.g., cytochrome and hydrogenase genes), and biodegradation of a range of organic contaminants, including long chain fatty acids. The SRS supplementation changed the overall community functional structure. Detrended correspondence analysis (DCA) incorporating all detected functional genes readily separated the communities in the seven downgradient wells four days after injection and thereafter from those in the same wells but before injection. In contrast, the functional structure in the upgradient control well remained similar throughout the entire experimental period and clustered closely with those in the seven downgradient wells before injection. Further analysis of a total of ~300 cytochrome genes detected revealed long-term enrichment of the genes derived from *Geobacter*, *Desulfovibrio*, and *Anaeromyxobacter* species, including some known-U(VI)-reducing organisms. This was indicated by increased relative abundance and diversity of the cytochrome genes in the greatly stimulated communities. The functional gene results were highly coincident with the supplement of electron donors ( $\text{H}_2$  and acetate) from biodegradation of the SRS and the subsequent reduction of multiple electron acceptors (U(VI),  $\text{SO}_4^{2-}$ , Fe(III), and  $\text{NO}_3^{-1}$ ) in the aquifer. GeoChip is a high-throughput technology that provides the advantage of directly targeting functional genes responsible for biotransformations of environmental importance.



## ***Federal Agency-Led Research***



## 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. However, subsurface environments are open, complex, and subject to multiple interpretations and conceptualizations. The approach of this project is to postulate multiple plausible conceptual models, calibrate each model to observations, and then make predictions with the calibrated model ensemble. Parametric and conceptual model uncertainty of the alternative models is jointly evaluated using Bayesian Model Averaging (BMA). The BMA method is being used with multiscale field and synthetic observations to test the hypotheses that model uncertainty dominates parametric uncertainty, and that BMA improves predictive performance.

The methodology is being applied to the Naturita UMTRA site to investigate how conceptual model uncertainty varies across scales ranging from column tests to the plume scale. The approach was applied to a set of previously published results on U(VI) transport in columns packed with well-characterized quartz. As in the initial study, seven different surface complexation models of varying complexity were calibrated against selected experiments and then used to predict U(VI) transport in four different experiments conducted with different experimental conditions. It was found that, of the seven postulated models, only three had a significant probability. Model probabilities calculated from calibration to different datasets were different. The probability of one conceptual model varied from 22 to 50 percent when calibrated to each individual experiment; however, the model probability was only 16 percent when all three experiments were used simultaneously in the calibration. 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. The approach is being applied to results from small-scale tracer tests conducted at the Naturita site. The models for the tracer tests include alternative representations of (1) the subsurface heterogeneity of hydraulic conductivity, (2) the U(VI) adsorption reactions, (3) the rate of adsorption and desorption and (4) geochemical processes affecting the key major ions such as calcium.

Simulations are also being applied at the Rifle 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 has been developed to simulate behaviors that will serve as a pseudo-reality for evaluating predictive errors in model probability estimates. The synthetic model includes all of the multi-component biogeochemical reactions described in a recently published model for the Rifle IFRC experimental conditions and characteristics. The synthetic data are being used to compare a sequential calibration approach with a joint calibration approach for inverse modeling and estimation of model probability. The former approach estimates parameters sequentially, for example, first estimating hydraulic parameters and dispersion, then the acetate oxidation rate by Fe(III), the parameters for the onset of sulfate reduction, the acetate oxidation rate by sulfate, and the acetate oxidation rate by U(VI). A joint calibration estimates all parameters at the same time and, while computationally more difficult, may provide better model probability estimates. Three alternative one-dimensional models of increasing complexity are being calibrated against the synthetic data to identify the optimal method of model calibration.



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

*Federal Agency-Led Research*

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The focus of this new 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. The project will use extensive datasets already collected at the uranium mill tailings site at Naturita, CO, at bench-, decimeter-, and tracer test-scales. It will utilize geophysical results that characterize facies in the Naturita subsurface at the plume scale. It is our hypothesis that physical and geochemical heterogeneities in the subsurface are one of the most important aspects that need to be understood to improve model upscaling to the plume scale. We have previously collected large U(VI) desorption datasets in experiments with known decimeter-scale physical and chemical heterogeneity (laboratory tanks with controlled grain size sediment packing structures) and in meter-scale, natural- and forced-gradient tracer tests. These experiments included temporal and spatial gradients in bicarbonate concentration, known to affect the rates of U(VI) desorption. Tracer test observations showed considerable physical heterogeneity in the subsurface at the Naturita site, with highly variable groundwater flow rates only a few decimeters apart and unusual breakthrough curves for nonreactive tracers.

Although we have datasets at these scales and the bench scale, we have thus far applied modeling studies only to bench-scale data. In the new project, we will investigate upscaling strategies by applying a multicontinuum surface complexation model to the decimeter, tracer-test, and plume scales. 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. High-resolution simulations will be conducted using the same numerical discretization for tank experiments as was used for column data, to provide a direct test of our bench-scale models in decimeter-scale experiments. We hypothesize that upscaled multicontinuum models (i.e., with larger grid discretization) will depend primarily on the interfacial area separating grain-size facies present within a single model gridblock. Up-scaled models for decimeter-level heterogeneity will be tested for an ability to describe observations made at the meter scale, based on a new geophysical study of flow path heterogeneity at the tracer test site. If a decimeter-scale model can describe these data without further adaptation, it will demonstrate whether intermediate-scale tank experiments sufficiently capture the upscaling factors introduced by heterogeneity at the meter scale, and whether heterogeneity at the meter scale can be adequately characterized by geophysical methods. Knowledge of the spatial distribution of sedimentary facies defined by geophysics and parallel characterization of U(VI) desorption kinetics by each facies will provide a better understanding of heterogeneity at the plume scale. 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. A sensitivity analysis will be conducted to determine which processes and parameters are most important in simulating U(VI) transport at the plume scale.

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



## Bacterial Toxicity of Engineered Metal and Metal Oxide Nanoparticles

*Joint DOE EPA-NSF Nanoparticulate Research*

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Nanomaterials are of tremendous interest to pursuits in biology, medicine, electronics, catalysis, and energy storage because of their unique size- and shape-dependent properties. Characteristics such as high surface-to-volume ratios, quantum confinement, and the ability to selectively mediate chemical transformations make them unique from their bulk counterparts. Besides size, composition, surface coat, and surface charges are properties that affect nanomaterial performance and may affect their fate and transport in the environment. The transformation of such nanoparticle catalysts in the environment is likely to be influenced through interactions with bacteria. Nanoparticle production, nanoparticle toxicity, nanoparticle binding and incorporation with bacteria have all been observed. However, basic knowledge that would allow prediction of the probable interaction between an engineered nanoparticle and bacteria is lacking. Our efforts seek to quantify and characterize interactions between engineered metal and metal oxide nanoparticles on selected microbial species. Initial efforts are focused on the effects of cerium oxide, zinc oxide and silver nanoparticles on the growth, viability, structural changes and genetic response of *E. coli* and *B. subtilis*. Well-characterized CeO<sub>2</sub>, ZnO, and Ag nanoparticles have been prepared and presented to bacterial cells in a dose-dependent manner. Apart from techniques such as disc diffusion tests, minimum inhibitory concentrations, viability assays, and colony forming units, advanced imaging techniques are also being used to evaluate the binding and fate of the nanoparticles on the bacterial cell. The results of these studies will provide a basis for understanding how nanoparticle size and composition influence their interactions with microorganisms, and how microorganisms may alter the fate and transformation of engineered nanoparticles in the environment.

# Transport of Engineered Nanoporous Silicate Particles and Its Effect on Uranium Fate and Transport in Porous Media

*Joint DOE-EPA-NSF Nanoparticulate Research*

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The objectives of this research are to: (1) characterize the fate and transport of engineered nanoporous silicate particles (ENSPs) that have been functionalized in their internal pore domains; (2) evaluate the impacts of functionalized ENSPs on the fate and transport of radionuclides (U) in the sediments under conditions relevant to DOE sites; and (3) provide microscopic insights into the mechanisms controlling the transport of ENSPs, as well as coupled transport of ENSPs and radionuclides in subsurface environments.

Engineered nanoporous particles have become an important class of nanostructured materials that have found their increasing applications in energy and environmental areas. The internal pore surfaces in the particles can be tailored to be covalently bonded with various specific chemical ligands that can be used for biomolecular sensing, energy storage and fuel-cell technology, nanocatalysis and photonics, and groundwater contaminant sequestration. In this research, an engineered material of ENSPs has been synthesized and functionalized as a model material to understand the fate and transport of the nanoporous materials and their effects on the fate and transport of DOE contaminants and radionuclides once the materials enter into the subsurface environments. The synthesized ENSPs material has high ion selectivity, dense population of absorbing sites, fast adsorption kinetics, and stable silicate structure, which yield a potentially efficient material for capturing contaminants from groundwater. A scanning optical fiber-laser excitation fluorescence profiler was developed to *in situ* track particle transport in a column system. The profiler directly detected the attachment of ENSPs to a sand collector even under thermodynamically unfavorable conditions and further, our *in situ* measurement results showed a spatial variability of ENSPs transport that deviated from a common one-dimensional assumption for colloidal and nanostructured particle transport in sand columns, implying that the attachment and detachment process of nanosized and colloidal particle transport is more complicated than previously hypothesized.

The fate and transport of ENSPs has a strong impact on the fate and transport of uranium in U-contaminated sediments. Our results showed that the competitive sorption of U by the sediments and ENSPs resulted in a fast mass transport of U from the sediments to ENSPs, once ENSPs enter into the groundwater and sediment systems. The ENSPs, however, can attach to sediment grains, with the attachment degree affected complexly by ENSPs concentration, ionic strength of groundwater chemical composition, and dynamics flow conditions. Our studies found that the ENSPs attachment and detachment qualitatively followed, but quantitatively deviated from the current particle transport theories, especially under dynamic flow conditions. Experimental micromodels and pore-scale numerical models are currently under development to provide microscopic insights into the mechanisms controlling the ENSPs fate and transport, and their impact on radionuclides in subsurface environments.

# Stability and Transport of Ligand-Coated Quantum-Dots Nanoparticles in Subsurface Porous Media

*Joint DOE-EPA-NSF Nanoparticulate Research*

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The rapid growth of nanotechnology has created substantial economic value as well as new applications for many industries and products, but the potential negative impact of engineered nanoparticles (ENPs) on human health and ecosystems has raised considerable concern. U.S. government agencies, the Environmental Protection Agency (EPA), the National Academy of Sciences, and the U.S. Department of Energy (DOE) have called for an increased understanding of the risks posed by ENPs. This research project focuses on ligand-coated quantum-dot (QD) NPs. The science and technology of ligand coating has brought exciting potential applications—for instance, quantum dots (QDs) and semiconductor nanocrystals with unique optical and transport properties are useful in electronics, solar energy conversion, biomedical imaging, and ultra-high-density data storage and quantum information processing. The hydrophobic metalloid crystalline core of QDs can be ligated with hydrophilic terminal functional groups to permit their stability in aqueous suspensions. Concern about the stability and mobility of QD NPs in the environment is growing, because they are potentially highly toxic. Compromising the coating can reveal the metalloid core, which may have a toxic impact as a composite core, but little is known about the environmental behavior of ligand-coating stabilized NPs.

*The effects of NP design (size, shape, and surface coating) on their stability in aqueous environments* were studied by using CdSe NPs modified with thiolate ligands. The concept of critical coagulation concentration (CCC) was used to quantify NP stability. Our results showed that NP stability is the function of the capping ligands' chain length. A linear relationship between particle surface area and the CCC was discovered and found to be independent of nanoparticle shape. The quantitative analysis of nanoparticle size, shape, and surface coating has demonstrated the importance of ligand stability and particle surface area for the prediction of nanoparticle fate and transport in the environment. (*manuscript submitted to JACS*)

*Transport of CdTe QD NPs in different types of porous media*, including pure-quartz sand, predominantly quartz sand (Accusand), and iron-coated quartz sand, at various solution ionic strengths, were studied using batch, column, and SEM/EDX methods. The measured mobilities of CdTe in pure-quartz and iron-coated sands were consistent with the predictions of classic filtration theories, but the results from Accusand were not. We concluded that the fast diffusive mass-transfer rate of QDs from bulk solution to the sand surface and the QD attachment in the primary or secondary energy minimum were the primary mechanisms causing the significant deposition and the delayed breakthrough curves observed in Accusand. We proposed that positively charged nanoscale sites of clay particles attached on the sand surface played a key role in QD deposition. (*manuscript submitted to JCH*)

*Effects of dilution on stability of ligand-stabilized NPs in aqueous suspensions* were studied by using CdSe and CdTe NPs. We found that dilution resulted in rapid aggregation of dispersed primary NPs that were originally stable at high concentrations. These observations, obtained by dynamic light scattering, were confirmed using sequential filtration and sedimentation. Measured decreases in NP surface charge density (zeta potential) with progressively higher dilution also indicated ligand loss and NP aggregation. Ligand dissociation from the metallic core, driven by decreasing the concentration of free ligands in the aqueous phase, is responsible for the observed destabilization. Because dispersal of engineered NPs in the environment invariably results in their entry into solutions having very low ligand concentrations, it is important to develop an understanding of the suspension destabilization resulting from ligand detachment. (*manuscript submitted to ES&T*)



## ***SciDAC Research***





## PFLOTRAN: Massively Parallel Subsurface Reactive Flow and Transport Code

*SciDAC Research*

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PFLOTRAN, a next-generation reactive flow and transport code for modeling subsurface processes, has been designed from the ground up to run efficiently on machines ranging from leadership-class supercomputers to laptops. Based on an object-oriented design, the code is easily extensible to incorporate additional processes. It can interface seamlessly with Fortran 9X, C and C++ codes. Domain decomposition parallelism is employed, with the PETSc parallel framework used to manage parallel solvers, data structures, and communication. Features of the code include a modular input file, implementation of high-performance I/O using parallel HDF5, ability to perform multiple realization simulations with multiple processors per realization in a seamless manner, and multiple modes for multiphase flow and multi-component geochemical transport. Chemical reactions currently implemented in the code include homogeneous aqueous complexing reactions and heterogeneous mineral precipitation/dissolution, ion exchange, surface complexation and a multirate kinetic sorption model. A new and promising class of discretization methods, dubbed Mimetic Finite Differences (MFD), is being implemented in PFLOTRAN. These methods are designed to mimic important properties of the continuum model, such as mass and energy conservation, but go beyond traditional Finite Volume methods. They are accurate on general polyhedral meshes with full tensor coefficients. This will allow PFLOTRAN to handle complex meshing techniques, including nonorthogonal, unstructured, and adaptively refined meshes, to simulate flows through highly anisotropic porous media with complex stratigraphy. PFLOTRAN has demonstrated petascale performance using  $2^{17}=131,072$  processor cores on problems composed of over 2 billion degrees of freedom. The code is currently being applied to simulate uranium transport at the Hanford 300 Area and CO<sub>2</sub> sequestration in deep geologic formations.



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



## Multiscale Mass-Transfer Processes Controlling Natural Attenuation and Engineered Remediation: Focus 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 Site 300 Area uranium (U) plume addresses multiscale mass-transfer processes in a complex hydrogeologic setting. A series of forefront science questions on mass transfer are posed for research which relate to the effect of spatial heterogeneities; the importance of scale; coupled interactions between biogeochemical, hydrologic, and mass-transfer processes; and measurement approaches needed to characterize and model a mass-transfer dominated system. The site has 35 instrumented wells and an extensive monitoring system. It includes a deep borehole for microbiologic and biogeochemical research that sampled the entire thickness of the unconfined 300 A aquifer, and that is now being used for down-hole biogeochemical studies. Significant, impactful progress has been made in CY 2009, with completion of extensive laboratory measurements on field sediments, field hydrologic and geophysical characterization, four field experiments, and modeling. The field experiments focused on: (1) physical characterization of the groundwater flow field during a period of low, stable Columbia River stage in early spring, (2) comprehensive groundwater monitoring during spring to characterize the release of U(VI) from the lower vadose zone to the aquifer during water table rise and fall, (3) dynamic geophysical monitoring of salt-plume migration during a summer, and (4) a U reactive tracer experiment (desorption) during the fall. Down-hole biogeochemistry studies were also initiated. Geophysical characterization of the well field was completed using the down-well Electrical Resistance Tomography (ERT) array. These measurements, along with hydrologic characterization, have yielded 3D distributions of hydraulic properties that have been incorporated into an updated and increasingly robust hydrologic model. All of the field tests, along with the field and laboratory characterization, are leading to improvements in the conceptual model of U(VI) flow and transport in the IFRC footprint and the 300 Area in general. During the fall U(VI) desorption field test, a potentially significant issue related to vertical flow in the IFRC wells was identified and evaluated. Both upward and downward flows were observed in response to dynamic Columbia River stage. The vertical flows are caused by the interaction of river-stage-induced gradients with our heterogeneous hydraulic conductivity field. These impacts are being evaluated with additional modeling activities to facilitate interpretation. The project moves into CY 2010 with ambitious plans for a drilling additional wells for the IFRC well field, additional experiments, and modeling.

## Uranium Isotopic Systematics in the 300 Area U Plume and the IFRC Plot: Progress Towards a Site U Isotopic Model

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Ongoing uranium (as well as Sr, O, and H) isotopic research is being carried out on the 300 Area groundwater U plume and sediments in general, with a specific focus on the IFRC experimental plot. This research has several different aims, including understanding the sources of U in the plume, the temporal dynamics of the U plume in its interaction with the Columbia River, the discharge rate of U to the river, the interaction between groundwater U and U held in vadose zone/aquifer sediments, and providing guidance in the selection of particular 300 Area wells as sources of groundwater for use in various experiments conducted within the IFRC experimental plot. Such experiments include adsorption/desorption of U within the IFRC experimental array, monitored in part through U isotopic analysis of groundwater samples from the array. In particular, the spring 2009 Passive U Mobilization experiment directly revealed the introduction of U from the lower vadose (“smear”) zone to groundwater.

Groundwater U and sediment leachate U isotopic compositions ( $^{234}\text{U}/^{238}\text{U}$ ,  $^{236}\text{U}/^{238}\text{U}$ ,  $^{235}\text{U}/^{238}\text{U}$ ) fall along similar arrays, indicating mixtures derived from processed-enriched U fuels and depleted U. Significant U isotopic differences are evident between different process ponds, along with U isotopic variations with depth. However, the spatial U heterogeneities at the scale of the IFRC plot are nearly as great, and consistent with the original layout of the former South Process Pond complex now overlain in part by the IFRC plot. Overall, comparison between 300 Area groundwater and sediments suggest that observed groundwater U isotopic variations are attributable in large part to local sediment U isotopic compositions, rather than simple mixing within the plume between two U isotopic end-member sources.

This effect is illustrated by groundwater samples taken during the Spring 2009 Passive U Mobilization experiment. During this experiment, rising of the water table into the lower vadose zone (the “smear” zone) due to spring river stage increases were found to be associated with rapid elevations in groundwater U concentration in bailed samples from well 3-30 (e.g., from 40 ppb to 270 ppb over two days—see poster this session by McKinley et al.). The U isotopic compositions of the samples with the highest U concentrations map to the U isotopic stratigraphy of sediment leachates from the immediately adjacent well 3-31, and point to a particular depth range. Intermediate concentration samples represent variable mixing between this added U, and up-gradient U represented by pre-rise groundwater U. This not only confirms that the source of the added U is in the “smear” vadose zone, but also provides constraints on its vertical and horizontal location, and mass balance. The spatial and temporal variations we observe in the U isotopic composition of 300 Area groundwater allows tracking of water masses independent of U concentration, and identification of local sources of U contamination to the Columbia River.

# Biogeochemical Redox Transition with Depth in the Hanford 300 Area Subsurface

Hanford IFRC (Principal Investigator: John Zachara)

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Joint IFRC-SFA research results on the microbial ecology and biogeochemistry of Hanford formation subsurface sediments obtained during the first phase of drilling for well installation at the 300A IFRC revealed a relatively abundant, active, and phylogenetically diverse subsurface microbial community. Molecular analyses, enrichment cultures, and sediment microcosm experiments indicated that total populations declined and community structure and function shifted upon transition from the Hanford formation sand and gravels to the underlying fine-grained Ringold Formation (Unit E). Motivated by these results, a joint IFRC-SFA *in situ* microbial ecology-biogeochemistry experiment was initiated in October, 2009. The objective of this experiment was to probe, at finer spatial resolution and in greater detail, the changes in microbial community structure and function, as well as Fe redox reactions with various iron-bearing mineral phases and sediments in relation to transitions in geochemical and hydrologic properties across the Hanford-Ringold textural and intra-Ringold redox boundaries.

Two IFRC wells (3-24 and 3-27) were selected as the screened interval for these two wells extending into the reduced region of the Ringold E unit. Down-hole microcosm MLS units containing site sediments, Fe(III) oxides, basalt coupons, synthetic magnetite, bio-sep beads for microbial capture, an *i*-chip for *in situ* cultivation of Fe(III)-reducing and Fe(II)-oxidizing microorganisms, and aqueous- and gas-phase diffusion cell samplers--were deployed at three depths in wells 3-24. The sampling depths included: (1) within the Hanford formation; (2) above the redox transition zone in upper Ringold formation; and (3) below the redox transition zone in the upper Ringold formation. The first set of samples was retrieved from well 3-24 on December 7, 2009.

Dissolved gas analyses revealed a clear redox transition trending from oxic in the lower Hanford formation to the bottom reduced fine-grained Ringold Unit E. These trends include a significant decrease in O<sub>2</sub> concurrent with increases in H<sub>2</sub>, CO, and CH<sub>4</sub> over the same interval. Note that the H<sub>2</sub> concentration measured in the reduced Ringold (7 nM) is consistent with thermodynamic controls on its concentration when CO<sub>2</sub> is the terminal electron-accepting process (i.e., methanogenesis) in microbial systems where organic matter fermentation is the primary energy source. Groundwater analyses revealed distinct changes in select inorganic constituents, including dissolved Mn and Fe, across the Ringold redox transition zone extending from ~ 58' to 67' in well 2-25. A peak (~1.5 mg L<sup>-1</sup>) in dissolved sulfide was also observed in this same region, coinciding precisely with the region of the reduced Ringold Unit E as determined from the direct inspection of cores during well drilling and logging. Analyses of core material from 2-25 revealed the presence of framboidal pyrite in the reduced region of the Ringold, and a pyritic sulfur concentration of 0.277 (wt. %) compared to the overlying oxidized region where pyritic sulfur was below detection and framboidal pyrite was not observed. Biomass and SEM analyses of BioSep beads clearly indicated microbial colonization of the beads over the 6-week interval. Geobacteraceae 16S gene copies, as determined by per of DNA extracted from the untreated beads, were detected throughout the interval, but were clearly highest in the reduced Ringold interval, accounting for up to 13% of the total Bacterial 16S gene copies. Most probable number (MPN) analyses of viable Fe(III)- and sulfate-reducing bacteria indicate that viable populations of these functional groups are 10<sup>2</sup>-10<sup>4</sup>-fold higher in oxide-coated sands incubated in the MLS section below the Ringold redox interface.

In summary, there is a well-defined redox transition zone in the Hanford 300A subsurface that extends from the base of the Hanford formation, through the upper oxidized Ringold Unit E, and into the underlying reduced Ringold. The electron acceptors utilized over this interval include O<sub>2</sub>, nitrate, possibly Mn(III/IV), Fe(III), sulfate and CO<sub>2</sub>. H<sub>2</sub> concentrations suggest the fermentation of sedimentary organic carbon as the primary energy source, but it is currently unknown whether H<sub>2</sub> may be outgassing from the underlying Columbia River basalt aquifer. While the areal extent of the reduced fine-grained Ringold Unit E is believed to be confined to the 300A, other, similar redox boundaries exist within the Ringold elsewhere on the Hanford Site and could have important implications for the subsurface migration of redox sensitive contaminants.



## Site Geochemical Reaction and Mass-Transfer Model for U(VI) Transport and Field Validation for the Hanford IFRC

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The Hanford IFRC Team's objective in this presentation is an integrated geochemical reaction and mass-transfer model for U(VI) transport that integrates multirate kinetics, surface complexation of sorbed U(VI), and dual-domain mass transfer. Uranium(VI) transport in groundwater within the Hanford 300 area is controlled by desorption of contaminant U(VI), present as surface complexes at sites distributed within the sediments across a range of distances from predominant flow paths at multiple scales. Surface and solution speciation of U(VI) are controlled primarily by aqueous carbonate activity, for which alkalinity can be used as a proxy given the relatively uniform pH values across the IFRC experimental domain. The 300 Area at Hanford has two predominant water chemistries: (1) a high-alkalinity groundwater and (2) a low-alkalinity Columbia River water. U(VI) sorbs more extensively in the presence of the low-alkalinity Columbia River water. These waters are present in different parts of the site and are transient in time. The impact of variable chemistry on equilibrium sorption can be described using a surface complexation model calibrated with laboratory experimental data. Diffusion-controlled sorption-desorption kinetics are best modeled using a lognormal distribution of rates that incorporates significant surface reaction over time scales from at least hours to thousands of hours, as supported by a sequence of batch and column experiments. Mass transfer between accessible (mobile) and relatively inaccessible (immobile) porosity is also required for representation of experimental data. It is expected that fits to field data may require an analogous distribution of mass-transfer rates, but lab experiments to date reveal the need only for a single mass-transfer rate.

Following thorough lab testing of the model, we propose a series of field experiments. The field experiments will test all elements of the model described above. We will first inject low-alkalinity water, representing Columbia River water, into the IFRC tracer-test array for a limited time. A well-doublet geometry may prove the best design to control, locally, the considerable variations in hydraulic gradients at the site. Following comparison of the results to the model, a second longer-term experiment will be conducted. The second experiment will make any needed adjustments to tracer or water chemistry. The injection will be carried out for sufficient time to test time scales of mass transfer on the order of tens to hundreds of hours.

## Placing the Hanford 300 Area IFRC Site in Perspective: Plume-scale Modeling of Uranium Attenuation and Its Flux to the Columbia River

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Understanding complicated processes involving natural systems generally requires collecting large amounts of data to support modeling of these processes. Such data support is becoming more burdensome as models become more sophisticated with increasing data needs. However, often it is not apparent which data and processes are most important and essential to the system under consideration; hence, resources can be wasted collecting data that are irrelevant to the problem at hand. Thus an important role for modeling geochemical systems is assessing the importance of various processes on system behavior, thereby potentially reducing the amount of data that needs to be collected. An important caveat, however, is that the model must be sufficiently accurate to capture all relevant processes in the system; otherwise false conclusions could be reached. This contribution takes a large-scale view of the Hanford 300 uranium plume through the use of plume-scale modeling to evaluate the importance of various processes that can influence uranium migration at the site. Processes investigated that can affect the rate of natural attenuation of uranium include sorption and desorption, presence of a continuous source term, the role of the hyporheic zone and Hanford sediment heterogeneity on flow velocity, and high frequency fluctuations in the Columbia River stage. The mathematical metric used to judge the importance of these processes is the cumulative flux of uranium into the Columbia River. This metric provides a time-averaged measure of the loss of uranium to the river that smoothes out high-frequency fluctuations and results in an approximately linear increase in mass of uranium released to the river over time. Through conservation of mass, the cumulative flux at the river boundary is related to the time-averaged leach rate of uranium from the source regions of contaminated Hanford sediments.

## The Deep Vadose Zone as a Source of Contaminant Uranium Recharge to the Near-Shore Aquifer at the Hanford Site, Washington

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The Columbia River exhibits large seasonal discharge variations, with the highest stage observed in the spring following mountain snowmelt. These stage variations propagate directly into the near-river groundwater aquifer in the Site's 300 Area in the form of seasonal water table fluctuations of ~2 m in depth. A persistent uranium (U) plume exists in the aquifer. It has long been speculated, but never documented, that rises in the water table solubilize sorbed contaminant U from the deep vadose zone (smear zone), and sustain persistent levels of groundwater U, further causing complex seasonal dynamics in concentration. This posit was evaluated by a comprehensive groundwater sampling and analysis campaign during spring 2009 over the DOE BER/CESD Integrated Field Research Center (IFRC) footprint. The IFRC, ~300 m from the river, is a well-instrumented field site containing 37 wells, some of which have been completed at multiple depths in the saturated Hanford formation. The uppermost 15 cm of the aquifer was sampled daily, April through June, by bailing, to trace the hypothesized U desorption front as the water table moved through the smear zone. More conventional samples were pumped from the center of the fully screened (6 m) Hanford Fm. Samples were analyzed for dissolved cationic and anionic components and dissolved carbon, along with conductivity and pH. In some wells, the aquifer-top U concentration varied modestly above the low water, mid-winter values (e.g., ~50 µg/L) over the course of the experiment. In others, dramatic concentration increases were observed (>300 µg/L) that were much higher (e.g., ~3x) than the paired pumped values, and coincided with seasonal river stage and water table fluctuations. This behavior was repeatedly observed during the multiple rise-and-fall cycles in river stage that occurred over the monitoring period. When the water table rose above the screened interval, pumped samples were collected still, particularly from wells with shallow completions; the U concentration in these wells (300 µg/L) indicated an even larger aquifer-top concentration (900 µg/L). U solubilized from the smear zone did not mix with deeper groundwaters of the Hanford formation during the monitoring period, yielding a stratified system. The overall concentration of U, shown by three evenly distributed shallow wells, increased across the site during the experiment, indicating that the contribution from the vadose sediments was broad, though uneven. These results indicated that the deep vadose zone provided a seasonal, spatially heterogeneous source for seasonal recharge of U to the aquifer.

In FY 2010, the experiment will be repeated with improved well control. The 2009 experiment showed some effects of borehole flow correlated with river stage fluctuations; these effects will be negated using intermediate-depth packers. Also, the resolution of U contributions will be improved by the installation of additional shallow wells prior to the Spring thaw. We are increasing our database of sediment characterization results for the smear zone, for comparison with the spatially heterogeneous contributions of U to the aquifer during water table rise.

## Hydrologic Characterization of the Hanford 300 Area IFRC Site, Observed and Simulated Tracer Test Results, and Plans for Future Refinements

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Aquifer tests, borehole geophysical logging, measurements on intact core samples, and multiple large-scale tracer tests have been performed for the Hanford 300 Area IFRC site. The objectives of these measurements and tracer tests were to provide critical datasets for characterization of the hydraulic conductivity and porosity distributions at the site. The parameter distributions that result from these measurements and inverse modeling of the tracer tests, performed by PNNL and collaborators, will be used for subsequent reactive transport modeling and testing of hypotheses related to reactive transport of uranium and mass-transfer processes.

Field hydrologic characterization at the 300 Area IFRC site has included short-duration, constant-rate injection and electromagnetic borehole flow-meter (EBF) testing, and multiple tracer tests performed under different hydrologic conditions. These data show the Hanford Fm sediments to be highly permeable ( $K > 6000$  m/d) and nonuniformly stratified, with the shallow and deep portions of the aquifer generally being more permeable than the middle portion. Tracer test results indicate that the deeper portion of the aquifer is more permeable than the shallow portion, and both are much more permeable than the middle portion. Gamma log data are positively correlated with porosity, which has an average value of  $\sim 0.2$  for the gravel- and cobble-dominated sediments at the site. Variogram analyses of gamma log data indicate different angles of anisotropy for different horizons within the aquifer. The characterization data yield simulation results that match observations from the field tracer tests reasonably well for the fully screened wells, albeit with less temporal variability that results, in part, from intrawell flow and mixing in the wellbores. Good correspondence between observed and simulated breakthrough curves for wells screened at discrete depths requires the use of pseudo-data or pilot points. Calibration results using STOMP with PPEST suggest that the EBF profiles are smoothed representations of the true variability of the  $K$  field.

Future data collection and associated model refinements include: (1) drilling new wells to provide improved boundary condition and structural information (i.e., elevation of contact between Hanford and Ringold Fm) outside the current well field, and more depth-discrete monitoring within the well field; (2) updating the EarthVision model of the 300 Area to incorporate the new structural information; (3) adding packers to existing, fully screened wells for future experiments to reduce or eliminate intrawell flow in order to isolate monitoring to selected portions of the aquifer; (4) incorporation of additional geophysical data (e.g., ERT, GPR, and petro-physical transforms) into model parameterization and inversion; and (5) continued analyses of intact core samples to measure physical, geochemical, and hydraulic properties, including  $k$ - $S$ - $P$  relations for future vadose zone modeling.

## Hydrogeological Site Characterization and Data Assimilation at the Hanford 300 IFRC Site using Stochastic Inverse Modeling

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The primary objective of our work in CY2009 was the development of multiple 3D realizations of the conductivity field at the Hanford 300 Site, based on the simultaneous inversion of the data obtained from (1) the constant injection rate pump tests, (2) the EBF data, and (3) the March 2009 tracer test focused on the hydraulic conductivity as target variable. We are also charged with preparing a framework for considering additional data as it becomes available, including borehole and tomographic geophysics. The results of the inversion are intended to provide the Hanford IFRC modeling teams with hydraulic conductivity fields that they could use for modeling and analyzing data from the transport experiments.

Using data obtained from Electromagnetic Borehole Flowmeter (EBF) tests and drawdown data collected during the constant rate injection tests, we obtained estimates of the statistical distributions of the geostatistical parameters of the conductivity, including the mean, the variance and the integral scales of the log-conductivity, in 3D. Using these distributions, we generated multiple realizations of the conductivity field, in 3D, conditional on the data and on the statistical distributions of the parameters. A cross-validation analysis was conducted and confirmed the improvement in the predictive capabilities of our models as additional information was incorporated into the inversion.

We developed, in collaboration with the PFLOTRAN modeling team, a detailed 3D flow and transport model for the IFRC site based on the PFLOTRAN computational platform. PFLOTRAN has the ability to run multiple realizations with multiple processor cores per realization. We created the software needed for running a Monte Carlo simulation of flow and transport at the site on the Franklin supercomputer at NERSC. This includes (1) generating multiple realizations of the geostatistical model parameters, (2) generating 3D realizations of the conductivity fields, (3) feeding these realizations to PFLOTRAN, (4) modeling the March 2009 transport experiment for each of these realizations, and (5) retrieving the data needed for post-processing.

The focus of our activity in CY2010 includes (1) completing the inversion based on the March 2009 data and revising it as necessary, and identifying where additional information is needed or where corrections to the database and to our inversion algorithm are warranted; and (2) expanding the inversion to include borehole geophysical data and cross-hole tomography, as well as additional types of information from the site as they become available. We plan to test the predictive capabilities of the aquifer model by evaluating field experiments that were not used for inversion.

## **River-Induced Wellbore Flow Dynamics in Hanford IFRC Monitoring Wells: Evidence, Implications, and Mitigation**

*Hanford IFRC (Principal Investigator: John Zachara)*

V.R. Vermeul (PI), J.P. McKinley, D.R. Newcomer, B.G. Rritz, R.D. Mackley, J.M. Zachara—*PNNL*

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. The objective of this study was to characterize the observed wellbore flows and their relationship to a nearby dynamic river boundary, assess the implications of wellbore flow on sampled aqueous concentrations, and evaluate an approach for mitigating these impacts.

Simultaneous measurement of (1) wellbore flow using an electromagnetic borehole flowmeter (EBF), (2) depth discrete hydraulic head, and (3) aqueous uranium concentrations were used to quantify wellbore flow and assess the associated impacts on measured aqueous concentrations. This study demonstrates the utility of continuous (i.e., hourly measurements for ~ one month) ambient wellbore flow monitoring and shows that relatively large wellbore flows (up to 4 LPM) can be induced by aquifer hydrodynamics associated with a fluctuating river boundary located ~250 m from the test well. The observed vertical wellbore flows were strongly correlated with fluctuations in river stage, alternating between upward and downward flow throughout the monitoring period in response to changes in river stage. Continuous monitoring of ambient wellbore flows using an EBF system allowed these effects to be evaluated in concert with continuously monitored river stage elevations (hourly) and aqueous uranium concentrations (daily) in a long-screen well and an adjacent multi-level well cluster. Observed variability in aqueous concentrations measured during active tracer transport experiments provided additional evidence of wellbore flow impacts and showed that the magnitude and direction of wellbore flow varied spatially across the wellfield. An approach to mitigate these effects, based on increasing hydraulic resistance within the wellbore by installing an inflatable packer, was evaluated. Comparison of measured wellbore flow, with and without a packer installed, demonstrated the effectiveness of this approach under IFRC site conditions, with vertical wellbore flows reduced by approximately 80 percent.

Efforts are ongoing to identify the best approach for cost effectively mitigating wellbore flow effects in Hanford IFRC fully screened wells. Appropriate packers will be identified and installed to support subsequent active experiments focused on the upper portion of the aquifer.

## Integration of Core, Log, and Electrical Resistivity Tomography Data to Improve Hydrogeological Characterization of the Hanford 300 Area IFRC Site

*Hanford IFRC (Principal Investigator: John Zachara)*

A. Ward (PI), R. Versteeg—*PNNL*; T. Johnson, *INL*; J. Greenwood, *PNNL*

A quantitative understanding of the impact of the spatial heterogeneity and anisotropy on solute transport is the key to developing models of large-scale groundwater contaminant transport. In this study, we introduce the concept of geophysical facies, in which multiple geophysical methods are integrated with core sedimentological analysis and borehole logs, of various vertical resolutions, to describe the physical heterogeneity at the 300 Area IFRC site. The resulting distributions of geophysical facies are translated into distributions of sedimentary facies using petrophysical transforms developed in collaboration with the PNNL SFA. The resulting facies distributions will be used as the basis of reactive transport models to test hypotheses on uranium transport and to evaluate alternative remediation strategies.

Grain size distributions measured on over 100 samples show a wide range of textures, ranging from clay to very coarse gravel, many with multimodal distributions, and size statistics that differ from those typically assumed for lognormal distributions. Regression of textural class on grain-size moments shows the strongest correlations between mud fraction (silt + clay), mean diameter ( $d_g$ ) and sorting index ( $S_o$ ). Radiometric (K, U, Th) analysis of the 100 samples and their fractions separated in 1-phi increments show distinct differences in the response between Hanford and Ringold formation sediments and strong but nonmonotonic relationships between K, Th, and  $d_g$ . Relationships between textural class and natural isotope activity are much stronger and form the basis of petrophysical functions for facies distributions. Surface and cross borehole electrical geophysical measurements made at the site were inverted using INL's parallel high performance ERT/IP inversion code with geostatistical constraints rather than the typical smoot inversion. The spatial covariance structure was estimated through geostatistical analysis of borehole electromagnetic induction logs, resulting in a probability distribution of semivariograms that were used to constrain realizations in the ensemble. Each realization is therefore geostatistically accurate, addresses the smoothness limitation of regularized inversion, and includes the probability distribution functions for each estimated parameter. The mean of 100 geostatistically constrained inverse estimates displays improved resolution in facies distribution compared to the regularized solution. Surface azimuthal resistivity soundings used to characterize anisotropy and heterogeneity measured differences in the anisotropy between the Hanford and Ringold units with a transition from an anisotropy-dominated to heterogeneity-dominated signal between the two units, and a strike angle that is consistent with a preferential path attributed to an erosional incision into the Ringold unit. Petrophysical transforms based on grain-size moments, spectral gamma response, and electrical conductivity are used to predict 3D facies distributions and the associated hydrophysical properties (porosity, specific surface area, permeability) that are in good agreement with laboratory and field measurements.

Subsequent efforts will focus on: (i) refining the 3D distributions of facies and the site-specific petrophysical functions for predicting multiscale reactive transport properties; (ii) quantifying temporal changes in the flow field and transport properties resulting from river stage fluctuations, and (iii) the explicit coupling of electrical geophysical codes to reactive transport codes for property estimation and enhanced tracking of a range of different passive and active experiments.

## Establishing a Geochemical Heterogeneity Model for a Contaminated Vadose Zone-Aquifer System

*Hanford IFRC (Principal Investigator: John Zachara)*

J. Zachara (PI), J. McKinley, C. Murray, Y.-J. Bott, D. Moore—*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 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. Key to the understanding and simulation of these experiments 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<sup>2</sup> 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 characterized for the following properties: total U, 1000 h bicarbonate extractable U, surface area, ammonium oxalate- and hydroxyl amine-extractable Fe(III). Sand, silt, and clay mineralogy was determined on 25 samples. Twenty-four-hour adsorption distribution ratios ( $K_d$ 's) were measured from synthetic groundwater on bicarbonate extracted sediments that had been washed repeatedly to remove residual bicarbonate. Desorption  $K_d$ 's, were measured in eight successive equilibrations with site synthetic groundwater. Numerous correlations between properties and variables were evaluated, and an uncertainty analysis was performed that involved Monte Carlo estimation of the spatial distribution of key properties and variables in the lower vadose zone and saturated zone of the IFRC site for reactive transport modeling.

General observations were as follows: High extractable U (> 7.5 ug-U/g of sediment) was localized to middle vadose zone hot spots that did not correlate with grain-size distribution. A secondary maximum of adsorbed U (~5 ug-U/g of sediment) occurred in the lower vadose zone with concentrations increasing upward to the maximum elevation of the current water table. Adsorbed U(VI) was low but detectable in the saturated zone where the plume exists. Adsorption affinity was difficult to measure because of the continued release of residual U from bicarbonate extracted sediments. Adsorption distribution ratios (< 2 mm- $K_d$  ranging between .75 and 25 L/kg) that were relatively free of artifact displayed a nonlinear increase with increasing mud fraction (silt plus clay comprised of detrital phyllosilicates), but did not correlate with extractable Fe(III) forms. Sequential desorption  $K_d$ 's revealed a generalized desorption function that was common to all sediments evaluated. The database and resulting correlations will assist in establishing a spatially variable, kinetic surface complexation model for the site.



## Modeling Coupled Transport and Geochemical Processes at the Hanford IFRC Site: Technical Approaches, Insights Gained, and Future Plans

*Hanford IFRC (Principal Investigator: John Zachara)*

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The modeling at the Hanford IFRC site has proceeded on a multi-tiered approach to cope with field experiments of increasing complexity and an increasing number of coupled processes. For the bromide tracer experiment conducted in March 2009, we applied the MODFLOW and MT3DMS codes to simulate conservative tracer transport and calibrate the hydraulic conductivity distribution. Several key insights have emerged from this part of our modeling efforts. First, the groundwater head data alone were entirely insufficient to constrain the model calibration because of the highly permeable Hanford Formation at the Hanford IFRC site. Second, the tracer plume movement was highly sensitive to the transient nature of the flow field induced by the Columbia River. This implies that great care must be taken to ensure the boundary conditions for the IFRC plot-scale model are properly constructed for successful analysis and understanding of the tracer tests. Third, there was a significant vertical flow component in the aquifer induced by the aquifer heterogeneity and highly dynamic fluctuations of Columbia River. This in turn caused significant intraborehole vertical flow with alternating upward and downward movements in the long-screened observation wells, impacting the representativeness of the water samples from these wells. The intraborehole vertical flow must be considered in the modeling analysis so that correct comparison could be made between the observed and calculated concentration values.

It is simpler and less expensive to measure the 3-D temperature distribution compared with a solute tracer. Thus, heat could be used as a good groundwater tracer to complement groundwater head and solute tracer data to better constrain the hydraulic conductivity estimation. In a follow-up to our Br tracer modeling work, we simulated heat transport to evaluate the utility and limitation of temperature data in conjunction with Br tracer data to calibrate the three-dimensional hydraulic conductivity distribution. Our modeling results suggest that the temperature data could serve as a cost-effective proxy for conservative solute tracers and help the further calibration of 3-D aquifer heterogeneity at the Hanford IFRC site. Extending beyond the Br and heat transport modeling efforts, we have developed a field-scale reactive transport model that accommodates the combined effects of physical and chemical heterogeneities by incorporating laboratory-characterized U(VI) surface complexation reactions (SCR) and dual-domain, multirate mass-transfer processes, and field-measured hydrogeochemical conditions. The field-scale model was used to assess the importance of multirate mass-transfer processes on U(VI) reactive transport under hypothetical scenarios, and to evaluate the effect of variable geochemical conditions caused by dynamic river water-groundwater interactions on U(VI) plume migration. The multirate SCR model appears to be a crucial consideration for future reactive transport simulations of uranium contaminants at the Hanford IFRC site and elsewhere under similar hydrogeochemical conditions. A detailed sensitivity analysis was further used to quantify the sensitivities of key physical and chemical model parameters in both laboratory- and field-scale models and to investigate the scaling issues associated with these model parameters as they are upscaled from the laboratory to field setting.

Currently, a reactive transport model that couples uranyl geochemistry, mass-transfer kinetics, and flow and transport heterogeneity at the Hanford IFRC site is under development in an attempt to understand the preliminary uranium tracer experiment conducted since summer 2009. In addition, we are exploring how the complex interactions among flow, transport and geochemical processes impact and ultimately determine the uranium fate and transport at the Hanford IFRC site. Furthermore, we are evaluating and comparing alternative modeling approaches that simulate coupled diffusional mass transfer and (non-linear) surface complexation processes to gain deeper understanding of these processes and their controlling factors.

## 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<sub>3</sub><sup>-</sup> 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: (i) 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; (ii) determine the rates and mechanisms of coupled hydrological, geochemical, and microbiological processes that control the natural attenuation of contaminants in highly diverse subsurface environments; (iii) explore novel strategies for enhancing the subsurface stability of immobilized metals and radionuclides; (iv) understand the long-term impacts of geochemical and hydrologic heterogeneity on the remobilization of immobilized radionuclides; and (v) 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 posters by Kostka et al., Gihring 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.

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

ORNL IFRC (Principal Investigator: Scott Brooks)

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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.

Our specific objectives are (1) to perform spatially resolved characterization of U-contaminated heterogeneous media, taking advantage of DOE user facilities; (2) to compare measurements of uranium release from contaminated subsurface materials with predictions based on results of microscale characterization and geochemical considerations; and (3) to predict and measure attenuation of dissolved uranium in response to spatial gradients in pH and carbonate, phosphate, and acetate concentrations, in heterogeneous media representative of the ORIFC site.

Our two hypotheses are as follows:

1. *Release of solid-phase U from ORIFC samples:* We posit that the rate and style of uranium release will depend on the molecular-scale mechanism by which it is sequestered, as well as by advective gradients in ligand concentration (phosphate, carbonate, or acetate) and pH, in qualitative agreement with thermodynamic predictions (U dominantly in impure mineral phases) or surface complexation models (high surface-area matrix; U dominantly adsorbed).
2. *Sequestration of dissolved U:* We posit that the mode of U sequestration (precipitation, sorption) in previously uncontaminated sediments will depend on the mineral assemblage at the pore scale, together with gradients in pH and ligand concentration (phosphate, carbonate, and acetate). Phosphate should stabilize uranium, whereas carbonate and acetate should enhance its mobility.

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. Microscale characterization, including x-ray fluorescence microprobe maps showing element correlations and the relative quantities of different uranium species, microbeam XANES, and microbeam X-ray diffraction of ORIFC samples will be performed at the synchrotron light sources operated by DOE, as well as the ShaRE SEM facility at ORNL. Dynamic uranium release experiments will be performed on a subset of these samples, imposing temporal gradients in concentration of key ligands and pH in column experiments. Uranium attenuation experiments will be performed with uncontaminated sediments and simple assemblages of relevant minerals exposed to spatially stable geochemical gradients employing the same solutions, but spiked with U(VI), followed by microscale characterization.

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.

## Characterization of Denitrifying Microbial Communities in the Subsurface Co-Contaminated with Uranium and Nitrate from the Molecular to the Watershed Scales

ORNL IFRC (Principal Investigator: Scott Brooks)

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Nitrate is a priority contaminant within the nuclear weapons complex managed by the U.S. DOE, due to the use of nitric acid in the processing of U(VI) and U(VI)-bearing waste, and the impact of nitrogen transformations on uranium speciation and mobility. We hypothesize that microbial denitrification, governed by pH and electron donor availability, is the primary geochemical mechanism for permanent removal of nitrate. This study investigates the attenuation of nitrate from the molecular to the watershed scale, with the following objectives: (1) quantify the relative roles of dilution and denitrification as mechanisms of nitrate attenuation, (2) identify and quantify the microbial populations that catalyze nitrate removal, and (3) directly link denitrification activity to the abundance and phylogenetic structure of nitrate-respiring organisms.

The rates and mechanisms of nitrate attenuation are examined using a suite of physico-chemical and isotopic tracers, while in parallel, denitrifying microbial communities are interrogated using a combination of cultivation and cultivation-independent analyses. Microbiological approaches include the analysis of bacterial and fungal ribosomal RNA (rRNA) genes using community profiling techniques (T-RFLP), isolation of denitrifying bacteria and fungi from highly contaminated, acidic groundwater and sediment, genome sequencing of representative denitrifiers, design of novel functional gene primers based on genome and metagenome sequence data, quantitative analysis of rRNA and nitrite reductase genes from watershed-wide groundwater and sediment samples, and deep sequencing of bacterial community structure (pyrosequencing).

The results from this study indicate that (a) pH is a key factor shaping the microbial communities, while electron-acceptor type and availability serve as secondary, but essential factors; (b) low pH groundwater and sediment samples have extraordinarily low bacterial diversity, with low bacterial abundance found in acidic sediments; (c) bacteria from the genus *Rhodanobacter* have a high relative abundance and are likely key denitrifiers within the acidic source zone, but are not targeted by commonly used functional gene primers; and (d) fungi are present in the contaminated subsurface and may play a role in denitrification. Future work will include the comprehensive characterization of microbial community structure across the watershed using deep sequencing of rRNA genes for bacteria and fungi. Geochemical and microbial parameters, including multidomain quantitative analyses of the *in situ* microbial community, will be fully integrated using multivariate statistical analysis.

## Changes in Microbial Community Structure During Amendment with Long-Term Electron-Donor Sources for Bioreduction of Groundwater Contaminants

ORNL IFRC (Principal Investigator: Scott Brooks)

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Longer-term maintenance of reduced zones is considered a primary impediment to a biostimulation approach to uranium-contaminated groundwater remediation. Here, we present initial analyses of an experiment conducted at the Oak Ridge Integrated Field Research Challenge (ORIFRC) site exploring the use of slow-release substrates (SRS) to achieve longer periods of sustained bioimmobilization. Our objectives were to track microbial populations as the manipulation zone transitioned through reduction and re-oxidation phases, correlate microbial changes with geochemical observations, and thereby improve the ability to predict the long-term effectiveness of remediation activities. SRS, consisting of 60% emulsified vegetable oil (triglycerides of long chain fatty acids; LCFA), was injected into a subsurface contaminant plume in Area 2 of the ORIFRC. The plume was sampled across space and time for ~12 months. Bacterial and archaeal communities were characterized using phylogeny-based between-sample comparisons and taxonomy-based measures on over 450,000 16S rRNA gene pyrosequencing reads from 80 groundwater and sediment samples. The abundance and metabolic activity of key populations is also being interrogated using sequencing and qPCR of functional gene DNA and RNA of sediment samples.

The subsurface amendment of SRS resulted in disappearance of nitrate in groundwater and an immediate decrease in community diversity and evenness in all wells down-gradient of the injection wells. Sequential reduction of nitrate, manganese, iron, and sulfate occurred over approximately the first 20 d and soluble uranium decreased to <80% of initial concentrations. During this initial reduction phase, members of the strictly anaerobic, fermentative genus *Anaerobaculum* (*Clostridiales*) dominated the bacterial communities in down-gradient wells, whereas they were largely absent up-gradient and prior to injection. During the mid-to-late reduction phase (ca. 0.5 to 4 mo.), acetate accumulated, then declined, likely due to consumption and flushing. After 1 month, members of the Deltaproteobacteria showed a high relative abundance and were dominated by taxa related to *Desulfotomaculum*, a LCFA-oxidizing, sulfate-reducing bacterium. A diversity of sulfate-reducers (*Desulfobulbaceae* and *Desulfovibrionaceae*) as well as members of the *Geobacteraceae* also increased in relative abundance. Results show *dsrB* genes from gDNA were more diverse than in cDNA from mRNA, and included many genes from Gram-positive bacteria, whereas no Gram-positives were recovered in cDNA, suggesting a difference between abundance and activity of these populations. A rebound in community evenness and diversity was observed during the re-oxidation phase (~4 to 8 mo.) as groundwater sulfate and uranium concentrations gradually rebounded and methane concentrations increased. However, community compositions were clearly divergent from pre-injection time points, demonstrating a lasting effect on the microbial ecosystem. *Desulfotomaculum* continued to show a high relative abundance of bacterial sequences, whereas  $H_2$ -oxidizing methanogens dominated the archaeal communities down-gradient in the re-oxidation phases.

We conclude that *Desulfotomaculum* and *Anaerobaculum* likely performed the initial degradation of LCFAs, and the long-term oxidation of high molecular weight substrates provided a continued supply of simple, labile electron donors (e.g.,  $H_2$ , acetate) to iron-, sulfate-, and uranium-reducers for over 4 months. The supply of lower molecular weight compounds diminished as SRS was depleted, although elevated  $H_2$  and  $CO_2$  concentrations persisted through the late reduction and re-oxidation phases. Methanogenic archaea appear to have flourished after other electron donors and acceptors were largely depleted, suggesting that highly reduced zones may in fact persist during the overall subsurface re-oxidation. We are currently analyzing new data from final time-point samples and applying multivariate analyses to link microbial measurements to key geochemical changes. Additional efforts to verify and track key populations using qPCR and microarray analyses, and to cultivate significant organisms responding to SRS treatment, are also under way.

## Subsurface pH Controls for the Immobilization of Uranium and Technetium

ORNL IFRC (Principal Investigator: Scott Brooks)

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The source zone soil and groundwater at the Integrated Field Research Challenge (IFRC) site in Oak Ridge, Tennessee, are characterized by a low pH (~3.5) but high levels of nitrate (up to 10,000 mg/L) and metal ions, including U (~60 mg/L), Tc (~5 µg/L), Al (~500 mg/L), Ca (~400 mg/L), Ni (~2 mg/L), Cr (~5 mg/L), and Co (~0.2 mg/L). Few effective treatment options exist to remove or immobilize these contaminant metals and radionuclides *in situ*. This research is aimed at evaluating and demonstrating the feasibility of controlled base addition (or pH manipulation) for immobilizing uranium (as uranyl,  $\text{UO}_2^{2+}$ ) and technetium (as pertechnetate,  $\text{TcO}_4^-$ ) *in situ* through the precipitation or co-precipitation with aluminum and other metal ions in the subsurface environment. Studies to date have shown 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 >83% of Tc(VII) can be immobilized at pH above 4.5 by the co-precipitation and/or adsorption with Al-oxyhydroxides. The presence of sediment minerals appears to facilitate the immobilization of these contaminants at lower pH values, and the immobilized U(VI) and Tc(VII) are found to be stable against leaching by relatively high levels of  $\text{Ca}(\text{NO}_3)_2$  (up to 50 mM), owing to the specific sorption or inner-sphere surface complexation between uranyl/pertechnetate and Al-oxyhydroxides. However, significant amounts of dissolution or desorption of U(VI) and Tc(VII) can occur in the presence of relatively high levels of carbonates (50 mM  $\text{KHCO}_3$ , pH >8), because of the formation of uranyl-carbonate species and the surface complexation between carbonate and Al-oxyhydroxides. A generic geochemical model based on aqueous complexation, precipitation, sorption and soil buffering with pH-dependent ion exchange is also developed to predict aqueous and solid phase concentrations of metals and anions during the batch titration and flow experiments. The acid-base behavior of the sediment-solution system under variable pH conditions is well described by the geochemical code, HydroGeoChem v5.0, and a close agreement is obtained between experimental data of major ionic species (e.g., U, Tc, Al, Ni, Co, Ca, Mg,  $\text{SO}_4^{2-}$ ) and the model results by treating the sediment solid as a polyprotic acid or alkali. A field plot of 7×8 ft at IFRC has also been completed, and our future work will demonstrate that subsurface pH control can offer an effective remedial option for *in situ* immobilization of U(VI) and Tc(VII), particularly for soils and groundwater contaminated with high levels of Al at contaminated DOE sites.

## Hydrogeophysical Quantification of Subsurface Architecture and Recharge Processes at the ORNL IFRC

*ORNL IFRC (Principal Investigator: Scott Brooks)*

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The impact of large episodic, seasonal, and annual recharge on remediation and natural attenuation of subsurface contaminants is not well understood and could represent a significant factor, especially at humid DOE sites. We explore this topic at the ORNL IFRC, where recharge adjacent to the source (S-3 ponds) and along the length of the groundwater plume is hypothesized to be the main hydraulic driver for groundwater flow and dilution of contaminants along the pathway. Analysis of wellbore datasets near the source region and at the distal end of the plume suggests that many processes may contribute to the system response as low ionic strength and less acidic rainwater (pH 5.6, poorly buffered) interacts with the high ionic strength, acidic (pH ~3.5, highly buffered) contaminated groundwaters near the S-3 source region. For example, a decrease in pH may increase desorption of uranium and cause dissolution of some mineral phases, which may release precipitated contaminants. Oxygen introduced during storm events may impact redox-sensitive species and may lead to metal precipitation. These geochemical processes occur within a multiscale hydrological template, which includes perched water bodies, matrix diffusion, and fast-path preferential pathways. For example, nitrate may initially increase as rainwater is flushed through lower permeability matrix material, but may be eventually be diluted in the preferential flow-paths. Our efforts to date have focused on extensive hydrogeochemical and geophysical data acquisition and on the development of joint inversion and coupled modeling approaches. Our overall objective is to use these datasets and inversion approaches to quantify system responses to recharge and the associated implications for natural attenuation.

We have developed and tested the following three approaches for integrating multiscale, hydrogeological, geochemical, and geophysical ORNL datasets to quantify major flowpaths and recharge-induced responses in the ORNL subsurface:

- 1) A Bayesian procedure was developed that permits the joint inversion of surface seismic refraction and wellbore data in the quantification of aquifer architecture. Synthetic case studies indicated that incorporation of local-scale depth constraints provided by wellbore datasets in the geophysical inversion procedure significantly reduced the uncertainty in the estimates of subsurface architecture obtained using surface seismic refraction datasets. Application of the inversion approach to ORNL datasets clearly delineated the location of a laterally persistent seismic low velocity zone that may serve as a preferential subsurface flowpaths along the plume axis.
- 2) We have developed and tested 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 moisture or nitrate concentration due to rainfall. The coupled model allows us to jointly honor time-lapse hydrological, geochemical, and geophysical datasets as we explore recharge-induced hydrogeochemical responses. Our objective is to gain confidence in the use of surface-based ERT measurements for monitoring recharge-related processes at the local scale and then apply our insights to surface-based ERT transects collected along the length of the plume and over time.
- 3) The utility of time-lapse surface seismic refraction datasets was explored for monitoring rainfall-induced vadose zone processes. Velocity error studies and residual trend analysis suggests that time-lapse surface seismic approaches hold potential for delineating the spatiotemporal evolution and distribution of perched water bodies in the ORNL subsurface.

Subsequent research will focus on use of the newly-developed novel approaches for exploring the impact of recharge-induced hydrogeochemical variations on overall system behavior.

## Multiscale Coupled Process Modeling at the Oak Ridge IFRC Site

ORNL IFRC (Principal Investigator: Scott Brooks)

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Multiscale modeling of coupled biogeochemical and hydrologic processes at the former S-3 Ponds waste disposal site at the Oak Ridge IFRC site has been performed to develop a better understanding of mechanisms that control the mobility and possible remediation of uranium and other contaminants at geochemically complex sites.

Aquifer pH is an important variable at the site due to co-disposal of acid in waste ponds and effects of pH on geochemical and microbially mediated reactions. We developed a model to characterize aquifer buffer capacity and pH-dependent cation and anion exchange capacity, with mixed mineral solids treated as an insoluble polyprotic acid along with specific aqueous acid-base reactions, aqueous complexation, precipitation-dissolution, and ion-exchange reactions. Simulations of batch and column experiments have successfully predicted solution pH and concentrations of Al, Ca, Mg, Mn, Ni, U and Co. Pilot-scale field pH manipulation tests are currently in progress.

The feasibility of U(VI) reduction with slowly degrading electron donor was investigated in microcosm tests using contaminated sediment and groundwater amended with calcium oleate. A kinetic bio-reaction model was developed to simulate the rapid removal of U(VI) from the aqueous phase associated with oleate degradation, acetate production, and sulfate reduction.

A travel-time based reactive transport model was developed to simulate an *in situ* bioremediation experiment in Area 3 for demonstrating enhanced bioreduction of U(VI) considering microbial reduction of nitrate, sulfate, and U(VI).

An investigation was designed to predict a major source of uranium-containing precipitates that have been observed in dolomitic gravel fill as a result of exposure to acidic groundwater contaminated with U, Al, SO<sub>4</sub><sup>2-</sup> and NO<sub>3</sub><sup>-</sup>. An equilibrium precipitation/dissolution reaction model was developed to simulate the groundwater geochemical composition and U removal resulting from exposure to high pH carbonate gravel. A field test involving oleate injection was simulated to evaluate effects of reaction kinetics versus transport-limited microbial growth on microbial community evolution and geochemical reactions. Refinements were made to a steady-state site-wide model encompassing the former S3 Ponds from the headwater of Bear Creek to tributary NT2. Simulations were also undertaken to evaluate the interactions between transient hydraulic conditions and physically or reaction-limited biogeochemical reactions.



## ***In Situ* Biostimulation of Uranium Reduction and Immobilization Using Emulsified Vegetable Oil as Electron Donor at the Oak Ridge IFRC Site**

ORNL IFRC (Principal Investigator: Scott Brooks)

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Bioreduction of U(VI) to U(IV) has been successfully achieved with ethanol as an electron donor to immobilize U *in situ* and control the migration of U in groundwater at the IFRC site, Oak Ridge, TN. However, in some subsurface environments, the immobilization of U is rapidly reversed when the U (IV) is exposed to oxidants as they migrate into the system from upgradient. Based on the results of batch-scale microcosm tests, slowly degrading substrates such as oleate and emulsified vegetable oil supported more sustained reduction of U(VI) to U(IV) than ethanol. A commercial emulsified vegetable oil product (SRS<sup>TM</sup>) containing (w/w) vegetable oil (60%), biodegradable surfactant (6%), and yeast extract (0.3%) was selected for injection during a field test.

A field biostimulation test was conducted in Area 2 where U contaminated groundwater flows to Bear Creek via a highly permeable gravel fill zone (hydraulic conductivity of ~0.038 cm/sec). Site groundwater contains 5–6  $\mu\text{M}$  U; 1.0–1.2 mM sulfate; 0.2–1.5 mM nitrate; 2.5–4.0 mM  $\text{Ca}^{2+}$ ; and a pH of ~6.8. Before injection of SRS<sup>TM</sup>, groundwater samples were taken from >50 wells to assess preinjection geochemical conditions. Microbial samples of groundwater and sediment were also collected from selected wells, and site hydrologic conditions were characterized by injecting bromide tracer (450 mg/L, 900 gal volume) simultaneously into three injection wells within a 1.5 h time frame on December 8, 2008. On February 9, 2009, diluted SRS<sup>TM</sup> solution (20% concentration, 900 gal total volume) was injected simultaneously into the three injection wells within 1.5 hours. The distribution of SRS<sup>TM</sup> injected and biogeochemical process was monitored through analysis of well fluid samples, surged samples of aquifer solids, and time-lapse surface electrical methods. Data suggested that much of the SRS<sup>TM</sup> injected was trapped or adsorbed in the subsurface, compared with the more soluble conservative bromide tracer.

The geochemical response to the SRS<sup>TM</sup> injection has been monitored over a period of more than a year. Geochemical analysis of site groundwater demonstrated that the sequential bioreduction of nitrate, Mn(IV), Fe(III), and sulfate occurred after SRS<sup>TM</sup> injection. Transient accumulation of acetate was observed as an intermediate in the oil degradation. An initial increase in U in groundwater was observed, but within 2–4 weeks of SRS<sup>TM</sup> injection, reduction and removal of U was observed in all monitoring wells previously shown to be connected to the injection wells during the bromide tracer test. U concentrations in groundwater were reduced to below 0.126  $\mu\text{M}$  (EPA drinking water standard) at several well locations during the initial 100 days. The U concentration in groundwater seeps flowing into Bear Creek was decreased by >80% within a four-month period and remained at less than 50% of original level after >365 days, indicating that the flux of U discharging to the creek was reduced substantially. Nitrate concentrations discharging to the creek were reduced to below detection limits and remained at the low levels. Dissolved methane concentration in monitoring wells increased after SRS<sup>TM</sup> injection, indicating the enhancement of methanogenic activity. After the oil was consumed, rebound of U in groundwater was observed, together with the rebound of sulfate concentrations as acetate production decreased and acetate concentrations approached detection limits. 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, first flowed into the field plot. Rebound of U and sulfate in downgradient wells were observed as the oil was sequentially consumed in upgradient locations.

U(VI) reduction to U(IV) *in situ* during the field tests was confirmed by x-ray near-edge absorption spectroscopy (XANES) analysis. The U valence change in sediment samples corresponded with the changes in groundwater geochemistry. Bacterial populations in field samples were analyzed using 16S rRNA gene libraries. Known U(VI) reducing microorganisms, including *Geobacter* spp. and a diversity of sulfate-reducing bacteria (SRB), were stimulated after the oil injection.

## Uranium Redox Status in an Alluvial Aquifer During Biostimulated & Non-Biostimulated Reducing Conditions: Results from the Integrated Field-Scale Subsurface Research Challenge Site at Rifle, Colorado

Rifle IFRC (Principal Investigator: Philip Long)

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The overarching goal of the U.S. Department of Energy's IFRC at Rifle, Colorado is to develop a mechanistic understanding of the physical, chemical, and biological processes and properties controlling uranium mobility in alluvial aquifers. Areas of study include geochemical and microbial controls on stimulated U(VI) bioreduction by iron- and sulfate-reducing microorganisms, U(VI) behavior under iron- and sulfate-reducing conditions, post-biostimulation uranium stability and removal from groundwater, and rates of natural bioreduction of U(VI). The Rifle IFRC has now completed five of eight planned field experiments using novel techniques ranging from proteomics to spectral-induced polarization to monitor subsurface metabolic and biogeochemical processes. A nonbiostimulated experiment conducted during 2009 examined the response of the subsurface system to amendment of Fe(II) in the form of ferrous sulfate (~45  $\mu\text{M}$ ). Results suggest that U(VI) is not strongly reduced abiotically by sorbed Fe(II) under conditions of the experiment. However, the experiment showed that transport of injected Fe(II) is not detected even over short distances (~1 m) in spite of complete breakthrough of nonreactive Br tracer over those same distances. These results suggest that significant loss of U(VI) during *in situ* acetate amendment experiments requires enzymatic microbial reduction or microbially mediated abiotic processes. Abiotic processes involving Fe(II) alone appear unable to decrease U(VI) in groundwater to the levels achieved during active microbial metabolism associated with electron-donor amendment.

Results from acetate amendment field experiments conducted at the site from 2007 to 2009 demonstrate the importance of acetate delivery for maintaining Fe and U reduction even under conditions of dominance by sulfate-reducing microorganisms. The 2009 experiment was the third injection of acetate within the same experimental plot, and the cumulative effect of pore clogging in or near the injection wells has emphasized the need to control biomass and mineral precipitates for application of biostimulation as a remedial approach at sites having geochemical conditions similar to those at Rifle.

During 2010, we will combine bioreduction and desorption experiments in the field under conditions of Fe(III)-reduction, tracking Fe cycling and the relationship between Fe and U redox status using *in situ* columns. We will also conduct experiments in a different experimental plot in which natural rates of U(VI) reduction will be assessed by a variety of techniques, culminating in a reactive tracer test measuring uranium isotopic exchange and fractionation in the subsurface. The combined results of these experiments are expected to significantly enhance our ability to predict both biostimulation and long-term natural attenuation outcomes using reactive transport models.

## Iron(II) Uptake During the River Rouge Field Experiment and Laboratory Experiments on Rifle Sediment

*Rifle IFRC (Principal Investigator: Philip Long)*

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In the course of biostimulation experiments designed to remediate subsurface uranium contamination through the creation of reducing conditions at the Rifle IFRC site, iron reduction by native bacteria produces large quantities of Fe(II) in the aquifer. Dissolved Fe(II) concentrations typically reach levels of 0.05-0.10 mM before the onset of sulfate reduction. Laboratory experiments on pure mineral systems suggest that Fe(II) may sorb to Fe(III) mineral surfaces and undergo electron transfer, such that the sorbed Fe(II) is oxidized to Fe(III) and the electron migrates to the interior of the Fe-oxide. In addition, several laboratory experiments in the literature suggest that U(VI) may be abiotically reduced to less soluble U(IV) phases by Fe(II) under certain chemical conditions. The goals of our experiments were to investigate the degree and mechanism of Fe(II) uptake on Rifle sediments and the possibility of abiotic U(VI) reduction in the Rifle aquifer. Results from a series of laboratory experiments performed over a range of chemical conditions (Fe(II) concentration and pH) are presented along with Mossbauer data collected on selected sediment samples to determine the ultimate chemical form of Fe(II) added to the sediments. A field experiment was performed in which 6 pulses of Fe(II) amended groundwater were injected into multi-level sampling wells in the Rifle aquifer. Background groundwater in this location is characterized by low levels of dissolved oxygen ( $0.6\text{--}2.5 \times 10^{-6}$  M), circumneutral pH (7.1-7.4), low dissolved U(VI) ( $1.8 \times 10^{-7}$  M), and low Fe(II) ( $<4 \times 10^{-6}$  M), although hot spots of elevated Fe(II) (0.02-0.08 mM) are occasionally observed. Fe(II) concentrations in the injection wells increased with each pulse of added Fe(II), reaching injection concentrations (0.045 mM) after the final injection. In shallower ports (18, 21 ft bgs), the Fe(II) concentrations remained elevated over the course of the 3-week experiment, while Fe(II) concentrations decreased more quickly (over 7 days) in deeper ports (24 ft bgs). Despite the appearance of a conservative tracer (Br) in wells 0.8-1.4 m downgradient of the injections and achieving sustained elevated Fe(II) concentrations in injection ports, no Fe(II) was detected in downgradient wells. A single pulse of high pH (pH 8.4) groundwater was injected following the Fe(II) injections in one of the wells. Decreases in dissolved U(VI) concentrations following the high pH injection provided evidence of abiotic U(VI) reduction in this well. However, no evidence of abiotic U(VI) reduction was observed in wells at ambient pH values (pH 7.2). Results from these field and laboratory experiments indicate that the sediment can take up very large quantities of Fe(II), and thus the dissolved Fe(II) represents only a fraction of the pool of Fe(II) in the aquifer. In addition, abiotic U(VI) reduction may be more favorable in the Rifle aquifer at higher pH values.

## Factors Controlling Microbial Dynamics During Biostimulation and Post-Biostimulation and Their Effect on U(VI) Stability

Rifle IFRC (Principal Investigator: Philip Long)

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There is a growing need for a better understanding of the biogeochemical dynamics occurring during biostimulation for the purpose of microbial U(VI) reduction. This includes Fe(III) bioavailability at different stages of the biostimulation, the microbial community changes throughout the biostimulation period, including the activity of iron reducers during the biostimulation period, even after sulfate reduction becomes the dominant TEAP. Column experiments were conducted with Old Rifle site sediments containing Fe-oxides, Fe-clays, and sulfate-rich groundwater. Half of the columns had sediment that was augmented with small amounts of  $^{57}\text{Fe}$ -goethite to track continuously minute goethite changes, and to study the effects of increased Fe(III) levels on the overall biostimulation dynamics. The addition of the  $^{57}\text{Fe}$ -goethite slightly suppressed the overall rate of sulfate reduction and hence acetate utilization; it did not affect the bacterial numbers of *Geobacter*-like species throughout the experiment, but did decrease the number of sulfate reducers in the sediments.  $^{57}\text{Fe}$ -Mössbauer analyses (a  $^{57}\text{Fe}$ -specific technique) confirmed that there was bioavailable iron present and being reduced throughout the biostimulation period, but that as Fe(III) phases became less amorphous, sulfate reduction became the dominant TEAP. Addition of the  $^{57}\text{Fe}$ -goethite to the sediment had a noticeable effect on the overall composition of the microbial population. 16S rRNA gene analyses of biostimulated sediment using TRFLP (terminal restriction fragment length polymorphism) showed that five TRFs dominated the community profile, comprising 25-57% of the total fingerprint. These TRFs were cloned, sequenced, and are related to *Geobacter* sp. (a known Fe-reducer). High molecular weight RNA analysis indicated these *Geobacter*-like species were active and growing during the period of significant sulfate reduction.

A separate study was conducted to determine the changes in the microbial community structure during the post-biostimulation period and how they might affect the stability of reduced uranium. Effluent groundwater from acetate-stimulated sediment flow-through columns was analyzed over 60 days after acetate amendment was discontinued. Only a minimal reoxidation of iron or uranium occurred in the presence of 1-2 mg/L  $\text{O}_2$  influent groundwater for the two-month period. Most uranium reoxidation occurred during the first two weeks after biostimulation with acetate was discontinued. Groundwater and sediment microbial community compositions suggested that two processes played important roles immediately after the cessation of acetate addition. The first process was characterized by a predominance of both sediment-bound and planktonic microorganisms most closely related to *Hydrogenophaga* sp. and *Thiobacillus* sp., which could oxidize a variety of reduced minerals. The second process was characterized by organisms closely related to *Lysobacter* sp. and *Sterolibacterium* sp., with the potential to metabolize complex organic compounds from biomass turnover. The presence of these bacteria and lack of uranium oxidation implied that after acetate addition was stopped, reduced inorganic compounds and dead biomass became electron donors for a microbial community capable of using low ambient oxygen as a terminal electron acceptor, contributing to the stability of biogenic U(VI).

## Microbial Community Dynamics at the Rifle IFRC: Influence of Acetate Additions in the Field

*Rifle IFRC (Principal Investigator: Philip Long)*

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Microbial community characterization of the Rifle, CO Integrated Field Research Challenge site began nearly 10 years ago. Early methodologies involved analysis of groundwater and sediments using clonal library approaches and demonstrated enrichment of *Geobacter*-like sequences. Recent research efforts at Rifle have focused on three subsequent field-scale acetate amendment experiments (Winchester [2007], Big Rusty [2008], and Buckskin [2009]) and on characterizing a naturally bioreduced area—La Quinta (2009). All of these field-amendments replicated results from earlier experiments, with uranium reduction in groundwater during biostimulation. However, additional molecular approaches have been employed to characterize the bacterial communities including PLFA, qPCR, TRFLP, and microarray analyses (Akonni and Affimetrix G3 phylochip). Quantitative PCR demonstrated significant shifts in *Geobacter* species during field amendment. TRFLP profiling also indicated *Geobacter*-like sequences represented nearly 50% of the bacterial community in groundwater at early stages of acetate amendment, with replacement by bacteria distantly related to *Acinetobacteria* and *Desulfobacter* with time. The Akonni microarray detected signals for *Geobacter*, *Pelobacter*, and *Geothrix*, in addition to *Dechloromonas* and *Dechlorosoma* for Winchester (2007). Furthermore, the 2007 profiles differed from 2008, which is supported by PLFA and qPCR data, indicating a residual biomass/stimulated community going into the Big Rusty experiment. The G3 phylochip documented how acetate-stimulated groundwater samples differed from background sediment samples by high amounts of *Geobacter* species and, to a lesser extent, *Desulfobacteraceae*. Both arrays showed a decrease in *Geobacter* species during the amendment as predominantly iron-reducing conditions transitioned to predominantly sulfate-reducing conditions. Later samples probed by the G3 chip contained high amounts of sulfate-reducing taxa bacteria, including *Desulfobacteraceae Desulfovibrionales*, *Desulfitobacterium*, and *Desulfotomaculum*. To ascertain the active bacteria at the Rifle IFRC, stable isotope probing methods were employed in groundwater and sediments during the Winchester experiment. Specifically,  $^{13}\text{C}$  acetate was used to assess the active microbes on three size fractions of sediments (coarse sand, fines [8–approximately 150 micron], groundwater [0.2–8 micron]) over a 24-day time frame. Results indicated differences between active bacteria in the planktonic and particle associated phases. with a *Geobacter*-like group (187, 210, 212 bp) active in the groundwater phase, an alpha Proteobacterium (166 bp) growing on the fines/sands, and an *Acinetobacter* sp. (277 bp) utilized much of the  $^{13}\text{C}$  acetate in both groundwater and particle-associated phases. Analysis of the microbial community in the naturally reduced sediment (La Quinta) indicated *Geobacteraceae* comprised 20% of the natural background community, 4 times greater than more oxidized sediment collected from the Rifle IFRC site. When La Quinta sediment was incubated with acetate, *Geobacteraceae* never became predominant, suggesting that the *Geobacteraceae* found in La Quinta may function differently from other organisms belonging to this family.

## Reductive Biotransformation of Fe in Rifle Alluvial Sediments: Implications of *In Situ* Bioremediation and Long-Term Behavior of Uranium(VI)

Rifle IFRC (Principal Investigator: Philip Long)

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The mineralogy of selected sediments from the Rifle IFRC was characterized using a variety of techniques to gain insights into the impact of biotransformed Fe-minerals on U(VI) attenuation in groundwater. Samples were obtained by drilling or backhoe and include natural- and acetate-amended materials (Fe- and/or sulfate-reducing conditions), and background sediment subjected to biostimulation in laboratory columns. Column experiments used Rifle groundwater containing 6-9 mM sulfate. The natural samples analyzed were: (a) typical background aquifer sediment and (b) *in situ* bioreduced sediments. In all the samples, Fe-oxides and Fe(III)-containing clays are the dominant Fe-minerals. Hematite, goethite, and magnetite comprise the Fe-oxide suite, while illite and chlorite are the dominant clays. Goethite is Al-substituted and superparamagnetic (<8 nm), while magnetite is crystalline, large particulate (>50 nm), and contains some Ti.

Comparison of naturally bioreduced zones, e.g., Winchester (relatively more reduced naturally conditions) versus La Quinta (relatively less reduced natural conditions) shows differences in total reducible inorganic sulfur (TRIS) content, Fe and sulfur (S) association, bioavailable Fe(III), and total sorbed U contents. Siderite, expected under these geochemical conditions, is only detected in the La Quinta samples. X-ray diffraction (XRD) indicated presence of some calcite. In the naturally bioreduced zone (pre-biostimulation Winchester), most of the S is associated with Fe as framboidal pyrites (containing U), and little or none of its precursor minerals, mackinawite and greigite. Applied-field Mössbauer studies are needed to quantify pyrite contribution to the samples. The Ti-magnetite Fe(II)/Fe(III) ratio is similar to that of the background sediment, but it contains small amounts of U. In the La Quinta sediments, Fe-sulfide precipitation is significantly lower, and most of its S is *not* associated with Fe. However, its Fe-mineralogy (including clay Fe(III)/Fe(II) ratio) and Fe and S association are similar to the sediments that were biostimulated to sulfate-reducing conditions (Big Rusty field experiment), implying that the biostimulation process may mimic natural conditions of the La Quinta samples. The marked differences between Winchester and La Quinta Fe-mineralogy may be due to biological activity and/or local Fe-oxide composition.

During the first 30 days of biostimulation of sediments in laboratory columns spiked with small amounts of  $^{57}\text{Fe}$ -goethite (<8 nm), Fe(III)-oxide and sulfate reductions were concurrent. During the first 30 days, loss of small particle fractions of the amended  $^{57}\text{Fe}$ -goethite was evident from Mössbauer measurements. After 30 days,  $^{57}\text{Fe}$ -goethite reduction subsided, and sulfate reduction predominated. Clay Fe(III) reduction was evident in Mössbauer spectra, similar to results for bioreduced field samples. Additionally, Fe-sulfides with chemical composition approximate to mackinawite and pyrite with U association were evident, based on SEM-EDX results.

Currently, nano-SIMS studies are under way to gain insight into the biotic or abiotic origin of pyrites. Also, because the role of pyrites and Ti-magnetites in attenuation of U is apparently highly variable, further characterization of these Fe-minerals (field and laboratory synthesized) is necessary to gain scientific knowledge supporting biostimulation strategies for attenuation of U in contaminated aquifers.

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

Rifle IFRC (Principal Investigator: Philip Long)

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Understanding where to use active remedial technologies and where passive or natural systems can be relied upon for remediation of uranium plumes requires knowledge of the subsurface natural attenuation capacity. An important component of understanding the natural attenuation capacity of a site is to quantify the extent and rate of uranium bioreduction occurring indigenously in the subsurface. One feature of the Rifle IFRC site is naturally occurring zones of bioreduction. The effect of these zones on the fate and transport of uranium in the subsurface is likely key to accurate prediction of natural attenuation of U in alluvial aquifers with high concentrations of organic matter. During the summer of 2008, geophysical surveys (induced potential) were done in order to locate zones of natural bioreduction. After exploratory drilling, a suitable bioreduced area was located and one 4-inch well (LQ-107) was completed. In 2009, three additional active multi-level sampler wells and four 4-inch monitoring wells were installed in the La Quinta naturally bioreduced test area. Out of seven wells installed in the La Quinta gallery, five contained readily observable natural zones of bioreduction. The strongest reducing gradient was down the center line of wells LQ-117, LQ-107, LQ-112, and LQ-115. Cross gradient to the East of the center line, there was a less reduced zone in wells LQ-116 and LQ-113, and to the West of the center line in wells LQ-114 and LQ-111, the sediments were not visually reduced.

An immediate question about zones of natural bioreduction was their possible role as net sources or sinks for uranium and other compounds. To address this question, ten Passive Flux Meters (PFM) were deployed in the five 4-inch La Quinta wells (LQ-107, LQ-114, LQ-115, LQ-116, and LQ-117). The PFM were retrieved and sampled in an onsite anaerobic glove bag after a deployment length of 21 days. Water and uranium flux increased with depth and were highest in the bioreduced zone. Flux-averaged uranium concentrations for each well were consistent with observed aqueous concentrations. This result was unexpected, in that the naturally bioreduced zones were thought to have a lower water and U flux.

A bench scale study was conducted to determine the differences in microbial acetate metabolism in the La Quinta reduced sediments versus more oxic Rifle background sediments, a  $^{14}\text{C}$ -acetate tracer was used to determine the turnover rate constants ( $k$ ) of acetate in these sediments. Hydrogen and acetate measurements were also taken to examine the redox state of the sediments and determine the acetate pool. Although the  $k$ -values for the background and reduced sediments were quite similar, the acetate pool was determined to be higher, as much as 5X higher, in the naturally bioreduced sediments. This was confirmed by higher hydrogen concentrations in the reduced sediments. Therefore, the actual turnover of acetate in the naturally reduced sediments was higher than in background sediments. This increased acetate pool is likely to be the product of fermentation. Clone libraries of the reduced sediments showed increased bacteria in the phylum *Firmicutes*, which comprises the bacteria most likely to be involved in fermentation. Furthermore, there was an elevated fraction of *Geobacter*, a known acetate-oxidizing and iron-reducing bacteria, in the naturally reduced sediments compared to the oxidized sediments.

The flux measurements and microbial activity experiments show that the naturally bioreduced zone in the La Quinta gallery is an active subsurface environment. Current work includes extensive microbial and geochemical characterization, and we have planned a series of *in situ* experiments, including reactive tracer tests, in order to investigate the field-scale attenuation capacity of these naturally bioreduced zones.

## Proteogenomic Analysis of Sediment and Groundwater-Associated Biomass Recovered During Bioremediation of U(VI)

Rifle IFRC (Principal Investigator: Philip Long)

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Proteomic samples recovered from two consecutive biostimulation experiments at the Rifle IFRC site in Colorado were analyzed using a multidimensional LC-MS/MS and LC-MS AMT approach. In the absence of metagenomic sequence, proteomic data generated from planktonic biomass was searched against a database consisting of a range of Fe(III)- and sulfate-reducing bacteria. This database was systematically constructed to ensure the highest numbers of unique peptides were detected while limiting ambiguous peptide detection. While results from the first acetate-amendment experiment demonstrated dominance of the community by members of the *Geobacteraceae*, a clear shift in both protein abundances and the community diversity was observed over the duration of the biostimulation. Detection of proteins involved in sulfate reduction in a later Fe(III)-reducing sample indicated that this analysis is an effective way to monitor changes in respiratory processes. Comparison of these data with samples recovered at the start of the 2008 field campaign revealed differences in the microbial community structure, suggesting that the acetate amendment alters the microbial population composition over an extended time period. Groundwater samples recovered later during biostimulation in 2008 contained lower amounts of biomass, suggesting that as the biogeochemistry in the system transitioned from Fe(III)-reducing conditions into sulfate-reducing conditions, an increasing fraction of the microbial community was attached to mineral surfaces. To test this hypothesis, sediments and pre-filters containing attached biomass were also recovered during these biostimulation experiments. Analysis of the pre-filters revealed abundant biomass associated with filtered precipitates, although when LC-MS/MS data was searched against a database consisting of over 1000 bacterial and archaeal isolate genomes, the majority of identified proteins had low sequence coverage. This suggests that the dominant species in these samples are not closely related to any currently sequenced strains. While the extraction of proteins from soil and sediments has long been a difficult challenge for metaproteomics, we have recently developed a novel technique, using SDS detergent-based lysis protocol coupled to a TCA protein precipitation cleanup. The detergents are able to lyse cells and solubilize proteins, thus aiding the extraction from sediments. While this technique was optimized using sediments spiked with isolate bacterial strains, analysis of sediments recovered during the 2009 field campaign identified ~ 1000 proteins, although again this analysis was severely hindered by the lack of suitable metagenomic information. Multiple metagenomic samples from the period of dominant sulfate-reduction at the site are currently being sequenced, and this data will greatly aid the interpretation of proteogenomic results.



## Biogeochemical Changes Accompanying Recurrent Acetate-Amendment: Implications for Sustainable U(VI) Remediation at the Rifle IFRC Site

Rifle IFRC (Principal Investigator: Philip Long)

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Research activities at the IFRC site in Rifle, CO are designed to integrate biological, geochemical, geophysical, and hydrological studies to enhance our understanding of subsurface uranium mobility. During successive summer experiments—"Winchester" (2007), "Big Rusty" (2008), and "Buckskin" (2009)—acetate was added to the aquifer to stimulate the activity of dissimilatory metal reducing bacteria capable of reductively immobilizing uranium. The experiments were conducted within the same flow cell; however, they differed in the length of injection, the maximum concentration of acetate injected, and the extent to which iron or sulfate reduction was the predominant metabolic process. In all cases, rapid removal of U(VI) from groundwater occurred at calcium concentrations and alkalinities where Ca-UO<sub>2</sub>-CO<sub>3</sub> ternary complexes were the predominant uranyl species. Complete consumption of acetate and increased alkalinity accompanying the onset of sulfate reduction corresponded to temporary increases in U(VI); however, by increasing acetate concentrations in excess of available sulfate, low U(VI) concentrations (<50 nM) were achieved for extended periods of time (>140 days). Sustained transcription of *Geobacter*-specific citrate synthase (*gltA*) during sulfate reduction indicated that members of the *Geobacteraceae* were still active during this phase and likely contributing to U(VI) removal; persistence of reducible Fe(III) oxides in sulfidic sediments supports this contention. Uniform delivery of acetate was impeded due to decreases in injection well permeability resulting from biomass accumulation and mineral precipitation, creating conditions where prolonged delivery of acetate and the ability to sustain the activity of uranyl-respiring *Geobacter* strains became compromised over long time scales (1+ years).

Surface-induced polarization and graphite electrode studies have accompanied each of the acetate amendment experiments. Spatial variations in the phase response between electric current and potential field correlate with changes in mineralogy and pore fluid composition associated with the accumulation of electroactive ions and mineral precipitates. Pronounced phase anomalies persist long after cessation of acetate injection, suggesting a spatially extensive, noninvasive means for monitoring post-stimulation changes in aquifer iron redox chemistry. Increases in current density ( $\leq 50$  mA/m<sup>2</sup>) resulting from electron transfer by anodophilic microbes were monitored using graphite electrodes; current flow tracked the delivery of acetate and the period of optimal uranium removal. Confocal microscopy of electrodes revealed firmly affixed biofilms, with community analysis detecting sequences dominated by *Geobacter* strains.

Fluorescent *in situ* hybridization (FISH) analysis of sediments recovered from in-well columns deployed during Buckskin were used to characterize firmly and loosely attached cell fractions using general 16S rRNA probes for Eubacteria and probes specific to *Geobacteraceae* and *Desulfobacteraceae*. Cells firmly attached to sediments (10<sup>9</sup> cells/gram) were one and two orders of magnitude greater than those loosely attached or planktonic, respectively. While members of the *Desulfobacteraceae* outnumbered those of the *Geobacteraceae*, the latter were present at elevated levels (10<sup>8</sup> cell/gram) even when sulfate reduction was the predominant metabolic process. Fe-XANES spectroscopic analysis revealed that abundant Fe(III) oxides (primarily goethite) were present at the time of sampling, with the results consistent with Fe-XANES analysis of drilling-recovered sediments obtained at the end of Big Rusty.

## Reactive Transport Modeling of Uranium Bioremediation Field Experiments at the Rifle IFRC Site

*Rifle IFRC (Principal Investigator: Philip Long)*

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The Rifle IFRC experimental and characterization studies continue to provide new knowledge on the processes, properties and conditions controlling subsurface uranium under *in situ* acetate biostimulation. While the original principle of stimulating indigenous dissimilatory metal-reducing bacteria (*Geobacter*) to immobilize U(VI) as solid-phase U(IV) has not changed over the course of the project, a more refined conceptual model of processes has emerged. Numerical simulation of subsurface flow and biogeochemical reactive transport provides an important framework for developing these conceptual process models, through systematic testing of our understanding of new processes and property characterizations in the context of observed experimental behaviors.

One important model refinement has been the simultaneous presence and activity of functional groups representing the metal and sulfate reducers throughout the biostimulation. While the role of metal reducers during sulfate reduction has been identified in previous Rifle IFRC studies, the identification of low-level sulfate-reducer activity during the initial iron-reduction phase was only recently identified through transcriptomics and metagenomics analyses. In this case, the model begins with an initially small amount of slow-growing sulfate reducers whose activity increases with increasing biomass. After approximately 30 days, the sulfate reduction becomes the dominant TEAP.

The products (e.g., bicarbonate, Fe(II), sulfide) of these biologically-mediated reactions significantly alter the geochemical controls on uranium mobility through increases in pH, alkalinity, exchangeable cations, and highly reactive reduction products. The modeling addresses surface complexation, ion exchange, mineral reactions, and aqueous uranium complexation to better represent the abiotic uranium behaviors. Where biostimulated sulfate reduction is dominant, this level of detail in the multicomponent reaction network is critical to capturing the rise in aqueous uranium concentrations that results from U(VI) desorption promoted principally by the increases in pH and alkalinity.

An important component of the uranium surface complexation process model is the density of reactive sites on the mineral surfaces, which is proportional to the bulk surface area. Facies-specific surface area, as well as hydrologic parameters (e.g., porosity, hydraulic conductivity, pressure-saturation) have been developed, based on lithologic logs, particle size distributions, slug tests, electromagnetic borehole flowmeter measurements, and geophysical data. Property transfer and petrophysical models are being developed to estimate modeling parameters from the geophysical log data, which include resistivity, density, neutron porosity, and spectral gamma logs. The culmination of the current characterization of the Rifle site processes, properties, and conditions is a three-dimensional simulation of multifluid flow and multicomponent biogeochemical reactive transport through physically and chemically heterogeneous subsurface sediments during the 2008 field biostimulation experiment. In addition to the abiotic uranium behavior, these simulations are being used to better understand the interplay of transport rates and TEAP reaction rates that determine the location and magnitude of key reaction products, particularly those that may occlude pore structure and alter hydraulic properties. Ongoing studies using this modeling framework include amendment density effects, enhanced groundwater oxygenation through seasonal water table fluctuation, and post-biostimulation oxidation processes.

## Characterization of Solid Phase and Secondary Mineral Formation Associated with Biostimulation at Old Rifle, Colorado

Rifle IFRC (Principal Investigator: Philip Long)

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Bioremediation through natural or stimulated microbial activity is an attractive strategy for remediating redox-sensitive metals and radionuclides, such as Cr and U, found at many of the Department of Energy's contaminated sites. To optimize the design of an effective remediation plan, it is necessary to understand the complex reaction pathways involved in the biostimulation processes and develop a quantitative understanding of the thermodynamic controls on the biogeochemical reaction rates. Using samples from a field-scale uranium bioremediation experiment conducted at a former uranium mill tailing site at Old Rifle, CO, the secondary minerals formed in the biostimulated sediments were characterized with synchrotron  $\mu$ -XRF, XANES, and XRD techniques to understand the reaction pathways involved. Chemical mapping at different energies around the Fe edge showed abundant ferric iron oxides, such as goethite, still remaining in the sediments following an extended period of sulfate reduction (55+ days). The Fe(III) oxides were found in close association with minor accumulations of reduced iron minerals, such as siderite ( $\text{FeCO}_3$ ) and mackinawite ( $\text{FeS}$ ). Using sulfur-XANES, we also detected elemental sulfur as another secondary phase in the biostimulated sediments, in addition to mackinawite, which was evidently formed through the reaction of biogenic sulfide with sediment Fe(III) oxides. These results suggest that sulfate reduction can commence even when significant pools of Fe(III) oxides remain in the sediments. Although iron reduction is energetically more favorable compared to sulfate reduction, this process is likely kinetically controlled and could not inhibit the use of sulfate as an electron acceptor by the microbes. The persistence of Fe(III) oxides following extended periods of sulfate reduction may explain the sustained activity of iron-reducing bacteria, such as *Geobacter sp.*, and the continued removal of uranium after the start of sulfate reduction. In addition to the enzymatic reductive dissolution of Fe(III) oxides, depletion of ferric iron also occurs through the abiotic reduction of Fe(III) linked to the oxidation of dissolved sulfide, which accumulates throughout the activity of sulfate reducing bacteria (SRB). In order to carry out accurate predictions of metal reduction by direct or indirect microbial processes, we must account for the consumption of ferric iron minerals as a result of both biotic and abiotic pathways, in reactive transport models describing stimulated biogeochemical processes at the Old Rifle site.

## ***Scientific Focus Areas (SFA)***



## The Argonne Subsurface Science Program 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 U., U. of Iowa, U. of Illinois at Urbana-Champaign, Stanford U., Hamilton College, Virginia Commonwealth U., 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 that is necessary for predicting contaminant reactivity and transport processes. This objective drives the development and optimization of synchrotron methods for measurements at the molecular level pertinent to understanding contaminant transformations in subsurface environments. Two scientific themes drive the development of hypotheses. These themes are: (1) Microbial metabolic activity—together with changes in solution chemistry, mineralogy, and solid-phase surface reactivity, and the presence of electron donors, acceptors, and shuttle—affects the formation and distribution of (bio)mineral phases, as well as the rate, extent, and mechanisms of biotic and abiotic molecular-scale contaminant transformation; and (2) multiple coupled biological, chemical, and physical processes can affect the molecular-scale transformation of contaminants in the vicinity of mineral-microbe microenvironments. Research is focused on the transformation of the contaminants uranium and mercury at different spatial scales and in the context of iron and sulfate reduction. Research strives to (1) determine which aspects of microbial metabolism, solution chemistry, and mineralogy are key to the distribution of mineral species and to contaminant transformations; (2) evaluate the reactivity of biogenic mineral phases with respect to the chemical transformations of UVI and HgII; (3) provide fundamental knowledge concerning the molecular-scale transformations of contaminants at the mineral-microbe microenvironment; and (4) evaluate the effect of diffusion and advective water flow on contaminant transformations. Research emphasizes laboratory-based experimentation with single-crystalline-phase iron oxide 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.*) respiring on the films, and uranium. These results identify at least two different interactions with uranium within five microns of the mineral-microbe interface.

## The Influence of Ligands on the Formation of Non-Uraninite U(IV) Phases During Biotic and Abiotic U(VI) Reduction

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

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Uraninite ( $\text{U}^{\text{IV}}\text{O}_2$ ) is a commonly assumed product in U(VI) immobilization approaches that utilize reduction. However, fully reduced non-uraninite U(IV) phases were found in biostimulated material from the Oak Ridge IFRC, both in spatially resolved microcosm experiments (Boyanov et al., 2009 PI meeting poster) and in surge material from Area 2 biostimulated wells (W.-M. Wu et al., 2010 PI meeting poster). Uranium phases with distinct atomic structures are likely to have different solubility and mobility, affecting the ultimate success of remediation approaches. It is therefore important to identify such U(IV) phases and define the conditions leading to their formation. We are employing a combination of techniques (U- and Fe-edge EXAFS, synchrotron XRD, uptake measurements, and pH titration studies) to study the phases formed during biotic and abiotic U(VI) reduction under several carbonate, phosphate, and pH solution conditions. Three reducing agents are used as representatives of U(VI)-reducing phases in the environment:

1. Strains of the gram-positive *Desulfitobacterium* spp. (detected at ORNL)
2. Dissolved and adsorbed Fe(II) phases in a carboxyl colloid suspension
3. A reduced soluble electron shuttle ( $\text{AH}_2\text{DS}$ , anthraquinone-2, 6-disulfonate)

The bioreduction experiments with five strains of *Desulfitobacterium* spp. showed that the organism can reduce 0.1 mM bicarbonate-complexed U(VI) to a mononuclear U(IV) product. Comparative experiments in the presence and absence of phosphate indicate that the individual U(IV) atoms are bound to phosphate and carbonate ligands, respectively. The strong solution control on the identity of the reduced U(IV) phase provides insight into the reduction pathways of gram-positive bacteria and suggests an extracellular electron-transfer mechanism.

The abiotic U(VI) reduction experiments also show a significant influence of ligands on the reactivity and end products. Increasing amounts of phosphate (0.1 to 5 mM) diminish the ability of Fe(II) or  $\text{AH}_2\text{DS}$  to reduce 0.1 mM U(VI) by forming U(VI)- $\text{PO}_4$  and Fe(II)- $\text{PO}_4$  minerals. Small amounts of  $\text{PO}_4$  did not inhibit U(VI) reduction, but led to the formation of mononuclear U(IV) and green rust as the Fe(II) oxidation product, as opposed to uraninite and magnetite under phosphate-free conditions. Increasing amounts of bicarbonate (0 to 30 mM) did not enhance U(VI)-Fe(II) redox at pH 7.5, which was hypothesized from the formation of Fe(II)-carbonate minerals. At pH 8.4, the appearance of Fe(II)- $(\text{OH})_2$ -Fe(II) edge-sharing linkages was observed, both in the presence and absence of carbonate. This polynuclear Fe(II) phase reacted readily with U(VI), producing nanoparticulate uraninite and U(IV) associated with Fe atoms. The presence of Fe(II)- $(\text{OH})_2$ -Fe(II) linkages appears to exert a strong control on reactivity. Our results provide mechanistic insight into the factors controlling U(VI) reduction during mineral nucleation, a stage of presumed high reactivity due to the exposed surface and highly strained bonds. This research is part of the SBR SFA at Argonne National Laboratory.

## Microbial Community and Biogeochemical Dynamics under Sulfate- and Iron-Reducing Conditions

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.

We hypothesize that the addition of an electron shuttling compound (2,6-anthraquinone disulfonate (AQDS)) to iron-rich material that is inoculated with sediments collected from the U.S. DOE SBR's Old Rifle IFRC site will result in changes to microbial community structure and function that are dependent upon the presence/absence of the electron shuttling compound. We characterized mixed batch experiments over a 60-day period by microbial community analysis (using 454 titanium-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. Specifically, we investigated evolution of natural microbial community structure, the production of Fe(II), the loss of sulfate, and the consumption of acetate within material inoculated with sediment collected from the Old Rifle SBR IFRC site, after it was biostimulated via the addition of acetate and augmented with AQDS. Geochemical characterization of the material identified an increase in the concentration of reduced iron and a decrease in the concentration of sulfate with time, concurrent with acetate consumption. The microbial community analysis indicated an expansion of the *Firmicutes* over time in both mixed batch experiments. An increase in the relative abundance of the *Deltaproteobacteria* was observed in the first half of the experiment when augmented with AQDS, compared to experiments absent of AQDS. Conversely, a greater increase in the relative abundance of *Bacteroidetes* was observed in the first half of the experiment for the batch not containing AQDS.

Current research is focused on determining how Fe(III) oxide mineralogy and the presence of 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, microbial community, and biogeochemical dynamics with populations from other DOE field sites under advective flow conditions.



## Abiotic Reduction of Uranium by Fe(II) in Soil

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

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The current DOE strategy for treatment of radionuclide and heavy metal contamination in the subsurface relies heavily on *in situ* immobilization and stabilization. It is well-established that hexavalent uranium (U(VI)) can be enzymatically reduced by a variety of microbial species, as well as abiotically reduced by many chemically synthesized Fe(II) containing Fe minerals. Despite the prevalence of Fe(II) under conditions where U(VI) is reduced, most studies to date have found little evidence for significant abiotic reduction of U(VI) by Fe(II) in pasteurized or sterilized natural materials. Here, we present evidence for U(VI) immobilization and reduction by Fe(II) present in a pasteurized, redoximorphic Iowa soil. We use data from U LIII-edge x-ray absorption spectroscopy (XAS) to confirm U(VI) reduction and <sup>57</sup>Fe-Mössbauer spectroscopy to confirm oxidation of the soil Fe(II). Soil characterization based on results from chemical extractions, Fe K-edge XAS, and Mössbauer spectroscopy indicate that the reactive Fe(II) phase is structural in nature and may be a green-rust like phase. Our work highlights the importance that abiotic reduction of U(VI) by structural Fe(II) may play in anaerobic environments.

## Uranium (VI) Interactions with Iron(II)-Bearing Minerals

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

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SBR-funded research has already significantly advanced our understanding of many of the basic microbial and geochemical processes affecting the speciation and distribution of U in subsurface environments. In particular, the reduction of U(VI) to U(IV) has been shown to dramatically affect the fate and transport of U under reducing conditions. Nevertheless, much remains to be learned about how highly coupled biotic-abiotic processes, such as the biogeochemical cycling of Fe and S, control the rate and extent of U transformations. Although many microorganisms can enzymatically reduce U(VI) to U(IV), U(VI) can also be reduced by reductants directly or indirectly formed by the metabolic processes of facultative and strict anaerobes, particularly iron- and sulfate-reducing bacteria. For example, the reduction of Fe(III) oxides by iron-reducing bacteria can result in the formation of various Fe(II) species, including soluble Fe(II) complexes, Fe(II) complexes with the surfaces of organic and inorganic solid phases—and a host of mineral phases containing structural Fe(II), including magnetite, siderite, vivianite, green rusts, ferrous hydroxy carbonate, and (under sulfate-reducing conditions) ferrous sulfide species, including mackinawite, greigite, pyrrhotite, and pyrite. The reactivity of Fe(II) species is highly dependent on speciation; therefore, we are evaluating the reactivity of specific Fe(II) species with U(VI) to gain insight into the potential for Fe(II) species to affect U(VI) fate and transport in subsurface environments.

A comparison of biogenic Fe(II) phases formed from the bioreduction of Fe(III) oxides revealed dramatic differences in U(VI) uptake and reduction. Solution-phase U(VI) concentrations decreased from 500  $\mu\text{M}$  to 1.5  $\mu\text{M}$ , 392  $\mu\text{M}$ , and 472  $\mu\text{M}$  in the presence of biogenic green rust, magnetite, and siderite, respectively. Analysis of the chemical speciation of U associated with the solids by U  $L_{III}$ -edge extended x-ray absorption fine structure spectroscopy (EXAFS) indicated that despite a stoichiometric excess of Fe(II), no more than 6% of U(VI) was reduced to U(IV) by biogenic siderite, and no more than 22% of U(VI) was reduced in the biogenic magnetite system. For comparison, in the green rust system, >99% of U(VI) was reduced to U(IV). Uptake of U(VI) by biogenic green rust and magnetite was accompanied by formation of nanoparticulate uraninite. There was no indication of uraninite formation in the presence of biogenic siderite. Instead, our results indicate that  $\text{U}^{6+}$  (and perhaps  $\text{U}^{4+}$ ) can substitute for  $\text{Fe}^{2+}$  in siderite, potentially providing another mechanism for U sequestration under Fe(III)-reducing conditions. The differential reactivity of biogenic Fe(II) phases and the corresponding implications for U speciation highlight the importance of understanding the processes leading to formation of specific Fe(II) phases in identifying the processes controlling the fate and transport of contaminants in subsurface environments.

Current and future work is focused on the kinetics and mechanisms of U(VI) interactions with non-stoichiometric magnetites, ferrous sulfide phases, siderite, vivianite, bioreduced whole sediments and natural iron oxides, and Fe(II) sorbed to common mineral phases.

## Amendment Transport, Mineral Precipitation, and Contaminant Immobilization in Subsurface Systems: An Overview of the Idaho National Laboratory Scientific Focus Area

INL SFA (Laboratory Research Manager: Mark Ankeny; Technical Lead: George Redden)

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The broad objective of the Scientific Focus Area at Idaho National Laboratory is to understand, and reveal methods to control, mineral precipitation reaction fronts in subsurface environments. Challenging topics that converge under the SFA include: the nature of reactant mixing in porous media; kinetically controlled reactions in reactive transport; important differences between dispersion and diffusion in the context of multicomponent reactions; coupling between reactions and changes in media properties that affect reactant transport and mixing; approaches for modeling dynamic behavior in porous systems that require volume averaging at some scales; and testing of geophysical methods for detecting and monitoring reaction fronts and changes in the temporal and spatial distribution of reaction products in porous media. In particular, the SFA is attuned to the study of early-stage transient events that are affected by the structure of mixing zones, and that in turn determine outcomes at later stages in engineered systems. The goal is to provide understanding to support the engineered formation of minerals such as calcium carbonates or phosphates that can immobilize metal contaminants (e.g.,  $^{90}\text{Sr}$ ).

The SFA is organized around a series of five experimental campaigns, each based on different approaches through which reactants involved in multicomponent reactions (mineral precipitation) can be mixed in porous or fractured media. Each mixing approach is characterized by different roles for dispersion and diffusion of reactants, and different ways in which reactions, reactant transport, and changes in media properties are coupled. We are currently focusing on an approach that involves *in situ* generation of one reactant at the solid-solution interface. This choice was based on opportunities to contribute to ongoing field-scale demonstrations that involve the microbial hydrolysis of urea to produce carbonate, which then can react to form carbonate minerals such as  $\text{CaCO}_3$ . Details of activities associated with the first research campaign are given in accompanying abstracts and presentations.

The reaction model for the first campaign is the urease-driven hydrolysis of urea to form carbonate, ammonia,  $\text{OH}^-$  and calcium carbonate when calcium is available in the solution phase. The kinetics of commercially available jackbean urease have been characterized, both in solution and immobilized on silica gel; immobilized urease is a surrogate for attached microbial ureolytic activity, which is generally considered to dominate over planktonic activity in the subsurface. Modeling platform options were assessed for the purpose of conducting pre-experimental simulations needed for hypothesis development and experimental design. The evaluation included TOUGHReact, STOMP, and a new platform developed at the INL called MOOSE; MOOSE was determined to be most appropriate for simulating kinetically controlled complex reaction networks. Pre-experimental modeling has included diffusion/dispersion control and the ability to couple mineral precipitation to changes in permeability. Column studies with immobilized urease are under way, as are preparations for experiments with 2-D flow cells. In support of current as well as future research campaigns, experimental efforts are also under way to examine phenomena that are common to all of the mixing scenarios: (1) the impact of ion activity ratios (e.g.,  $\text{CO}_3^{2-}:\text{Ca}^{2+}$ ) on precipitation kinetics and co-precipitation of strontium, and (2) the dynamic nature of precipitation zones in the absence of advective transport, using gels and granular media (“double diffusion” experiments). Finally, geophysical methods (electrical) are being evaluated for their ability to track reaction fronts in the experiments, and efforts are also under way to link electrical measurements with pore-scale models of reaction chemistry.

## Dynamics of Mineral Precipitation in Diffusion-Controlled Mixing Zones

*INL SFA (Laboratory Research Manager: Mark Ankeny; Technical Lead: George Redden)*

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Engineering the precipitation of mineral phases in subsurface environments will often involve transient events in multicomponent mixing zones. The near-term distribution of mineral precipitates in mixing zones can influence: (1) the long-term outcomes of reactions in the affected region, and (2) whether models that employ volume averaging at some scales can predict changes in local or volume-averaged properties of a system, such as extent and rates of reactions. Chemical gradients within the mixing zones will determine local reaction rates, and consequent changes in hydrodynamic characteristics of the porous media. To illustrate this point, and in support of the primary experimental campaigns planned for the INL SFA, mineral precipitation dynamics for calcium carbonate and calcium phosphate are being studied using double diffusion experiments and reactive transport modeling. The aim of this study is to investigate the effects of reduction in permeability on: (1) the reactions that can change local chemical conditions, hydrodynamic conditions, and therefore the rate and distribution of precipitation; (2) narrowing (“focusing”) of the precipitation zone; and (3) migration of the precipitation zone due to asymmetry across the mixing zone with respect to precipitation rates and/or local chemical conditions. Three hydrogel media (gelatin, agarose and polyacrylamide) and two boundary solutions (calcium/carbonate and calcium/phosphate) are being used in the experiments as model systems. The mixing interface and position of the precipitation zone are determined by the diffusion of the reacting components, their resulting local concentrations, and particle nucleation/growth kinetics. Results to date appear to show differences in the induction period for precipitation, and the position and migration of the precipitation zone, depending on the type of hydrogel used and reacting components. Experimental results concur with observations from the reactive transport simulations. One interesting observation is the generation of multiple bands in the calcium phosphate system, while only a single precipitation band has been observed in the calcium carbonate system. Precipitation band width and position were found to change with time and to nearly eliminate further reactions between the reactants at the boundaries. Some differences may be due to differences in nucleation kinetics between the gels, which may have implications for developing model simulations for natural media. The spacing between the precipitations bands in the calcium phosphate system appear to be influenced by the hydrogel type used. In experiments with granular media where advective transport is possible (unlike in these experiments), local changes in density and the resulting unstable conditions caused by precipitation can create more complex mixing zones.

## Reactive Transport Modeling of Ureolytic Calcite Precipitation in a Column Using a Parallel, Fully Coupled, Fully Implicit Approach

*INL SFA (Laboratory Research Manager: Mark Ankeny; Technical Lead: George Redden)*

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One potential remediation strategy for trace metal and radionuclide contaminants in the subsurface is to induce the formation of mineral precipitates that immobilize metals by co-precipitation or isolation from the fluid phase. The objective of the INL SFA is to understand how reactants are, or can be, transported and mixed in subsurface environments, in order to control the spatial distribution of mineral phases and their ability to sequester contaminant metals. The design of experiments and interpretation of experimental results depends on model simulations that can represent kinetically controlled precipitation events that are, under some conditions, coupled to changes in porosity and permeability.

The first SFA experimental campaign involves calcium carbonate mineral precipitation that results from urea hydrolysis and carbonate production. This is a model for immobilizing contaminants such as strontium-90 by co-precipitation. Multiple processes, including fluid flow, dispersive/ diffusive transport of reactants, biogeochemical reactions, and changes in porosity-permeability are involved and tightly coupled at multiple scales. Numerical modeling can be used to investigate the nonlinear coupling effects of these processes, which are extremely difficult to explore experimentally. Most existing subsurface reactive transport simulators typically employ a de-coupled solution approach, often referred to as operator-splitting, where transport equations and batch chemistry reactions are solved sequentially. However, such an approach has limited applicability for many biogeochemical systems with fast kinetics and strong medium property-reaction interactions, such as the ureolytically driven calcite precipitation scenario noted above. We have developed a massively parallel, fully coupled, fully implicit reactive transport simulator (RAT) based on a parallel multi-physics object-oriented simulation framework (MOOSE) developed at the Idaho National Laboratory. Within this simulator, the system of transport and reaction equations is solved simultaneously in a fully coupled, fully implicit manner, using the Jacobian Free Newton-Krylov (JFNK) method with additional advanced computing capabilities, such as (1) physics-based preconditioning for solution convergence acceleration, (2) massively parallel computing and scalability and (3) adaptive mesh refinements for 2D and 3D structured and unstructured mesh. The simulator was first tested against analytical solutions, then applied to model reactive transport in a one-dimensional column where conditions that favor calcium carbonate precipitation are generated by urea hydrolysis catalyzed by urease enzyme. The modeling results were used to guide the design of physical experiments conducted within the INL SFA. The RAT simulation results for the spatial and temporal distributions of precipitates, reaction rates, and major species in the system (and also for changes in porosity and permeability) were compared to both laboratory experimental data and computational results obtained using the reactive transport simulator STOMP. The comparisons clearly demonstrate the advantages of fully coupled approaches for accurate simulation of processes such as engineered mineral precipitation in the subsurface.

## Geophysical Sensing of Reaction Fronts Associated with *In Situ* Mineral Precipitation

*INL SFA (Laboratory Research Manager: Mark Ankeny; Technical Lead: George Redden)*

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The high-level objective of the INL Scientific Focus Area is to develop the scientific understanding required for field application of subsurface remediation strategies based on engineered mineral precipitation. In order to monitor and eventually to control the evolution of precipitation fronts, tools that provide near-time information on the spatiotemporal evolution of subsurface systems are needed. The objective of the geophysical sensing component of the INL SFA is to develop and enhance geophysical monitoring methods, such that they can provide information on this evolution with the spatial and temporal resolution required for effective, real-time decision making during remedial efforts. Given the sensitivity of spectral induced polarization (SIP) methods to both fluid chemistry and surface properties, we are particularly focused on developing and enhancing SIP methods.

Enhancing the capabilities of SIP to provide information on the evolution of subsurface processes requires the capability to investigate realistic field-based scenarios. While validation efforts are possible in laboratory settings, realistic field-based investigations of spatiotemporal resolution cannot be conducted in the laboratory due to the difficulty in reproducing field-realistic boundary conditions. Field-based scenarios can be investigated synthetically with the appropriate modeling and inversion capabilities. Our efforts to date have focused on developing this capability through a high-performance SIP modeling and inversion code, and coupling this code with the reactive transport model (RAT) developed as part of the SFA modeling effort. Ultimately, these coupled codes provide a framework whereby a variety of field-based monitoring scenarios may be investigated. Leveraging petrophysical relationships developed during the experimental campaigns, the reactive transport code will simulate time-lapse changes in electrical properties based on corresponding changes in chemical and physical properties. These simulated changes in electrical properties will then be used to investigate SIP imaging capabilities under a variety of field scenarios. The tight coupling between reactive transport and SIP codes also gives added value to the experimental campaigns, as these can be used to validate each other under a variety of conditions, providing independent measures of success.

## Urea Hydrolysis and Calcium/Strontium Carbonate Precipitation in a Homogeneous Media System with Flow

INL SFA (Laboratory Research Manager: Mark Ankeny; Technical Lead: George Redden)

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The first major experimental campaign for the INL SFA is focused on a mineral precipitation approach that involves *in situ* generation of one reactant at the solid-solution interface. The model system chosen for study is the enzyme (urease) catalyzed hydrolysis of urea to form carbonate ions in a background solution containing calcium. This particular approach for inducing calcium carbonate precipitation is currently the subject of SBR-funded field studies. The proposition is that trace metal or radionuclide contaminants such as strontium-90 can be immobilized by co-precipitation with calcium carbonate phases such as calcite or aragonite. The same process has also been promoted as a method for increasing the geotechnical strength of soils and sediments. The ability to immobilize a metal contaminant using *in situ* mineral precipitation, and the mechanism by which a contaminant is immobilized, can be affected by changes in porosity and permeability associated with the precipitation reactions, and therefore reactant transport (mixing) and reactions are coupled to changes in media properties.

As prelude to 2-D and 3-D flow cell experiments with heterogeneous media, a quasi 1-D column experiment was conducted in a microporous silica gel medium. A commercially available jackbean urease enzyme was immobilized within a defined interval of the column, and urea+calcium+strontium solutions were injected, to determine where (and whether) calcium carbonate forms along the flow path, and whether strontium is immobilized. Two alternate hypotheses guided the experimental plan:

1. If the reaction rate for calcium carbonate precipitation is slow relative to the rate of carbonate ion production and transport, calcium carbonate will accumulate in a narrow region downstream of the head of the enzyme zone and eventually reduce permeability. Under constant head conditions, a steady reduction in flow will result from decreasing permeability, and the zone of precipitation will be extended upstream.
2. If the mineral precipitation reaction rate is fast relative to carbonate ion formation and transport, calcium carbonate will initially form near the head of the immobilized enzyme zone. Mineral formation on the solid media surface will reduce the net enzyme reaction, and the reaction zone for urea hydrolysis and  $\text{CaCO}_3$  precipitation will be extended downstream without a large reduction in permeability.

Pre-experimental model simulations were conducted using both STOMP and a multi-physics reactive transport code developed at INL. Enzyme kinetics were characterized for use in model simulations and to determine or verify enzyme activity levels in the column. The column was constructed with sampling ports to measure changes in the chemistry profile along the column (and within the urease zone) and electrode ports for electrical geophysical measurements to be correlated to changes in the chemistry profile.

The first column experiments provided results that can be compared to the preliminary model simulations for the purpose of interpretation and further model development. Chemical (e.g., pH) gradients formed that correspond to expectations and modeling predictions for urea hydrolysis and calcium carbonate precipitation system. Permeability of the column was dramatically reduced (supporting Hypothesis 1 above), and evidence for a precipitation zone that started downstream but gradually migrated upstream was observed. The pH buffering capacity of the silica gel media was slowly exceeded, resulting in a high pH zone that was initially limited to a portion of the enzyme zone, but eventually extended the length of the column.

Variations involving changes in flow, initial conditions, and injectate concentrations will follow, with continued integration of the multiscale geophysical and reactive transport modeling components of the SFA. The results will be used to support the design of larger 2-D flow cell experiments with constructed permeability heterogeneities.

## Overview of the LBNL Sustainable Systems SFA

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

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The overarching premise of our *Sustainable Systems SFA* is that improvements in understanding how hydrological, biological, and geochemical processes and their couplings impact larger-scale system behavior are needed to guide sustainable environmental stewardship of subsurface resources and problems. The “Systems” term emphasizes the need to focus on the integrated and macroscopic manifestation of smaller scale component behavior. The “Sustainable” term recognizes that to be effective over stewardship time frames, subsurface strategies (such as active remediation or natural attenuation of metals and radionuclides) must be compatible with hydrological and biogeochemical conditions that prevail in their immediate environment. The SFA is composed of the following three Challenges, each of which is being advanced through multidisciplinary team-based research:

1. The “Unraveling Biogeochemical Pathways” Challenge focuses on quantifying critical and interrelated microbial metabolic and geochemical mechanisms associated with chromium *in situ* reductive immobilization and reoxidation, from the molecular to the pore scale, as needed to assess long-term sustainability of chromium bioremediation. The four hypotheses associated with this Challenge focus on: the dominant reduction pathway (direct versus indirect), the rate and extent of Cr(III) reoxidation, the retention of organic carbon in the aquifer, and the role of hydrogeological formation on redox stratification and Cr mobility. This Challenge is being carried out through interrelated tasks using biomolecular, spectroscopic, isotopic, and reactive transport modeling approaches, and is being performed 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 how biogeochemical transformations modify the connectivity of the pore structure; how those pore-scale changes impact flow dynamics at the larger scale in porous media; discovery of diagnostic (isotopic and geophysical) signatures that are diagnostic of critical system transitions; and the impact of the feedbacks on overall system response to the introduced treatment. The three hypotheses associated with this Challenge are being carried out using integrated tasks using synchrotron, isotopic, geophysical, and reactive transport modeling approaches. This Challenge is aligned with and leverages on the Rifle IFRC team field experiments that are being conducted at the uranium-contaminated Rifle, Colorado.
3. The “Predicting Contaminant Mobility at the Plume Scale” Challenge is motivated by the recognition that predictions of contaminant evolution, migration, and remediation efficacy at the plume scale often fail because of the great simplifications that are typically made in the representation of subsurface heterogeneity and coupled hydrobiogeochemical processes. The Challenge strives to tractably identify spatially variable precipitation/sorption behavior as a function of “reactive facies” using combined laboratory and field experimentation, and uses mechanistic reactive transport models to identify the minimal but necessary level of detail that is required for adequate plume-scale predictions. The research is being carried in collaboration with EM-supported SRNL scientists at the Savannah River Site F-Area, where natural attenuation is a desired closure strategy.

These three SFA Challenges explore the linkages between fundamental processes and overall system responses, from molecular to field scales. An Exploratory Project avenue also exists within the SFA to solicit new investigators and new ideas. The Sustainable Systems SFA is facilitating the development of system-behavior insights relevant to a variety of DOE environmental stewardship areas, with a particular focus on environmental remediation of metals and radionuclides.



## Using Biomolecular Signatures to Elucidate Biogeochemical Processes Associated with Cr(VI) Reduction at the Hanford 100H Site

LBNL SFA (Laboratory Research Manager: Susan Hubbard)

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We are developing the use of biomolecular signatures as part of the LBNL SFA challenge “Unraveling Biogeochemical Reaction Networks Mediating Sustained Chromium Reduction,” which focuses on *in situ* reductive immobilization of Cr at DOE’s Hanford 100H site. There are two primary research components of biomolecular signatures in this challenge: (a) biomolecular signature discovery, which involves development and testing of meta-transcriptome-based gene expression microarrays, and (b) quantifying biomolecular signatures, which involves assessing relationships between microbial metabolic activity and specific biomolecular signatures. Our biomolecular signature work is being integrated into an interdisciplinary effort involving spectroscopic, isotopic, and reactive transport modeling approaches.

**Meta-transcriptome-based gene expression microarrays:** We are developing a high-throughput approach that uses the meta-transcriptome to design high-density oligonucleotide microarrays, which can be used to identify highly expressed genes in a specific community under conditions of interest, without requiring any *a priori* sequence information or hypotheses about which genes the community might be expressing. To generate initial meta-transcriptome samples, we inoculated anaerobic microcosms with groundwater from the Cr-contaminated Hanford 100H site and supplemented them with lactate and electron acceptors present at the site, namely, nitrate, sulfate, and Fe(III). The microcosms progressed successively through various electron-accepting conditions. Cr(VI) was rapidly reduced initially and again upon further Cr(VI) amendments. To date, samples representing denitrifying and fermentative/sulfate-reducing conditions have been sequenced using 454 Titanium technology. From four libraries, >130K reads were obtained totaling >32 Mb of sequence with mean length of ~250 bp. Of the non-rRNA related reads for the denitrifying sample (which was also actively reducing chromate), ~8% were associated with denitrification and ~0.9% were associated with chromate resistance/transport, in contrast to the fermentative/sulfate-reducing sample (in which chromate had already been reduced), which had zero reads associated with either of these categories but many hypothetical proteins associated with sulfate-reducing bacteria. Ongoing work includes further sequence analysis, improvement of rRNA removal methods, and sequencing of more microcosm samples and additional samples from flow-through columns constructed with Hanford 100H aquifer sediments.

**Activity-signature relationships and physiological studies:** Research on metabolic activity-biomolecular signature correlations has been conducted with two bacterial species isolated from Hanford 100H groundwater: strain RCH1 (a sulfate-reducing bacterium similar to *Desulfovibrio vulgaris* Hildenborough) and strain RCH2 (a denitrifying strain similar to *Pseudomonas stutzeri*). Cell suspension studies with strain RCH2 and lactate demonstrated that Cr(VI) reduction could occur under either denitrifying or aerobic conditions (at comparable rates), and that reduction was much more rapid when the terminal electron acceptor (i.e., nitrate or O<sub>2</sub>) was present. It appears that, under both aerobic and denitrifying conditions, the chromate reductase gene(s) are not inducible by Cr. Continuous culture (chemostat) studies showed strong correlations ( $r^2$  values > 0.93) between denitrification rate and either *nirS* or *narG* transcript copy number, suggesting that monitoring expression of such genes in groundwater could provide valuable information on *in situ* biogeochemical dynamics. Chromium isotopic fractionation experiments with strain RCH2 demonstrated fractionation for Cr(VI) reduction under aerobic conditions (~2 per mil), but no significant fractionation under denitrifying conditions, possibly suggesting different reduction mechanisms under aerobic versus denitrifying conditions. Cell suspension studies with sulfate-reducing strain RCH1 showed that it can reduce Cr(VI) enzymatically just as rapidly in the absence of hydrogen sulfide as in its presence; this is an important result for distinguishing abiotic reduction from enzymatic reduction. Also, unlike strain RCH2, strain RCH1 does not require that the terminal electron acceptor (in this case, sulfate) be present to reduce Cr(VI).

## Using Cr Isotopic Measurements Together with Reactive Transport Modeling to Monitor Stimulated Bio-Containment at the 100H Test Site, Hanford, Washington

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

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The isotopic composition of Cr can be fractionated during reduction from Cr(VI) to Cr(III) and so has the potential to be used as a monitor of hexavalent Cr reduction. This would then provide a direct signature of Cr(VI) reduction, distinguishable from simple attenuation by dilution. To fully exploit Cr isotopic measurements of field samples from remediation projects, the effects of open system behavior, and complex reaction networks need to be taken into account. Here, we present reactive transport modeling and Cr isotopic data for a field demonstration experiment of a slow-release polylactate amendment (HRC<sup>TM</sup>, Regenesys, Ltd.). The HRC<sup>TM</sup> was injected into a groundwater Cr(VI) plume in the 100H area at the Hanford Site to stimulate bacterial activity to produce conditions promoting the reduction of dissolved Cr(VI) to insoluble Cr(III) complexes. During the course of the experiment (August 2004 to present), groundwater samples were collected from the HRC injection well, from multiple depths of three down-gradient wells, and from an up-gradient well. Samples from down-gradient wells have Cr that is isotopically fractionated relative to samples from the up-gradient well, indicating continued Cr(VI) reduction up to 40 months from the time of the initial HRC<sup>TM</sup> injection. With depth in the down-gradient wells, the  $\delta^{53}\text{Cr}$  data outline two separate zones of isotopically fractionated Cr: the deeper larger peak is centered ~1 m above the Hanford/Ringold contact, the shallower peak ~3 m above. Comparing  $\delta^{53}\text{Cr}$  to [Cr(VI)] indicates a range in [Cr(VI)] with depth of 44–84 ppb prior to reduction, de-convolving effects of dilution from Cr(VI) reduction. No Cr isotopic difference is seen between the 100H up-gradient well and a sample from the high Cr(VI) concentration (58,000 ppb) source in the 100D area, suggesting that essentially no Cr(VI) reduction takes place during transport between the 100D area and the 100H experimental site.

A reaction-transport model that was developed to investigate the HRC<sup>TM</sup> promoted reduction of Cr(VI) in the groundwater of the 100H aquifer was extended to include the effects of Cr isotopic ( $^{53}\text{Cr}/^{52}\text{Cr}$ ) fractionation via reduction of Cr(VI) to Cr(III) and precipitation of Cr(OH)<sub>3</sub>. Reduction of Cr(VI) and Cr isotopic fractionation was captured using a mixed thermodynamic-kinetic approach, using modified Gibbs free energies of the reactions while considering a wide range of other aqueous complexes and mineral-water reactions. The model is able to capture simultaneously the observed ranges in Cr(VI) concentration and isotopic composition in the down-gradient wells. With adjustment of the effective surface area of the HRC<sup>TM</sup>, the model can also reproduce the observed long-term (years) Cr(VI) isotopic evolution. The short-term (days to months post-injection) is controlled by fast-reacting lactic acid and glycerol that make up a currently unknown proportion of HRC<sup>TM</sup>.

## Variations in Biomineral Morphology as Imaged by Synchrotron Microtomography: Implications for Pore-Structure Evolution

LBNL SFA (Laboratory Research Manager: Susan Hubbard)

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

Two classes of potentially important biominerals in both artificial and naturally bioreduced regions are  $\text{CaCO}_3$  and U compounds. Using integrated geophysical and synchrotron expertise, we have developed and tested a first-of-a-kind, flow-through, instrumented (electrical, seismic, and pressure) mini-reactor vessel, as well as imaging algorithms required for quantifying the pore-scale changes caused by induced perturbation. The novel micro *in situ* reaction cell (cell size: ~5–7 mm in diameter and ~15 mm in height) has been tested for interrogating the evolution of a few different reaction end-products as a function of initial mineralogy and culture, including the use of *S. pasteurii* for urea hydrolysis in a material comprised of glass beads. This and other recent work shows that the new approach can yield images of precipitates over time and in a noninvasive manner. The recent experiments also indicate that the micromorphology of the calcite precipitation varies, depending on whether it is biotically or abiotically induced, and that a reasonable fraction of microbes associated with biotic experiments survive the x-ray synchrotron energy.

Progress has also been made on understanding the impact of grain morphology on the patterns U precipitation using chemically resolved synchrotron tomography. Application of the dual-energy imaging method to permeable reactive barrier (PRB) bone char apatite material identified variations in U precipitation morphology not resolved by x-ray fluorescence microprobe. Two specific morphologies were identified. Grains with the first pattern contain an outer layer of evenly distributed U that is highest near the exterior of the grain and diminishes quickly with distance from the surface. Grains of the second type contained small semi-spherical precipitates of high U concentration distributed throughout the grain on the edges of pores. While speculative, one potential explanation for the two different patterns is that the first pattern represents nonspecific sorption of U to the apatite surface and/or the formation of uranium phosphate surface precipitates, while the second pattern is consistent with localized precipitation of U as  $\text{UO}_2$ . These results are some of the first direct observations of variations in U precipitate morphology in field materials.

## Reactive Transport Modeling of Biostimulation at the Old Rifle Site: Reaction Pathways and Porosity Reduction

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

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Reactive transport modeling of biostimulation at the Old Rifle site in Colorado focused on understanding the system response of laboratory flowthrough column experiments using Rifle sediment and groundwater spiked with two different concentrations of acetate. The results demonstrate that the abiotic reduction of iron hydroxide by hydrogen sulfide (H<sub>2</sub>S) is an important if previously unrecognized pathway. Based on an electron balance, as much as 95% of the acetate was consumed by Sulfate Reducing Bacteria 20 days before the breakthrough of dissolved sulfide from the column. The “missing sulfide” balances the pool of poorly crystalline iron hydroxide as determined by hydroxylamine extraction. The presence of elemental sulfur, which is a product of this abiotic reaction, was confirmed by HPLC analysis and by XANES spectroscopy. Reactive transport modeling using thermodynamic functions suggested a Gibbs energy threshold (or bioenergetic energy quantum) of  $\leq 4 \text{ k e-mol}^{-1}$  for sulfate reduction. It also suggested that the suppression of the microbial reduction of more crystalline iron oxides could be a thermodynamic effect that disappears once the poorly crystalline pool is depleted.

We also examined the effects of physical and geochemical heterogeneities on the spatial distributions of mineral precipitates and biomass as a result of biostimulation at the Old Rifle UMTRA site in Colorado. Field bromide breakthrough data were used to infer a heterogeneous distribution of hydraulic conductivity through inverse transport modeling, while the solid phase Fe(III) content was determined by assuming a negative correlation with hydraulic conductivity. A defensible reaction network was developed by simulating laboratory column experiments involving Rifle sediment and groundwater. Validated then by field aqueous geochemical data, reactive transport modeling was used to explicitly track biomass growth and to estimate the spatial distribution of precipitates and biomass. The results showed that the maximum mineral precipitation and biomass accumulation occurs in the vicinity of the injection wells, occupying up to 5.4% volume of the pore space, and is dominated by reaction products of sulfate reduction. Accumulation near the injection wells is not strongly affected by heterogeneities present in the system, due to the ubiquitous presence of sulfate in the groundwater. However, accumulation in the down-gradient regions is dominated by the iron-reducing reaction products, whose spatial patterns are strongly controlled by both physical and geochemical heterogeneities. Heterogeneities can lead to localized large accumulation of mineral precipitates and biomass, increasing the possibility of pore clogging. Although ignoring the heterogeneities of the system can lead to adequate prediction of the average behavior of sulfate-reducing related products, it can also lead to an overestimation of the overall accumulation of iron-reducing bacteria, as well as the rate and extent of iron reduction. Surprisingly, the model predicts that the total amount of uranium being reduced in the heterogeneous 2-D system was similar to that in the 1-D homogeneous system, suggesting that the overall uranium bioremediation efficacy may not be significantly affected by the heterogeneities of Fe(III) content in the down-gradient regions. Rather, the characteristics close to the vicinity of the injection wells may be crucial in determining the overall efficacy of uranium bioremediation.

## Geophysical Quantification of Remediation-Induced Biogeochemical Transformations

LBNL SFA (Laboratory Research Manager: Susan Hubbard)

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Part of the LBNL SFA "Evolution of Pore Structure and Flowpath Challenge" focuses on exploring the potential of geophysical methods for quantifying hydrological controls and induced biogeochemical transformations associated with *in situ* bioremediation from column to local field scales. This task will help to address a key Evolution Challenge hypothesis that the cumulative impacts of *in situ* remediation-induced transformations are significant enough to impact flowpaths at the field scale. Our efforts are focused on the Rifle IFRC site, where bioremediation experiments are being conducted within a shallow, unconfined aquifer to immobilize uranium in tailings-contaminated groundwater. Our geophysical objectives associated with this Challenge include: (1) measuring and modeling the geophysical signatures of remediation-induced biogeochemical transformations; (2) developing and testing frameworks that can integrate time-lapse geophysical and geochemical datasets for the estimation of remediation end-products; and (3) quantifying hydrogeological controls and induced biogeochemical transformations at the field scale using our petrophysical insights, estimation frameworks, and Rifle field datasets.

To meet the first objective, we are performing laboratory column experiments to measure and model geophysical signatures of remediation-induced transformations. Through co-collection of geophysical, biogeochemical and hydrological data, we are investigating how the evolution of mineral precipitates (e.g.  $\text{CaCO}_3$ ,  $\text{FeS}$  and  $\text{S}^0$ ) as well as changes in electroactive ions (e.g.  $\text{Fe}^{2+}$  and  $\text{HS}^-$ ), impact these signatures. The suite of experiments will refine our ability to geophysically differentiate between the multiple and often competing processes that occur during biostimulation at the Rifle site. Recent results revealed anomalous complex resistivity (CR) signatures associated with calcite precipitation; experiments are currently under way to explore the geophysical signatures from mixed precipitation of calcite and iron sulfide, as occurs during biostimulation at the Rifle site. The contribution of electroactive ions, such as  $\text{Fe(II)}$ , to the overall CR response is also being investigated, with preliminary lab and field results indicating the importance of such species in mediating the magnitude of the polarization response. To meet the second objective, we have developed a state-space Bayesian framework that permits integration of time-lapse geophysical, geochemical, and other types of datasets with petrophysical relationships in the estimation of biogeochemical parameters. We have applied it to CR data collected during biostimulation column experiments and have illustrated how the CR data and Bayesian approach can be used to quantify the volume fraction of the remediation-induced disseminated precipitates, mean radius of aggregated clusters, and permeability reduction over time. Our results suggest the developed state-space approach permits the use of geophysical datasets for providing quantitative estimates of the evolution of end-products and permeability reduction associated with biogeochemical transformations at the column scale. We are extending the estimation formalism to enable joint consideration of time-lapse, multidimensional geophysical and geochemical field datasets by treating the remediation-induced transformations as a spatially smoothed evolution process that contains temporal structure.

In collaboration with the Rifle IFRC team and to address our third objective, we have assembled an extensive field geophysical database as part of the Winchester, Big Rusty, and Buckskin field experiments at Rifle, including surface spectral induced polarization (SIP) data, tomographic radar and seismic data, and wellbore logs, as well as accompanying hydrological (tracer and wellbore) data. A sequential Bayesian-inversion approach is being used that will honor all key datasets and provide estimates of initial hydrological zonation and changes in flow properties due to the biostimulation at the field scale. Related to this effort, we are developing a deterministic 3D inversion framework for SIP data. This framework is also capable of jointly inverting for EM, electrical, and magneto-telluric data. We demonstrated this framework on SIP data collected in conjunction with bioremediation activities performed as part of the Winchester field experiment.

## Characterization and Simulation of Plume Mobility at the SRS F-Area Using a Reactive Facies Approach

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

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Predictions of contaminant migration to assess remedial alternatives at plume scales often fail because of the simplifying assumptions that are typically made in the representation of heterogeneity, as well as the coupled hydrological and biogeochemical processes taking place within the subsurface. Indeed, contaminants at most polluted sites are subject to a wide range of scale-dependent processes, following complex biogeochemical reaction networks, and affected by feedbacks of heterogeneity and biogeochemical-hydrological processes on flow and transport. Therefore, there is a significant need to develop methods to guide the characterization and prediction of plume mobility accurately, tractably, and over field-relevant scales. To meet this challenge, we are exploring the concept of *reactive facies* to bridge hydrogeochemical measurements at multiple scales towards the predictive understanding of contaminant mobility at the plume scale. We hypothesize that a limited number of sediment groups can (1) individually display unique geochemical and physical characteristics relating to sorption/precipitation processes, and (2) adequately represent the spectrum of geochemical behavior in the subsurface when considered as an ensemble. We then explore whether reactive transport properties and mechanisms can be represented using such reactive facies, capitalizing on the coherent spatial distribution associated with depositional processes and the linkage between hydrological and geochemical properties that often exist in nature.

The reactive facies concept is being tested at the Savannah River F-Area, where contaminant plumes were created by the disposal and leakage of low-level acidic radioactive solutions into seepage basins. Primary focus is given to the natural attenuation of uranium. Geophysical, geochemical, and hydrological data from field investigations, together with laboratory-based geochemical/mineralogical/textural characterization (see poster by J. Wan) are being used to determine if specific reactive facies can be distinguished with confidence and ultimately "mapped" at the field scale. Seismic and radar tomograms have been acquired at two "local-scale" sites, inside and outside the main U plume. The delineation and extrapolation of reactive facies at various scales is also being tested using trend analyses, self-organizing maps, and Bayesian methods. These statistical approaches are being applied to explore links between lithofacies (e.g., mud content) and permeability, integrate borehole data with tomographic information (greater spatial extent), and develop hydraulic conductivity realizations for input into plume-scale modeling. These efforts are being complemented with "data mining" of the historical discharge and groundwater monitoring records, isotopic measurements, and preliminary geochemical and reactive transport simulations to help understand processes at play and constrain boundary conditions, including poorly known bulk discharge rates and compositions from impoundments to the subsurface. Preliminary analysis of the radionuclide data suggests seepage velocities could be faster than previously thought. Isotopic measurements reveal three distinct U contaminant end-members, and a possible U isotopic "stratigraphy" for the aquifer that may be mappable to sediment facies. Mixing simulations indicate that the pH rebound at the site could be significantly retarded by the precipitation of Al hydroxides upon neutralization of the acidic plume with regional groundwater. Ongoing efforts have also been directed towards advancing reactive transport simulation capabilities, notably the development of a parallel simulator (tough+RT) and enhancements to our existing in-house simulator (TOUGHREACT).

## Geochemical Behavior of Uranium in Acidic Waste Plumes at F-Area, Savannah River Site

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

Jiamin Wan (PI), S.S. Hubbard—*LBNL*

The Savannah River Site (SRS) F-area was one of DOE's processing facilities for extracting plutonium (Pu). The low-level radioactive process waste solutions were discharged to a series of seepage basins in the area during the years of 1955–1989. The plumes containing U,  $^{90}\text{Sr}$ ,  $^{129}\text{I}$ , and  $^{99}\text{Tc}$  extend from the vadose zone to groundwater and towards a stream that recharges to the Savannah River. The basins were closed and capped in 1991. Although the site has gone through many years of active remediation, including pump-and-treat remediation and alkaline-solution injection to neutralize the acidic groundwater, the groundwater remains acidic, with pH values as low as 3.2 near the basins, and the concentrations of U,  $^{90}\text{Sr}$ ,  $^{129}\text{I}$  are up to ten times higher than their MCLs. The overall objective of this research is to identify and quantify the major geochemical factors that control the sorption/precipitation and mobility of radionuclides (U,  $^{90}\text{Sr}$  and  $^{129}\text{I}$ ) at the Savannah River Site (SRS) F-Area, in order to assist with the DOE's remediation decisions at the site. Our specific goal at this stage is to characterize borehole sediment samples, providing plume- scale geochemical data for reactive-transport modeling and reactive-facies modeling.

Four vertical boreholes were drilled in August 2008 by the EM-funded project at the F-Area—one located in the background area and three within the plume along the groundwater flow path. Thirty-two borehole samples were collected and characterized. Pore waters were extracted from all the borehole samples by centrifugation. The chemical compositions of the pore waters were measured, including pH, ionic strength, cations, and anions. Uranium concentrations in pore waters, total U in sediments, and extractable U from the sediments (bicarbonate extraction) were measured, and the partitioning coefficients of U (field  $K_d$ ) were determined. To determine adsorption capacities of the sediments, selected borehole sediments from both the background area and plume are being used to conduct batch sorption experiments.

Typical sediments are composed predominantly of fine quartz sand, with  $4.4 \pm 1.1\%$  clay. Kaolinite and goethite are the major minerals of the clay size fraction, residing primarily as coatings of varied thicknesses on the quartz grains, and providing reactive surfaces for contaminant sorption. Goethite occurs as well-crystallized 100-500 nm long needles, commonly in rosette clusters. Micro- to nanoporosity exists within the grain coatings. The coatings are relatively thin, so that the local equilibrium is unlikely to be significantly diffusion-limited. The current plume aqueous phase contains U concentrations up to  $3.39 \mu\text{M}$  (highest measured value). Many samples have U concentrations higher than MCL ( $0.12 \mu\text{M}$ ), and the U concentrations are strongly pH dependent. For the samples of  $\text{pH} > 5$ , the aqueous U concentrations are below the MCL ( $0.12 \mu\text{M}$ ). U(VI) adsorption onto SRS sediments increases sharply as pH increases. The results are supportive of goethite in the SRS sediments playing the dominant role in uptake of U(VI). A surface complexation model will be developed to describe U(VI) adsorption onto these SRS sediments.

## Uranium Immobilization in Oxidizing Environments: Testing Interactions with Vanadate

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

Tetsu Tokunaga (PI), Y. Kim, J. Wan—*LBNL*; S.R. Sutton, M. Newville, Y. Choi, *U. of Chicago*

Over the past several years, a growing body of evidence is indicating that bioreduction-based methods for remediation of most U-contaminated sediments and groundwaters are unsustainable. The high cost of continuing electron-donor supply without a clear closure time, and the remobilization of U upon return to prevailing oxidizing conditions, are obstacles for permanently stabilizing U(IV) in most shallow groundwater systems. Therefore, it is important to understand the factors controlling U(VI) concentrations in oxidizing contaminated environments, especially in sediments containing not only high levels of total U, but also elevated levels of other components associated with formation of U solid phases. In this exploratory project, we seek to develop better understanding of these controls on U(VI) concentrations, especially in environments containing elevated levels of other elements from either the contamination process or from remediation efforts. Determining likely concentrations of U in pore waters equilibrating with regional groundwaters is important for obtaining reliable predictions on natural attenuation.

Vanadium is a trace element found to accumulate with U in some environments because of similarities in redox behavior. Understanding interactions of both elements in groundwater and with sediment surfaces is important, because of the very low solubility of some uranyl vanadates, and because both are strongly sorbed on mineral surfaces. Our calculations for vanadate, V(V), and U(VI) in equilibrium with tyuyamunite,  $\text{Ca}(\text{UO}_2)_2\text{V}_2\text{O}_8$ , predict that concentrations of both elements are very low (sub- $\mu\text{M}$ , and below the U MCL) in many neutral to slightly acidic groundwaters. Laboratory tests of calcium uranyl vanadate precipitation are in qualitative agreement with equilibrium predictions. These new results are similar to our previous study demonstrating control of U(VI) concentrations through precipitation of amorphous potassium uranyl vanadate (rather than carnotite,  $\text{K}_2(\text{UO}_2)_2\text{V}_2\text{O}_8$ ). The very low solubilities of both Ca- and K- uranyl vanadates at  $\text{pH} \approx 6$  suggest that their precipitation reactions could be utilized in water treatment and recovery of U and V.

Sorption and mobility of vanadate in the subsurface are also being investigated, with and without U present as a co-contaminant. In calcareous Oak Ridge sediments ( $\text{pH} 7.9$ ), V sorption isotherms are approximately linear up to  $[\text{V(V)}] \approx 20 \mu\text{M}$ , with progressively nonlinear (weaker) sorption at higher concentrations. Nonlinear sorption over broad ranges in a solute's concentration is predicted to result in self-sharpening of its diffusion front during transport through sediments. This self-sharpening front behavior was confirmed for V diffusion into U-contaminated Oak Ridge sediment using micro- X-ray fluorescence mapping to measure V and U concentration profiles. Micro-XANES spectroscopy confirmed that neither V nor U reduction occurred during the diffusion experiment. Even in the absence of V and U reduction, their complex interactions (sorption and precipitation) can impose significant controls on transport through oxidizing environments.



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

*LLNL SFA (Laboratory Research Manager: Annie Kersting)*

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This new SFA program is designed to better understand the biogeochemical processes that control plutonium transport at environmentally low concentrations and field scales. The major challenge in predicting the mobility and transport of plutonium (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. Most laboratory experiments have been performed at Pu concentrations orders of magnitude higher than those observed in the field ( $10^{-9}$  mol/L compared to  $10^{-12}$ – $10^{-16}$  mol/L observed at DOE sites).

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. We are conducting laboratory experiments on colloids at environmentally relevant concentrations, using state-of-the-art facilities at LLNL such as the accelerator mass spectrometer (AMS), a NuPlasma HR IsoProbe mass spectrometer (MC-ICPMS), the Cs-corrected Titan Transmission Electron Microscope (SuperSTEM), and the nano-secondary ion mass spectrometer (NanoSIMS). Controlled experiments in the laboratory are coupled with characterization of samples collected from known Pu-contaminated field sites—the Nevada Test Site, Mayak, Russia (in collaboration with S. Kalmykov), and Hanford (in collaboration with A. Felmy).

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
5. Direct and indirect microbial interactions with Pu and colloids.

Initial results and planned experiments will be presented for each of the research elements, including Pu sorption to iron oxide at femto-molar concentrations, Pu–humic acid complexation, and *ab initio* modeling of aqueous Pu(IV) complexes.

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

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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Mercury is a key metal contaminant on the Oak Ridge Reservation (ORR) due to its historic release to soil, sediment and stream environments. Biogeochemical factors controlling inorganic mercury transformation to the more toxic organomercuric species are not well understood at ORR and other contaminated sites globally. The ORNL Science Focus Area program centers around understanding coupled biogeochemical processes that determine net production of methylmercury ( $\text{CH}_3\text{Hg}^+$ ). The main objectives are to (1) elucidate the rates, mechanisms, and controls of abiotic and microbial processes affecting Hg speciation and transformation, (2) resolve the critical Hg precursors that are produced and subsequently methylated, and (3) develop and validate subcellular models to understand the biochemical and biophysical mechanisms of transformation between Hg species and methylmercury.

We take a systems approach, examining processes occurring from the field scale down to the molecular scale. Field studies are focused on establishing the range of geochemical conditions in which critical transformations occur (see poster by Brooks et al.). Our work shows the importance of kinetics in systems that receive a constant source of inorganic mercury input (see poster by Gu et al. and Miller et al., *Environ. Sci. Technol.* 2009, 43, 8548). Both kinetics and speciation data will be critical in examining the cycling of mercury and the production of methylmercury in contaminated systems. These efforts are performed in parallel with, and contribute directly to, complementary studies of abiotic and microbial mercury transformation.

Studies of the mechanisms and geochemical controls on mercury speciation and transformation reveal that natural dissolved organic matter (DOM) plays an important role in the complexation, reactivity and redox transformation of mercury (see poster by Gu et al.). Even at low DOM concentrations (< 3 mg/L), DOM appears to dominate mercury speciation and reactivity in the UEFPC by forming strong Hg(II)-DOM complexes through the reactive thiol functional groups in DOM (Dong et al., 2010, *Environ. Chem.*, in press). The complexation is kinetically hindered. Reduced DOM is also found to be capable of reducing the mercuric ion Hg(II) to Hg(0) and forming strong Hg(0)-DOM complexes. Work is currently in progress to evaluate the potential impact of the formation of such complexes on biological production of toxic methylmercury (see poster by Schaefer et al.) and abiotic demethylation in the environment. Recent results show that Suwannee River NOM did not affect methylmercury production by the known methylator *Desulfovibrio desulfuricans* ND132 (see poster by Brooks et al.).

In our investigations of Hg methylating microbial communities, field samples have been collected from a known methylating area of the contaminated stream at ORR. Microbial community characterization using both a functional gene array (FGA) and 454 amplification and sequencing of 16S genes revealed pronounced phylogenetic and functional differences that appear to be related to seasonal trends (See poster by Elias et al.). With its draft genome sequence, the known methylating sulfate-reducing bacteria, *D. desulfuricans* ND132, is being used to elucidate the genes responsible for mercury methylation (See poster by Kucken et al.). The targeted approach has not yet identified a methylase, and we are in the process of creating a random Tn5 transposon mutant library with an attainable goal of >5000 mutants. We are also designing a second generation FGA that will allow detection of MerA and MerB genes, as demethylation is a key reaction in global mercury cycling.

Molecular, or subcellular, studies include OmcA, a dissimilatory metal reducing enzyme from *Shewanella oneidensis* MR-1, as well as various proteins and enzymes encoded in the *mer* operon, which confers bacterial mercury resistance. We have collected initial X-ray diffraction data to 2.6 Å resolution for OmcA. Small-angle X-ray scattering (SAXS) and molecular dynamics (MD) simulations have been used to characterize the structure and interdomain motions of the metalloregulator, MerR, and the mercuric reductase, MerA (See poster by Liang et al.). Quantum mechanical calculations have been used to show how the organomercurial lyase MerB catalyzes the demethylation of methylmercury (Parks et al., *J. Am. Chem. Soc.* 2009, 131, 13278).

Future work will involve functional genomics techniques to determine key microbial groups that influence methylmercury production under varying geochemical conditions. Investigation of structure and dynamics at the molecular level will reveal regulation mechanisms and the role of various subcellular components. Molecular simulation will be applied to determine key enzymatic mechanisms and extended to elucidate microbial methylation mechanisms identified by advanced genomic techniques. These studies will help in understanding the oxidation-reduction and methylation-demethylation transformations that determine the fate of Hg in sediment-water environments.

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

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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ORNL

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

*Site investigations and source characterization.* Total Hg concentrations decrease while MeHg concentrations increase with increasing downstream distance. Greater than 90% of the Hg in streambanks, streambed sediments, gravel, and biofilms is extracted only with relatively aggressive chemical extragents (12 M HNO<sub>3</sub>, aqua regia). The majority of the MeHg is associated with biofilm or suspended solids, becoming more pronounced with increasing distance downstream. In the upper reaches of the creek Hg concentration increases with sediment depth; dissolved Hg(0) approaches saturation with metallic Hg. Surface water rapidly exchanges water with the hyporheic zone to depths  $\geq 70$  cm delivering a significant load of Hg to the surface water of the creek (see below). Other geochemical parameters are indicative of microbial activity – NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup> decrease, Mn increases, with increasing depth. Total sulfide concentration ranged up to 100  $\mu$ g/L while dissolved sulfide was less than 3  $\mu$ g/L. These results are consistent with an active sulfate-reducing community; sulfate reducers are dominant microbes responsible for Hg methylation. To the extent that dissolved sulfide plays an important role in Hg methylation, the low dissolved sulfide concentrations may be one factor to explain why MeHg concentrations at this site remain low in the presence of the highest total and dissolved Hg concentrations in the creek.

To better understand the role of buried metallic Hg as a contaminant source, studies were conducted to measure Hg dissolution. Water containing traces of residual chlorine contacting metallic mercury, as could occur with the discharge of chlorinated cooling water or potable water leaks, greatly accelerates dissolution. The measured oxidative dissolution rate at the surface of an Hg bead in water without residual chlorine was 0.5 ng/cm<sup>2</sup>/h, but increased to 17,000 ng/cm<sup>2</sup>/h in the presence of residual chlorine concentration typical of potable water.

*Methylation bioassay using pure cultures of methylating bacteria.* Cultures of *Desulfovibrio desulfuricans* ND132 grown on a pyruvate/ fumarate media without sulfate were inoculated into bottles containing inorganic Hg or Hg plus Suwanee River NOM (SRNOM). Sacrificial samples were collected at various times during the growth curve for quantification of MeHg, filter-passing (0.2  $\mu$ m) and total Hg, Hg sorbed to bottle walls, organic acids, and cell enumeration. ND132 incompletely oxidized pyruvate to acetate and reduced fumarate with stoichiometric production of succinate. Methyl mercury production was not influenced by the presence of SRNOM at any sampling time. MeHg increased from 0.058 picograms MeHg per 10<sup>6</sup> cells (pg/Mcell) at mid-log phase to 0.09 pg/Mcell at late stationary phase. When Hg or Hg-SRNOM was added at late stationary phase, MeHg production increased  $\sim 4\times$  (0.35 pg/Mcell). No MeHg was produced from cell-free spent media harvested at this stage, suggesting that ND132 in this stressed condition is a more active methylator.

Future efforts will include *Desulfobulbus propionicus* (identified in our previous survey of multiple local creeks and sites; see microbial study of the SFA) or related isolates from EFPC in experiments, and targeted experiments to identify the role of sulfate reducers in MeHg production in EFPC.

## Mercury Methylation: Genes and Communities Involved in Hg Transformations (Hg SFA at ORNL, Microbial Genetic Study Task)

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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In microbial transformation and genetic studies, we have examined the genes and microbial communities involved in mercury methylation in streams contaminated as a result of 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 by sampling and characterizing Hg-contaminated streams and background sites. Water and sediment samples from transects of a methylating area of the stream were characterized geochemically. They were analyzed for the community complement of microorganisms using both a functional gene array (FGA) and phylogenetically via 454 amplification and sequencing of the V4 region of 16S genes from the total community. 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), and (4) specifically, the number of *Deltaproteobacteria* (the group involved in methylation) will positively correlate with MeHg concentrations. Analysis of 60 samples as above revealed pronounced phylogenetic and functional differences that appear to be 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 as determined by RDA. 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. Hence, enrichment and isolation performed using propionate and sulfate has resulted in a pure culture that morphologically resembles this type strain. The metabolic and Hg methylation/demethylation characteristics are currently being assessed. We are also designing a second generation FGA with many known *mer* gene sequences, along with consensus qPCR primers, for testing the presence of *merA* and *merB* in field samples.

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

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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As part of the ORNL Science Focus Area (SFA), this research is aimed at fundamental understanding of the mechanisms and geochemical controls on the speciation and transformation of mercuric ion  $\text{Hg(II)}$ , elemental  $\text{Hg(0)}$ , and methylmercury ( $\text{CH}_3\text{Hg}^+$ ) at the Upper East Fork Poplar Creek (UEFPC) site in Oak Ridge, Tennessee. Our initial focus is centered on the effects of particulates and dissolved organic matter (DOM), which have been shown to play a dominant role in the complexation, redox chemistry, reactivity, and transformation of mercury and  $\text{CH}_3\text{Hg}^+$  in aquatic systems.

Using a modified ion exchange technique and geochemical speciation modeling, we show that, even at relatively low concentrations ( $< 3 \text{ mg/L}$ ), DOM determines  $\text{Hg(II)}$  speciation at equilibrium by forming strong  $\text{Hg-DOM}$  or  $\text{CH}_3\text{Hg-DOM}$  complexes through the reactive sulfur or thiol functional groups in DOM. Studies using reducible  $\text{Hg(II)}$  titration and  $\text{C}_{18}$  solid-phase extractions on waters collected from UEFPC and simulated DOM solutions demonstrate that the interaction of  $\text{Hg(II)}$  with DOM is kinetically hindered. The strength and reactivity of the  $\text{Hg(II)-NOM}$  complex also change over time. The high fraction of reducible  $\text{Hg(II)}$  ( $\text{Hg}_R$ ) ( $>90\%$ ) at the headwaters of UEFPC and the persistence of  $\text{Hg}_R$  in the upper 2.5 km of the creek suggest that the slow formation of  $\text{Hg(II)-DOM}$  complexes is important in the geochemical cycling of mercury, and that equilibrium models cannot be assumed in predicting the complexation and speciation of  $\text{Hg(II)}$  in this contaminated ecosystem. Furthermore, studies of the effect of DOM on the redox transformation of mercury reveal that reduced DOM, particularly the high molecular weight humic acid (HA), is capable of rapidly reducing the ionic  $\text{Hg(II)}$  to elemental  $\text{Hg(0)}$  under anaerobic conditions. More importantly, the reduced humics are found to form strong complexes with  $\text{Hg(0)}$  with an estimated partitioning coefficient up to  $10^6 \text{ mL/g}$  and a binding capacity up to  $3.5 \text{ mmol Hg(0)/g HA}$ . These findings suggest that, apart from strong complexation between  $\text{Hg(II)}$  and DOM,  $\text{Hg(0)-DOM}$  complexes are likely prevalent in anoxic sediments and water columns, and constitute an important yet unexplored pool of Hg, which could potentially impact biological production of toxic methylmercury and/or abiotic demethylation under such a reducing environment.

New findings of the nonequilibrium complexation between  $\text{Hg(II)}$  and DOM in the EFPC and the complexation between  $\text{Hg(0)}$  and DOM are significant, because both of these processes could greatly influence the net methylation or demethylation process, which will be the subject of our future studies. Our future work will also attempt to elucidate detailed mechanisms of the complexation through approaches such as competitive ligand interactions, molecular simulation, and detailed characterization of reduced sulfur functional groups in DOM. Furthermore, we will study the interactions between Hg and particulates, a process that could alter the partition and kinetics of  $\text{Hg(II)-DOM}$  complexation and dynamics in the EFPC ecosystem.

## Mercury Methylation: Genetic Determinants of Methylmercury Production in the Sulfate-reducing Bacterium *Desulfovibrio desulfuricans* ND132 (Hg SFA at ORNL, Microbial Genetic Study Task)

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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J.D. Wall, *U. of Missouri*

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). We have focused our attention on the anaerobic DSRB *Desulfovibrio desulfuricans* ND132. This strain was chosen for its high methylation rate and phylogenetic similarity to the lost *Desulfovibrio desulfuricans* LS, for which methylation pathways were partially defined (Choi et al. 1994). Strain ND132 was isolated from estuarine mid-Chesapeake Bay bottom sediments where measured MeHg production rates are high. Methylmercury production by ND132 depends on Hg concentration and complexation in the culture medium. Methylation rates by ND132 are strongly dependent on the Hg concentration and medium chemistry, but under optimal conditions in the absence of sulfide ND132 can methylate about 30% of 10 ng/mL HgCl<sub>2</sub> during batch culture growth. We found that small thiol ligands did not alter the amount of MeHg produced by this organism. We propose to establish ND132 as a model organism for mercury methylation and have characterized its physiology and established various molecular approaches to determine potential genes involved in mercury methylation.

ND132 is an incomplete substrate oxidizer that utilizes a narrow range of electron donors and acceptors for respiratory and fermentative growth. It is an estuarine, mesophilic organism, with NaCl and pH optima of 1% and 7.8, respectively. ND132 is one of only two mercury methylating sulfate-reducing bacteria to have its genome sequenced, and we expect a finished genome from JGI within 3-6 months. The ND132 genome is comprised of 3478 candidate protein-encoding genes, 65.2% G+C, has a total size of 3.8Mb and is currently at 45 contigs. To elucidate the genes responsible for mercury methylation, we isolated potential ND132 methyltransferase genes and transformed each into a nonmethylating strain of *Desulfovibrio*, and then measured MeHg production. This targeted approach has not yet identified a methylase; therefore, we have initiated the creation of a random Tn5 transposon mutant library with an attainable goal of >5000 mutants. Currently, we have over 1300 individual mutants ready for assay. Transposon mutants will be tested for lack of MeHg production, and a collection of nonmethylating mutants will be sequenced for identification of gene interruptions. Development of a high-throughput assay to identify non-methylating clones is also under way.

## Molecular Structure and Dynamics of Mercury Biotransformations

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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As a part of the ORNL SFA, this study emphasizes subcellular processes, including transfer of Hg(II) between specific enzyme components as well as enzyme-catalyzed reactions involved in bacterial Hg resistance and methylation. The initial focus has been on the biomolecular structure and solution conformations of several key proteins and enzymes, which confer mercury resistance in bacteria that 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. Previous *in vivo* and *in vitro* biochemical work showed that binding of Hg(II) by MerR induces a significant conformational change that ultimately results in the transcription of the structural genes. Although structures exist for several activated MerR-family regulators, no structure has been determined for MerR or any non-activated MerR-family protein. Small-angle X-ray scattering (SAXS) on apo- and Hg(II)-bound forms of MerR in aqueous solution revealed that specific binding of Hg(II) transforms apo-MerR from a flattened, compact state into an elongated conformation that ultimately initiates transcription by RNA polymerase. Molecular dynamics (MD) simulations were performed on a homology model of MerR-Hg(II) to characterize the conformational dynamics of Hg(II)-MerR. MD revealed large amplitude interdomain motions involving fluctuations on the nanosecond time scale. The nature of these motions suggests a role in propagating allosteric changes from the metal-binding site to the DNA-binding site.

The mercuric reductase MerA catalyzes the reduction of Hg(II) to Hg(0). Each monomer of the MerA homodimer consists of a catalytic core domain, a flexible linker, and an N-terminal domain, NmerA, which acquires Hg(II) and transfers it to an active site in the core homodimer for reduction. Using SAXS and molecular dynamics simulations, we have studied the structure and dynamics of MerA to elucidate the role of its N-terminal domain.

Bacterial reduction of Hg(II) can also occur distinct from *mer* operon activity by a dissimilatory reduction mechanism in metal-reducing bacteria such as *Shewanella* and *Geobacter*. We hypothesize that this process is linked to the activity of outer-membrane multiheme cytochromes. We have isolated and purified the decaheme outer-membrane cytochrome OmcA from *Shewanella oneidensis* MR-1 and determined its solution structure by SAXS. Neutron reflectometry revealed its interaction with hematite surfaces, where it assembles into a monomolecular layer. We have also obtained x-ray diffraction data of OmcA to a resolution of 2.6 Angstrom. Reduction experiments *in vitro* have shown evidence for a direct electron transfer between Hg(II) and OmcA. Ongoing studies focus on the Hg(II) reduction capacity of OmcA *in vivo*.

Future efforts of this SFA Task will address atomic and subcellular mechanisms relevant to mercury biotransformation using structural biology and computer simulations. This work will probe macromolecular dynamics, enzyme reaction pathways, and mechanisms of intracellular mercury transfer to gain a fundamental understanding of biomolecular processes impacting mercury speciation.

## Specific Uptake of Hg-thiol Complexes in *Geobacter Sulfurreducens* as Compared to *Desulfovibrio Desulfuricans* ND132

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 US DOE Oak Ridge Reservation, is the species of Hg(II) available for uptake by methylating bacteria. Little is known about the mechanism of Hg(II) uptake and methylation in bacteria; however, Hg(II) uptake studies with *Geobacter sulfurreducens* suggest the involvement of an unknown facilitated transport mechanism of Hg(II)-thiol species that has greater substrate specificity than observed in the sulfate-reducing bacterium, *Desulfovibrio desulfuricans* ND132. In short-term washed cell assays, *G. sulfurreducens* displayed enhanced Hg(II) uptake and methylation rates of specific Hg-thiol complexes (e.g. Hg-cysteine) at thiol concentrations found in the environment. Cells were able to discriminate between related thiols (penicillamine and cysteine), which vary only on the thiol carbon chain, as observed by the lack of Hg(II) uptake and methylation of Hg-penicillamine complexes. In contrast, changes to the amino group of cysteine, such as its removal (e.g. thioglycolate) or changes in chirality (L- vs D-form), did not appear to affect Hg(II) methylation. Similar to *G. sulfurreducens*, the sulfate-reducing bacterium, *Desulfovibrio desulfuricans* ND132, showed a modest increase in the Hg(II) methylation rate in the presence of cysteine relative to no-thiol or sulfide-added controls. However, the uptake and methylation of these Hg-thiol complexes lacked the specificity displayed by *G. sulfurreducens*. For instance, similar Hg(II) methylation rates were observed regardless of the thiol added (glutathione, penicillamine, or cysteine). These data suggest that a facilitated uptake mechanism for Hg(II)-thiol complexes is present in both sulfate- and iron-reducing bacteria, but with quite different patterns of specificity. As part of the collaborative research with the Science Focus Area (SFA) of the Oak Ridge National Laboratory, our future plan will attempt to determine the specific Hg(II) species that are involved in bacterial uptake, and elucidate possible mechanism(s) of methylation by *G. sulfurreducens* and *Desulfovibrio desulfuricans* ND132.



## 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 for field-scale application. The SFA builds on established areas of PNNL expertise in geochemistry, microbiology, and multiscale modeling. The SFA consists of approximately 15 PNNL investigators and 12 external collaborators with expertise in: (1) microbial ecology, (2) molecular scale mechanisms of microbiologic and geochemical processes, (3) pore-scale experimentation and modeling, (4) reactive transport science, (5) multiscale reactive transport models, and (6) characterizing *in situ* subsurface structures and reactive transport properties. Cohesiveness and integration will be 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 SFA relies strongly on the Environmental Molecular Sciences Laboratory (EMSL) for fundamental science capabilities 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 contains multiple transition zones that are the focus of current and future research, including: (1) a dramatic redox interface, (2) a fluctuating water table, (3) a spatially dynamic zone of groundwater-river mixing within the aquifer, and (4) a hyporheic zone where groundwaters discharge to surface water.

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

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

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

This project focuses on the identification of the changes in the chemical form of plutonium (Pu) at different locations and with depth in the sediments at the Hanford site and how these chemical forms are altered in response to changes in redox conditions. The overall objective is to provide a baseline of knowledge on Pu contamination, reactivity, and the potential for future subsurface migration. Specific activities include: (1) obtaining and archiving samples from field studies of Pu contamination at Hanford, (2) detailed molecular-level characterization of the chemical form of Pu in the sediments, and (3) experimental laboratory studies of the transformations of these different chemical forms in response to changes in redox conditions that could occur at the site.

Sediment samples from two different disposal locations (Z-9 and Z-12 cribs) and as a function of depth have been obtained and archived from the Hanford 200 west area. XAFS characterization of these materials shows that the surface sediments at the Z-9 disposal site are dominated by the presence of  $\text{PuO}_{2+x}$  which was either formed by contact of the acidic waste solutions with the sediments or directly disposed from Z-plant operations. However, as Pu migrated deep into the subsurface, the chemical form of Pu changed, and no longer shows the characteristic Pu-O or Pu-Pu distance characteristic of  $\text{PuO}_{2+x}$ , although the XANES still shows Pu is present as Pu(IV). The surface sediments at the Z-12 disposal site, which did not receive the same acidic waste, also does not show the presence as  $\text{PuO}_{2+x}$ . Instead, Pu appears to be present as Pu(IV) but either in monomeric form or as very disordered solid phase, since there is no Pu-Pu backscatter in the XAFS. Samples of these materials are undergoing further XAFS analysis. In addition, sediment samples from the deep subsurface have been sent to our collaborators at LLNL and elsewhere for analysis by NanoSIMS and other methods, to gain further insight into the chemical speciation of Pu. Studies have also been initiated on the redox transformation of  $\text{PuO}_{2+x}$  with Fe(II), focusing on the influence of the iron reaction products. Specifically, we are examining the changes in  $\text{PuO}_{2+x}$  solubility influenced by the formation of aqueous Fe(III), ferrihydrite, goethite, and hematite. Unraveling the influence of redox processes will be the key to determining the potential for Pu solid phase remobilization into groundwaters.

## Biogeochemical Activities and Microbial Community Diversity Associated with Hanford 300 Area Subsurface Sediments

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

J. Lee (PI), X. Lin, D. Kennedy, A. Pymale, A. Konopka, R. Kukkadapu, J. Zachara, and J. Fredrickson—PNNL;  
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Borehole sediments from the Hanford Integrated Field Challenge (IFRC) site were collected during well installation (August 2008) to probe potential biogeochemical reactions impacting contaminant behavior. The samples included Hanford formation gravels and fine-grained sediments from the Ringold Formation Unit E. In the Hanford 300 Area subsurface, there is a sharp redox transition within the fine-grained Ringold indicated by a color change from tan to blue-green and an associated increase in acid-extractable Fe(II). Three sediment composites (Hanford <5 mm fraction, Ringold oxidized, and Ringold reduced) were used for characterization and microcosm experiments. Electron-acceptor utilization was evaluated by incubating subsurface sediments in synthetic groundwater (SGW1), formulated based on Hanford 300 Area groundwater chemistry, and an organic carbon mixture.

Hanford formation sediment was incubated with sulfate (0.63 mM) and without. Biogenic iron reduction was faster with sulfate than without, suggesting that Fe(III) reduction was facilitated by abiotic reaction with biogenic sulfide. The heat-treated sediments did not show any obvious Fe reduction either with or without sulfate. Ringold oxidized and reduced sediments did not exhibit Fe or sulfate reduction under the specific experimental conditions. Sulfate was reduced in Hanford sediment, and the sediment-associated sulfide accounted for 63% of the initial sulfate-sulfur. Mössbauer analysis was used to characterize iron phases associated with Hanford formation sediments before and after bioreduction in the presence or absence of sulfate. Changes in the spectra following bioreduction were subtle and indicative of a minor decrease in an oxide component. Nitrate was also reduced in all three sediments, albeit at different rates. The reduction of U(VI) and Tc(VII) were also investigated in Hanford sediment microcosms. Microbial U(VI) reduction was observed in the Hanford formation sediments both with and without sulfate, while Ringold oxidized and Ringold reduced sediments showed adsorption of U(VI), but not obvious microbial reduction. From a series of microcosm incubations with U(VI), microbial reduction was not observed above a concentration of 120  $\mu\text{M}$  U(VI), while the lower concentrations of 20 and 60  $\mu\text{M}$  U(VI) were reduced in Hanford formation sediments regardless of organic carbon stimulation although at different rates. There was no detectable Tc(VII) reduction in Ringold oxidized sediment, while Tc(VII) was reduced to Tc(IV) in the organic carbon-stimulated Hanford formation sediments. Pertchnetate reduction experiments using the bioreduced Hanford formation sediments were conducted to probe the redox reactivity of the bioreduced sediments. The reduction rates of Tc(VII) increased with increasing sediment mass [i.e., Fe(II)] concentrations. In general, sediments exhibiting sulfate and Fe(III) reduction exhibited slightly higher rates of Tc(VII) reduction compared to those where only Fe(III) was reduced. It is currently unclear whether sulfides are directly contributing to Tc(VII) reduction.

Dissimilatory sulfite reductase (*dsr*) genes were detected by PCR of DNA extracted from bioreduced Hanford formation sediments (104 d). *Clostridia* and  $\beta$ -*proteobacteria* were commonly detected phylotypes while sulfate-reducing and sulfur-disproportionating were also relatively abundant in the sediment incubated with sulfate. *Symbiobacterium* was common in the sediment incubated without sulfate.  $\delta$ -Proteobacteria were also more common in microcosms without sulfate; *Geobacter* and *Desulfuromonas* were both detected.

In summary, the reduction of nitrate, native Fe(III) and sulfate were readily stimulated in Hanford formation sediments while only nitrate was reduced in Ringold sediment microcosms. These terminal electron accepting processes along with the capacity for U(VI) and Tc(VII) reduction were consistent with the microbial community phylotypes determined by cultivation-independent sequence-based analyses. Additional laboratory and field investigations are under way to evaluate the nature and reactivity of Fe(II) in bioreduced sediments and the *in situ* microbial activities and community structure across the Hanford-Ringold redox transition zone.

## Proteome Analyses of Subsurface Microbial Systems

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

Mary S. Lipton (PI), H. Zhang, M.J. Marshall—PNNL

Proteomic characterization represents a powerful technology to elucidate the relevant biological processes when microbes and the communities they live in interact with metal contaminants in Hanford subsurface sediment and groundwater. In-depth proteomic characterization is currently hampered by several factors including: (a) the lack of genomic sequence from indigenous soil and groundwater communities, (b) the potential diversity and heterogeneity within communities at different depths of the Hanford strata, and (c) the lack of relevant environmental isolates from these materials. To overcome these barriers, we focused on studies with defined systems to build a biological knowledge base to characterize the microbial function(s) important to biogeochemical processes at the site. We also tested new incubation, sample preparation, and sample handling methods that are necessary for the eventual characterization of the microbial communities from the soils and groundwater.

Laboratory culture conditions often poorly reflect the field, and hence the state of cells under natural conditions may be very different from those grown under laboratory conditions. Thus, biological conclusions from laboratory conditions may be biased by this disconnect. We present a novel concept for the development of an *in situ* culture system using electron donor- or electron acceptor-infused biobeads and a semi-permeable containment system housing axenic cultures of a model organism or consortia in an apparatus that permits groundwater to permeate and flow through, while simultaneously preventing the emigration and immigration from the containment system. Cultures would react to the natural groundwater, site-relevant nutrients, temperatures, and gradients in redox chemistries. We anticipate building a prototype culture apparatus this next year, followed by laboratory and field testing with both media and groundwater from the site, and ultimately installation onto the 300 Area well field.

*c*-Type cytochromes play a central role in microbial respiration and the electron-transfer reactions between microbes and minerals. These heme-containing proteins have remained intractable to proteomic characterization mainly because of inability to detect the post translational modifications and the potential low abundance of the heme-containing peptides. In an effort to overcome these issues, we developed a novel enrichment technology that will selectively bind the iron in the heme. Our initial studies have shown that this technology selectively enriched for the heme containing peptides of bovine cytochrome *c* spiked into a protein lysate from human plasma (one of the most complex protein mixtures studied to date). We plan to expand this technology to characterize putative *c*-type cytochromes from *Anaeromyxobacter dehalogenans* 2CP-C. Genomic analysis of this model organism has shown genes predicted to encode a wealth of multiheme *c*-type cytochromes. One protein from this organism possesses 33 C-x-x-C-H motifs that can serve as putative heme binding sites. While it is unclear whether these motifs are bound to heme, this protein represents an excellent opportunity to verify the effectiveness of our enrichment technology. In conjunction with the Shi SFA project, we have expressed a recombinant form of this protein for detection studies in complex protein mixtures. Future work will extend this approach to the characterization of other *c*-type cytochromes from *Anaeromyxobacter*, *Geobacter*, or other model organisms isolated from groundwater or soils at the Hanford site.

Although the proteomic characterization of the microbial communities isolated from the Hanford site is hindered by the lack of genomic sequence information, proteomic characterization of selected microbial isolates can proceed using the genomic information of sequenced organisms that are near neighbors to the isolates, as determined by 16S DNA analysis. In conjunction with the Konopka SFA project, we will determine which genomes are candidates for genomic searches as well as submit organisms for sequencing at the JGI. While this method is not ideal for the characterization of these communities, it will provide some baseline information about the biological processes represented in the community. Subsequent studies will characterize the proteome of relevant Hanford isolates as they become available. To transition from groundwater to sediment communities, we have also developed novel methods for the extraction of microbial proteins from soils after *in situ* lysis.

## Microscopic Reactive Transport and Its Effect on Scale-Dependency of Geochemical and Biogeochemical Reaction Rates in Subsurface Porous Media

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

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The objectives of this research are to: (1) characterize coupled mass transfer/transport and geochemical/biogeochemical reactions in microenvironments within subsurface porous media; (2) investigate the effect of pore- and subpore- scale mass transfer/transport on the apparent rates and the scale-dependency of geochemical and biogeochemical reactions that control the fate and transport of metal and radionuclide contaminants in heterogeneous porous media; and (3) derive scaling relationships of transport and reaction parameters from the molecular to the pore, and from the pore to the continuum scales in porous media.

The scale-dependency of uranyl [U(VI)] desorption rates has been observed in a contaminated sediment containing intragrain U(VI) from Hanford 300A. The apparent rate constants of U(VI) desorption from the sediment varied over three orders of magnitude, as estimated using a continuous scale, macroscopic reactive transport model from the measured effluent chemical compositions in stirred flow-cell, small column, and large column systems. Pore-scale investigation using x-ray tomography showed a complex heterogeneous structure of pore size, geometry, and connectivity in the column systems, even in the column with the ideal macroscopic transport behavior of nonreactive tracers. A lattice Boltzmann (LB) model was developed to evaluate the effect of pore-scale heterogeneity of mass transfer/transport processes on the scale-dependency of U(VI) desorption rates. The LB pore-scale model was constructed based on the macroscopic properties (i.e., porosity, dispersivity, and grain-size distribution) and x-ray tomography measurements of pore structure and grain orientations in the column systems. The grain-scale kinetics of U(VI) desorption determined from the stirred flow-cell system was explicitly incorporated in the LB model to investigate its kinetic manifestation in the column systems. Grain-scale U(VI) desorption kinetics resulted from the coupling of intragrain diffusive mass transfer and local equilibrium surface complexation reactions. Simulations using the pore-scale model showed that the flux-averaged effluent chemical composition in the column systems primarily reflected the aqueous chemical composition in the faster pore-velocity domains, while desorption occurred preferentially in the slower pore-velocity domains, where there was a higher solid-surface-area to pore-volume ratio. The difference in dissolved U(VI) concentrations between the higher and lower pore-velocity domains drove pore-scale mass transport, leading to a complex coupling between the grain-scale desorption kinetics and pore-scale mass transport. This pore-scale reactive mass transport was at the scale below the macroscopic model resolution, and consequently its kinetic effect was lumped into the reaction-rate term within the macroscopic reactive transport model, resulting in the scale dependency of the apparent rate constants for U(VI) desorption. Our results implied that such a pore-scale reactive mass transport will also affect the apparent rates of other geochemical/ biogeochemical reactions in heterogeneous porous media, and consequently require a careful consideration in both experimental investigation and numerical simulations in determining and extrapolating reaction rates.

## Microbial Community Structure, Diversity, and Heterotrophic Activity within the Saturated Zone of the 300 Area, Hanford Site

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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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 and activity, potential functionality, and phylogenetic diversity is a first element in 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). We hypothesize important roles for sediment physical (e.g., pore-size distribution, permeability) and chemical (e.g., organic matter content, electron acceptor type and availability) properties in structuring microbial community composition.

One important effort has been the detailed analysis of 21 sediment samples recovered primarily from well 399-2-25, a well drilled down to the basalt (52 m bgs), hence encompassing the saturated zone and basalt flow-top beneath the 300 Area. To assess phylogenetic diversity, we analyzed ~8000 bacterial and archaeal near-full length 16S rRNA gene sequences 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. We detected 1233 and 147 unique bacterial and archaeal operational taxonomic units (defined as >97% sequence homology). Microbial community structure and richness varied substantially across the different geological strata. A high beta diversity was found in this subsurface, with a distance-decay halving distance of 5.4 m. Bacterial OTU richness was highest (>700) in the smear zone and the upper Hanford formation, and declined to about 120 at the bottom of the Hanford formation. Just above the Ringold oxic-reducing interface, richness was about 325 and dramatically declined to less than 50 in the deeper reduced zones. The bacterial community in the oxic Hanford and Ringold formations contained members of nine major well-recognized phyla, as well as unusually high proportions of three poorly characterized candidate divisions (GAL15, NC10, and SPAM). The deeper strata had ca. 90% *Proteobacteria*, with substantive differences in specific OTUs between samples. The presence of specific phylotypes at particular depths was identified. The smear zone within seasonal fluctuating water table depths contained more diverse phylotypes belonging to *α-proteobacteria*, *Bacteroidetes*, and *Actinobacteria* than other depths. Microbial communities from 1 m below the smear zone contained higher proportions of candidate division NC10 and *Acidobacteria*. The oxic Ringold sediments harbored the broadest set of divisions and were dominated by *Nitrospirae*, GAL15, and *Chloroflexi*. The upper reduced Ringold sediments contained a higher relative abundance of putative denitrifiers, *Ochrobactrum anthropi* and *Achromobacter xylosoxidans*, than any other depth. Distinct archaeal OTUs were also found to reside at different strata, with a dominance of *Thaumarchaeota* in the Hanford formation and oxic Ringold.

Phylogenetic analysis suggested that the microbial community in the saturated zone was primarily organotrophic. Bacterial heterotrophic activity was assessed by <sup>3</sup>H-Leucine assimilation; microbial activity was about three times higher in the oxic Ringold sediment than in the upper Hanford formation. Bioassay experiments amended with combinations of ammonia, phosphate, and organic carbon indicated that organic carbon limited microbial activity in the saturated zone.

In summary, ecological analyses emphasize elevated microbial diversity and substantial heterotrophic activity in the transition zone (vadose/saturated and Ringold oxic/reduced), which will be a focus for future work to determine if microbial redox cycling operates in this region. The study has greatly expanded the intralinear phylogenetic diversity within some major microbial divisions. Furthermore, these subsurface sediments contained substantial proportions of novel microbes (only 6% of bacterial OTUs were closely related to cultivated bacteria). The prevalence of novel lineages at high relative abundance provides unique opportunities to relate their physiological ecology to the geochemistry and sediment mineralogy of the distinct strata in which they occur.

## Visualization of Hydrated Bacterial Structures by Complementary Electron Microscopy Techniques

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

M.J. Marshall (PI), A.C. Dohnalkova, B.W. Arey, J.K. Fredrickson—PNNL; K.H. Williams, LBNL

Electron microscopy (EM) -based imaging and analyses provide excellent details for studying how microbes interact with minerals in the environment. Conventional EM sample processing includes dehydration as a prerequisite for imaging in a vacuum-based EM. This process can cause substantial ultrastructural damage, due to the constriction of delicate structures such as membranes, and can ultimately disrupt the true interaction with mineral surfaces, consequently leading to inaccurate interpretations. In an effort to minimize these artifacts, we have used cryogenic (cryo) sample preparation in which cells are flash-frozen in amorphous (vitrified) ice. The preparation of frozen-hydrated samples is an emerging method for visualizing a biological material in its closest-to-natural, fully hydrated state, since these can be generated and visualized entirely in vitreous ice without dehydration steps. Vitrified samples can be prepared for both cryo-transmission and scanning EM (cryoTEM and cryoSEM, respectively).

Recently, we reported that a highly hydrated extracellular polymeric substance (EPS) produced by dissimilatory metal-reducing bacteria (DMRB) has been observed to bind (or nucleate) nascent nanoparticles of reduced uranium (i.e., uraninite,  $\text{U(IV)O}_2$ ). In *Shewanella oneidensis* MR-1, the  $\text{UO}_2$ -EPS was found to contain polysaccharide, two outer membrane *c*-type cytochromes (OMCs) involved in  $\text{U(VI)}$  reduction, and other integral OM proteins. Although the discovery of  $\text{UO}_2$ -EPS could have major implications on the fate and transport of U in subsurface waters, the massive collapse of EPS that was observed during conventional EM preparations proved problematic in understanding the true structure (and the potential importance) of EPS during the bioremediation of  $\text{U(VI)}$ . Therefore, a better understanding of the EPS produced by *Shewanella*, *Anaeromyxobacter*, and other DMRB may provide insight into other processes, including electron-transfer mechanisms between microbes and minerals, such as iron and manganese (hydr)oxides.

Here we present a novel, interrelated approach of both cryo- and traditional TEM/SEM to elucidate the high-resolution, close-to-natural, hydrated state interactions of bacterial EPS with metals, radionuclides, or even other bacteria. We investigated the correlated use of cryoTEM/SEM as an enhanced visualization method for the fully hydrated, fine-scale interactions of *Shewanella* cells and EPS associated with the newly formed biomineral phases. Samples were imaged in their hydrated state and directly correlated with images from identical samples subjected to dehydration and drying prior to imaging. Our methodology also produced extremely accurate measurements of the region exterior to the cell membrane: the location of the electron-transfer proteins essential for metal reduction. We also employed cryo- and traditional SEM to visualize a mature biofilm produced during biostimulation activities at DOE's Integrated Field Research Challenge (IFRC) site near Rifle, CO, to demonstrate the applicability of these techniques with natural biofilms present during *in situ* biostimulation activities. Our observations with field-relevant biofilms may influence how we perceive a microenvironment such as the pore-scale flow of subsurface waters through soils at contaminated field sites.

This research was performed at the Environmental Molecular Sciences laboratory (EMSL), a national scientific user facility sponsored by the U.S. Department of Energy's (DOE's) Office of Biological and Environmental Research (OBER) and located at Pacific Northwest National Laboratory (PNNL). Financial support was provided through an EMSL Research and Capability Development Proposal.

## Reactions of Tc with Fe(II) and O<sub>2</sub> in Hanford Redox-Sensitive Sediments

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

T. Peretyazhko (PI), J. Zachara, R. Kukkadapu, C. Liu, T. Resch, A. Plymale—PNNL;  
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Technetium-99 (<sup>99</sup>Tc) is a fission product of uranium-235 and plutonium-239, and has been introduced into the Hanford site mainly as a result of disposal of spent nuclear fuel and tank waste leakage. Under oxic conditions, Tc exists as the pertechnetate anion (TcO<sub>4</sub><sup>-</sup>) which is weakly sorbed. Because of its mobility and long half-life ( $t_{1/2}=2.13 \cdot 10^5$  years), Tc is a high-risk contaminant with a potential to migrate to the Columbia River. The reduced form of technetium, Tc(IV), is stable in anoxic environments, and often forms a sparingly soluble precipitate TcO<sub>2</sub>·nH<sub>2</sub>O. Therefore, Tc redox transformations are crucial in controlling its migration in subsurface environments. The objectives of this PNNL SFA research are to (1) investigate abiotic electron-transfer reactions of Fe, O<sub>2</sub>, 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 sediment reduction and/or oxidation; and (3) formulate rate expressions for describing the potentially multi-component redox reaction network.

Technetium(VII) reaction experiments were performed with anoxic sediments collected from Hanford's unconfined aquifer (~18 to 55 m bgs) within the Columbia River corridor. Wet chemical, X-ray diffraction, Mössbauer, X-ray absorption and electron microscopy measurements were performed to identify the ferrous minerals that might control Tc(VII) reduction. The results revealed complex Fe(II) mineralogy, including Fe(II)-smectites, pyrites, magnetites, and still-unidentified Fe(II) discrete phases. Batch experiments with 10 µM Tc(VII) and six different sediments demonstrated that all anoxic sediments reduce Tc(VII), but at different rates. All Tc(VII) was reduced to Tc(IV) and immobilized as TcO<sub>2</sub>·nH<sub>2</sub>O. The residual Tc(IV)-containing sediment suspensions were used in stirred flow reactors to study their reaction with dissolved O<sub>2</sub>. Ongoing experiments reveal that O<sub>2</sub> reacts preferentially [over Tc(IV)], albeit slowly, with sediment Fe(II). Effluent Tc solution is at a nanomolar concentration level and consists of Tc(VII) and up to 5% Tc(IV). The data suggest that both nonoxidative and oxidative dissolution of TcO<sub>2</sub>·nH<sub>2</sub>O occur. Post-reaction solids will be characterized for Fe by x-ray absorption and Mössbauer spectroscopy, and Tc by EXAFS spectroscopy. Kinetic models under development describe the observed redox reactions between the various valence states and mineral forms of Tc, Fe, and O<sub>2</sub> present in this system, with an ultimate goal of predicting Tc behavior in Hanford aquifer sediments.



## Mapping the Iron-Binding Site on the Small Tetraheme Cytochrome: Implications of Soluble Iron Reduction in *Shewanella oneidensis* MR-1

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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*Shewanella oneidensis* MR-1 has the metabolic capacity to grow anaerobically under Fe(III)-reducing conditions, in which it produces a variety of periplasmic *c*-type cytochromes that are thought to be involved in the Fe(III) respiratory process. Among those is the small tetraheme cytochrome (SoSTC) with unknown physiological role, although its homologous form from *Shewanella frigidimarina* has been shown to participate in iron respiration. In this work, biochemical studies were conducted to characterize the iron-binding affinities and identify the iron-binding site on SoSTC. SoSTC has been heterologously expressed in *Escherichia coli* containing heme maturation genes. We use Isothermal Titration Calorimetry (ITC) to measure binding affinities between purified recombinant SoSTC and various Fe (III)-chelates, including Fe (III)-EDTA\*, Fe (III)-NTA\*, and Fe (III)-Citrate. The ITC measurements demonstrate that SoSTC has a preferential binding with Fe (III)-EDTA and Fe(III)-NTA, with dissociation constant ( $K_d$ ) around 2  $\mu$ M over Fe(III)-Citrate with  $K_d$  larger than 1 mM. The iron-binding constants have also been quantitated by iron-dependent transient kinetics using stopped flow. Both ITC measurements and transient kinetics have shown comparable binding affinity values. Interestingly, there is little binding between SoSTC with NTA alone, suggesting that the binding event occurred between the SoSTC and Fe(III)-NTA complex. In contrast with ferric species, ferrous chelates have shown about 10-100 times less binding affinity with SoSTC.

The surface charge of SoSTC is predominantly negative (Leys et al.), and thermodynamic characterization of each heme reveals that Heme I serves as the electron entry gate with heme IV as the electron exit (Paquette et al.). Assuming SoSTC binding with the charged iron complex, we propose that the iron-binding site should consist of charged amino acids. Through chemical modification of amine groups and carboxyl groups on SoStc, using 2,4,6-trinitrobenzenesulfonic acid (TNBS) and 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide (EDC), respectively, we have found that SoStc with carboxyl residues modified to amine groups shows no binding with Fe(III)-EDTA by ITC measurements. Moreover, the second-order rate constant of carboxyl-group modified SoSTC, when reacting with Fe (III)-EDTA, was retarded 50 fold. Carboxyl-group scanning studies were used to probe the putative iron-binding site by site-directed mutagenesis of glutamate and aspartate on SoStc. ITC and stopped flow were then used to characterize the binding between SoStc purified from each mutant with Fe (III)-EDTA. SoStc (D79N) shows no binding with Fe(III)-EDTA measured from calorimetric measurements. The disparity of the binding of SoSTC (D79N) with Fe (III)-EDTA was further confirmed by transient state kinetics of reduced SoSTC (D79N) reacting with Fe (III)-EDTA, which demonstrates an approximate 10-fold decrease in second-order rate constant. Thus, we suggest that Asp<sup>79</sup> is involved in iron binding on SoSTC.

It has been shown that soluble iron reduction mostly occurs in the periplasm of *S. oneidensis* MR-1 (Ruebush et al). With biochemical characterization of periplasm-localized SoSTC binding with iron chelates, we are able to probe the critical amino acid involved in iron binding. This finding may help elucidate the physiological role of SoSTC in iron respiration, as well as the soluble iron-reduction process of *S. oneidensis* MR-1.

\* EDTA: ethylenediaminetetraacetic acid; NTA: nitrilotriacetic acid.

## Rates and Mechanisms of Heterogeneous Pertechetate Reduction by Mixed Valent Iron Oxides

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

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Sediments at the Hanford Site contain significant total Fe, of which a large percentage exists in the reactive ferrous state. The Fe(II) component in part arises in the form of magnetites crystallized within the basaltic source material that are present in the sediments, both as subhedral to euhedral micron-sized free crystal grains and as mineral components of basaltic lithic fragments. These magnetites naturally contain variable amounts of impurities in the form of isostructural alio- and isovalent metal substitutions. Ti is a dominant constituent, and the resulting metal oxide can therefore be described with the titanomagnetite formula  $\text{Fe}_{3-x}\text{Ti}_x\text{O}_4$ , which falls between endmember magnetite ( $x = 0$ ) and ulvöspinel ( $x = 1$ ). In the absence of surface passivation, Fe(II)-bearing minerals such as these have been shown to be redox-active polyvalent metal reductants. At Hanford, it has been speculated that grain interfaces of such minerals are reducing microenvironments, especially if oxygen is scavenged by associated oxygen-consuming mineral reactions or by microbial activity. Of particular importance in this regard is the availability of electron equivalents for metal reduction by the Fe(II)-mineral phases. Titanomagnetites have the feature that substitution of Ti(IV) into the metal sublattice is charge compensated by a proportional increase in the Fe(II) content. This built-in natural "tunability" of the Fe(II)/Fe(III) ratio in principle adjusts the reducing capacity of the solid, and is therefore central to its redox reactivity with respect to contaminant reduction. This project has been focused on developing a molecular-level understanding of rate-controlling factors in the heterogeneous reduction of pertechetate by such phases, including surface oxidation, passivation, and Fe(II) resupply towards improved predictability of the reducing potential in Hanford-specific microenvironments.

Using EMSL facilities, we developed successful procedures to synthesize compositionally controlled  $\text{Fe}_{3-x}\text{Ti}_x\text{O}_4$  (1) bulk powders; (2) nanoparticles to provide a high surface area pristine material compatible with complementary batch studies; and (3) thin films to provide crystallographically oriented well-defined surfaces for detailed characterization. The Fe(II), total Fe, and total Ti content of the nanoparticle samples was determined by acid digestion, followed by a combined ferrozine assay/ICP-MS approach. Characterization also involved *in situ* XRD, Mössbauer spectroscopy, XANES/EXAFS, and *ex situ* techniques such as TEM, XPS, 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 pertechetate 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, sorbed Fe(II), and Fe(II) in other discrete phases.

Nanoparticle titanomagnetite was found to 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. Spontaneous Fe(II) release from the solid into solution increases in extent systematically with both Ti-content and decreasing pH. At pH 8, where Fe(II) release from  $x = 0$  (magnetite) is minimal, Fe(II) release kinetics display a fast initial increase followed by slower reabsorption of Fe(II) equivalents back into the lattice for  $\text{Fe}_{3-x}\text{Ti}_x\text{O}_4$  compositions at or below  $x = 0.35$ . Reaction with 10 and 30 mM Tc(VII) solution yields fast exponentially decaying reduction kinetics with rates that increase with increasing Ti-content. From the reaction extent, both solubilized Fe(II) and lattice Fe(II) are found to participate in Tc(VII) reduction, although not all lattice Fe(II) is accessed. Beyond residual titanomagnetite, no crystalline reaction products were detected. In preliminary studies, Tc(VII) was also found to be efficiently reduced by oriented thin films of composition  $x = 0$  but not by analogous  $x = 1$  films due to more facile passivation of the latter by pre-oxidation. The collective measurements point to a picture of condition-sensitive dynamic exchange of reducing equivalents between bulk lattice Fe(II), more accessible surface-associated Fe(II), and solubilized 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.

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

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

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Reactive transport simulation models (RTMs) serve as a critical integrating element among the various experimental elements of the SFA, in particular integrating information from fundamental process scales (molecular, subpore, and pore) to phenomenological prediction scales (column, field). Our work is focused on developing pore-scale models of processes critical to uranium transport at the Hanford IFRC, and integrating information from the pore-scale models into a field-scale model (in cooperation with IFRC researchers). Our approach will provide a means for systematically incorporating information from SFA experiments at smaller scales into the field-scale model. Currently, there remains a gap between scientific understanding at fundamental scales and simulation at field scales that leads to an unsatisfactory degree of model and parameter empiricism in many field-scale simulations. Quantitatively linking across this wide range of physical scales with minimal empirical calibration is an ambitious goal being enabled by strong coordination of SFA research and ongoing computational advances. At the Hanford IFRC, surface complexation and intragranular diffusion are two critical pore-scale mass-transfer processes. We have incorporated a model of intragranular diffusion into our parallel 3D smoothed particle hydrodynamics (SPH) code. Using this code, we are performing a suite of pore-scale simulations to identify those pore-scale features that give rise to apparent linear single-rate and multirate mass-transfer models at the porous medium (Darcy) scale. These simulations will explore the effects of variable grain size, variable intragranular diffusion rates (reflecting variable grain types), and the relative importance of intragranular diffusion versus diffusion-limited transport into small intergranular pore spaces. An effective Darcy-scale transport model will be developed for surface complexation in this system using the volume averaging method of upscaling. Such an approach has been used previously to examine mass-transfer limitations in biofilms in porous media, with significant success. The case of U transport in heterogeneous porous networks has many similarities to this problem, and it suggests that an approach similar to that outlined in Wood et al. (2007) will be a useful one. The practical outcome of this approach will be a model that incorporates a reaction-rate effectiveness factor that modifies the microscale reaction rate to account for the influence of mass transfer. The upscaled models will be validated against column-scale reactive transport experimental data and applied to simulate field-scale reactive transport at the Hanford IFRC. Model uncertainties associated with sparse characterization data will be evaluated using stochastic simulation methods, and field-scale predictions will be compared to IFRC experimental observations (without empirical parameter fitting) to test the models' predictive power.

## Microbial Fe-phylosilicate Redox Metabolism in Hanford 300 Area Sediments

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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The potential role of phyllosilicates in the redox biogeochemistry of Hanford 300 Area sediments was investigated using microbiological methods. First, the ability of phyllosilicates to serve as a source of electron acceptor or donor for Fe(III)-reducing or Fe(II)-oxidizing microorganisms was evaluated using the fine silt/clay size fraction of oxidized and reduced Ringold sediments. Both sediments are rich in illite-smectite, illite, kaolinite, and vermiculite; the reduced Ringold sediment is more enriched in illite compared to the oxidized material. Oxidized Ringold sediment was repeatedly reduced by *Geobacter sulfurreducens* under growth conditions with 10 mM acetate as the electron donor. The extent of Fe(III) reduction was 14–17% of total Fe(III). Reduced Ringold sediment was repeatedly oxidized by the lithotrophic Fe(II)-oxidizing, nitrate-reducing enrichment culture reported by Straub et al. (*Appl. Environ. Microbiol.*, 1996) with 5 mM nitrate as the electron acceptor. The extent of Fe(II) oxidation was 1.5–2.7 % of total Fe(II). Similar extents of Fe reduction and oxidation were observed in enrichment cultures containing suspensions of oxidized and reduced Ringold sediments inoculated with fresh groundwater from the overlying Hanford formation. Collectively these results show that Fe-bearing phyllosilicate phases in Hanford 300 Area sediments are susceptible to microbial redox transformation. In order to isolate indigenous microorganisms capable of phyllosilicates redox metabolism, parallel *in situ* incubation and standard liquid enrichments were set up using groundwater from Hanford 300 Area as a source of microorganisms. For *in situ* cultivation, i-chips designed by Slava Epstein were loaded with groundwater and either Ringold sediment or model clay minerals serving a source of electron donor or acceptor. The i-chips targeting Fe(III)-reducing microorganisms were deposited in the reduced zone of Ringold formation, while i-chips targeting Fe(II)-oxidizing microorganisms were deposited in oxidized Hanford formation sediments. The i-chips will be recovered in March 2010.

Laboratory enrichment cultures with purified NAu-2 specimen smectite as electron acceptor recovered organisms related to *Geobacter* and *Azospira* spp., whereas enrichments with chemically reduced NAu-2 as electron donor were dominated by organisms related to *Acidovorax* sp. Strains of *Geobacter bremerensis* (98% 16S rRNA gene sequence similarity) and *Acidovorax defluvii* (99% similarity) were isolated from the Fe reducing and oxidizing enrichment cultures, respectively. The latter culture was able to grow on reduced NAu-2 without any organic matter addition, and was able to grow on soluble Fe(II) with 0.2 mM acetate as a carbon source. These results, together with the fact that *Acidovorax* is not known for lithoautotrophic metabolism, suggest that the *Acidovorax defluvii* isolate grew using Fe(II) as an energy source and small amounts of organic matter in the NAu-2 as a carbon source.

## Functional Characterization of Microbial Proteins Involved in Biogeochemical Electron-Transfer Reactions

PNNL SFA (Laboratory Research Manager: Harvey Bolton)

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Microbial proteins involved in extracellular electron transfer (ET) reactions play critical roles in metal redox transformations. As part of the PNNL SESP SFA's Biogeochemical Electron Transfer Science Theme, this research project focuses on developing 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). The redox proteins of Hanford-relevant and model microorganisms are being investigated using complementary laboratory experiments and modeling to elucidate the ET reactions that affect the availability and supply of reactive Fe(II). The results of these studies will contribute to resolution of PNNL SESP SFA hypotheses pertaining to the role of subsurface microenvironments and transition zones as dominant regions of contaminant oxidation-reduction reactions at Hanford.

As part of the overall SFA effort to enhance Hanford relevance, we have initiated the characterizations of UndA-HRCR-6, a *c*-type cytochrome (*c*-Cyt) identified from a *Shewanella* strain isolated from the Hanford Reach of the Columbia River (HRCR). UndA-HRCR-6 was purified as a heme-containing protein from the membrane fraction of *S. oneidensis* MR-1 cells after its overexpression, which is consistent with the prediction that UndA-HRCR-6 is an outer membrane (OM) cytochrome. *In vivo*, UndA-HRCR-6 complemented the impaired phenotype of a *S. oneidensis* MR-1 mutant lacking MtrC and OmcA in both Fe(III) oxide- and U(VI)- reduction assays. In *S. oneidensis* MR-1, the recombinant UndA-HRCR-6 was translocated across the bacterial OM by the type II secretion system. Collectively, these results indicate that UndA-HRCR-6 most likely has a functional role in extracellular metal reduction.

To understand how electrons are translocated across bacterial OM, we characterized a trans-OM icosaheme complex, MtrABC of *S. oneidensis* MR-1. The results showed that an isolated MtrABC complex could transfer electrons across a lipid bilayer after incorporation into proteoliposomes. A model is proposed for the modular organization of the MtrABC complex, in which MtrC is an extracellular element that mediates electron transfer to extracellular substrates, and MtrB is a trans-OM spanning  $\beta$ -barrel protein that serves as a sheath to embed MtrA subunit in the membrane, where it forms a trans-membrane electron-delivery module that services MtrC. This is the first molecular model of electron translocation across the bacterial OM, which could also apply more widely to mechanisms of electron exchange between related proteins present in a number of other Gram-negative bacterial genera and extracellular electron sources and sinks. Consistent with this model, protein cross-linking results and a structural model of MtrA show that (1) MtrA physically interacts with MtrB and MtrC, and (2) ~50 % of the MtrA heme chain is embedded within MtrB. At present, full datasets at less than 4 Å resolution are also obtained for MtrF (a MtrC homologue) of *S. oneidensis* MR-1 and indicate that the overall fold of MtrF is totally novel.

In collaboration with the Rosso SFA project, we measured the direct electron transfer between purified *S. oneidensis* MR-1 *c*-Cyt STC (locus tag SO\_2727) and hematite ( $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>) with cyclic voltammetry. Although it did not form a stable film on the hematite surface, STC could still rapidly transfer electrons to hematite, most likely via transient interactions with the oxide particles. In addition to metal reduction, investigation of microbial proteins involved in extracellular oxidation of Fe(II) is being initiated.

## A Pore-Scale Analysis of the Electrical Properties of Partially Saturated Porous Media and Implications for Scaling Reactive Transport

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

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Electric and electromagnetic geophysical measurements are becoming more widely used for characterizing flow and reactive transport properties, but still rely heavily on empirical relationships for data interpretation. These relationships are difficult to calibrate for heterogeneous unconsolidated porous media, especially when they are partially saturated. Our objective was to develop a pore-scale model for predicting the electrical properties of partially saturated, heterogeneous unconsolidated porous media. This model is used to quantify the effect of particle shape and size distribution, pore geometry, grain surface charge, and pore fluid composition on the hydroelectrical properties and their scaling behavior.

Synthetic pore-scale models, including unit cells, and assemblages of isotropic and anisotropic sands were constructed to represent homogenous and heterogeneous porous media with and without dispersed clay and clay coatings. These models were used to simulate infiltration, redistribution, reactive transport, and mineral precipitation with a numerical model based on smoothed particle hydrodynamics (SPH). Simulation results show that the SPH, Lagrangian particle method is an effective tool for studying pore-scale flow and transport allowing complex physical processes such as diffusion, reaction, and mineral precipitation to be simulated with relative ease. Output of the SPH model was used with a fully explicit pore-scale geometrical model to calculate the d.c. and frequency-dependent electrical response of the porous media. Simulations of d.c response were used to evaluate empirical models of the electrical behavior of shaly sand including Waxman and Smits, Sen, and Clavier Dual-Water models, whereas frequency dependent simulations were used to evaluate the Dias model. Results show that the pore-scale electrical response of unconsolidated media is sensitive to variations in structure, the distribution of clay minerals, the distribution of fluids, and precipitates in the pore space. Aspherical particles result in an anisotropic response that is well described with a cementation index,  $m$ , tensor. The degree of anisotropy also appears to be dependent on saturation. Under partially saturated conditions, the saturation exponent,  $n$ , is lower than the typical value. Desaturation results in a shift from a parallel-dominated to a series-dominated electrical conductivity as the pore space becomes more tortuous, giving rise to a two-arc response at high frequencies. Simulations of the displacement of water of one conductivity with that of a different conductivity show that conductivity is dependent on the composition history, with the displacement of low conductivity water by a high conductivity water giving different  $m$  and  $n$  values than vice versa. The ability of the pore-scale models to correctly predict the electrical response during flow and transport validates the consistency of the approach and will allow the investigation of the effects of several system parameters on the electrical response, thereby providing new insights into constitutive relationships for use at the field scale. Further calibrations are planned with broadband data being collected on IFRC cores at INL.

## Understanding Grain-Scale Diffusion Processes and Quantifying Mass-Transfer Parameters in Hanford 300A Sediments

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

M.B. Hay, D.L. Stoliker, J.A. Davis—USGS; J.M. Zachara (PI), PNNL

The mobility 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 intragranular U diffusion in the 300A sediments is complicated by multiple factors that are each poorly understood, including the complex aqueous and surface speciation of U, the precise nature of the diffusion process (e.g., aqueous-phase versus surface diffusion), anion exclusion effects, and the heterogeneous nature of the intragranular/intra-aggregate pore network. The objective of our work is to independently address the effects of chemical speciation/retardation, physical diffusion, and diffusion domain heterogeneity in order to obtain a better overall understanding of the intragranular diffusion process.

To characterize the grain-scale physical diffusion regime, uptake/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 results suggest a high degree of heterogeneity in the diffusive regime, whereas results using bromide indicate a narrower range in diffusion rates and a smaller intragranular pore volume, consistent with anion exclusion of bromide.

To better quantify the diffusion mechanism (aqueous phase vs. surface) and to understand effects of chemical speciation, U adsorption/desorption and nonreactive tracer diffusion experiments are also being performed using model porous sorbents. Model phases currently under study include silica gel and activated aluminum oxide chromatography substrates, both highly porous, large grain-size materials with intragranular pore diameters of ~6 nm. Preliminary results with these materials include predictable kinetic trends with varying chemical conditions and solid:solution ratio. Future work on these substrates will involve nonreactive tracer experiments and high-resolution microscopy to characterize the physical diffusion regime and resolve physical diffusion and sorptive retardation effects.

## Investigating the Impact of Microenvironments on Contaminant Biogeochemical Reactive Transport

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

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Pore-scale processes play a key role in biogeochemical cycles occurring in subsurface environments. The objective of this project is to study the impact of subsurface microenvironments on contaminant biogeochemical reactive transport in microfluidic pore structures (micromodels). Specifically, we plan to investigate (i) bacterial growth and distribution in transition zones and (ii) the influence of mineral surface properties on bacterial growth and geochemical reactions. Multiple inlets/outlets created in etched-silicon micromodels will be used, along with precise fluid delivery, to control chemical gradients resulting in the formation of transition zones. Bacterial cell colonization and growth at these transition zones within the micromodel will be visualized using fluorescent protein expression vectors and fluorescent microscopy. The fabrication of micromodels with a metal oxide (e.g., hematite) coating on the surface will allow us to study biogeochemical redox cycling of key contaminant metals present at the Hanford site, including U(VI) and Tc(VII), using advanced characterization techniques such as X-ray absorption spectroscopy. The flexibility in the design of the micromodels, in combination with advanced characterization techniques, will allow us to probe a wide range of pore structures with distinctive physical, chemical, and biological properties. This project will provide a better understanding of biogeochemical processes occurring in subsurface microenvironments and their impact on the transport of key contaminants.



## Speciation and Reactivity of Reduced Uranium: Collaborative Field Research at the Old Rifle IFRC Research Site

SLAC SFA (Laboratory Research Manager: John R. Bargar)

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Understanding the roles and function of molecular-scale processes at the field scale is an important goal of the SLAC SFA program. Laboratory-based structure-speciation-reactivity studies are being complemented by field investigations at the Rifle IFRC field site to test conceptual models and evaluate rate and stability constants under field conditions. The Rifle site is ideal for such investigations because of the availability of wells with contrasting dissolved oxygen concentrations, extensive historical groundwater data, and good site infrastructure.

*Rates and mechanisms of biogenic uraninite corrosion in ground water.* Biogenic uraninite is of potentially great significance to naturally and artificially bioreduced sediments. However, the stability of this phase is believed to be compromised by its nano size. Since other forms of U(IV) are thought to be more prone to oxidation than uraninite, the stability of this solid phase can be viewed as a limiting case, a first-order scientific question likely to impact the success of stimulated bioremediation approaches. Trace and major-ion solutes strongly moderate uraninite stability, and their spatial/temporal variability in groundwater is difficult to capture in laboratory analog studies. Evaluation of kinetic parameters for bio-uraninite dissolution in the field and meaningful testing of hypotheses developed from previous laboratory work by the SLAC-SFA team therefore requires studies of uraninite stability under *bona-fide* groundwater conditions. Consequently, biogenic uraninite corrosion by molecular oxygen was studied in the Rifle aquifer. Uraninite synthesized by microbial U(IV) reduction by Gamma proteobacteria isolated from Rifle groundwater was deployed in anoxic and oxic wells and subsequently analyzed to assess dissolution rates and compositional/structural changes. The net dissolution rates are remarkably slow (*ca*  $10^{-11}$  moles/m<sup>2</sup> sec), 100-fold slower than those obtained in laboratory experiments. *Impact:* This study indicates that biogenic uraninite is relatively stable in groundwater, even under oxic conditions.

*Speciation of uranium in sediments following acetate-stimulated bioremediation.* The chemical and physical form of bioreduced uranium in Rifle sediments remains unknown. We initiated a new experiment in July 2009 to obtain spectroscopically analyzable subsurface sediment uranium concentrations from the Buckskin acetate-stimulated bioreduction experiment. Columns were filled with fresh biologically active sediments and placed in the aquifer directly down gradient of the Winchester acetate injection gallery. Surface pumps were used to draw groundwater, to which 10  $\mu$ M U(VI) and 2.5 mM acetate was added, through the columns. Harvested sediments are being analyzed by XAS, x-ray microprobe analysis, electron microscopy, and microbial community analysis. *Impact:* Knowledge of uranium speciation in bioreduced sediments is informing the development of geochemical models at Rifle and sites with similar groundwater characteristics.

## Fundamental Biogeochemical Controls over the Speciation and Reactivity of Uranium in Reducing Sediments

SLAC SFA (Laboratory Research Manager: John R. Bargar)

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Reducing conditions are common in the subsurface where solute supply is locally limited and oxidants become exhausted. Uranium reduction in such sediments is an important natural attenuation mechanism that has inspired amendment-based *in situ* bioremediation technologies. Reduced sediments can act as long-term sinks or sources for contaminants and exhibit rich redox biogeochemistry that profoundly impacts uranium stability and fate. Our limited knowledge of the biogeochemical processes governing uranium speciation and stability in such sediments is a significant barrier to understanding natural and stimulated attenuation processes.

The SLAC-SFA program is investigating fundamental biogeochemical processes and critical species that control uranium fate and transport in sediments under metal-reducing conditions. By characterizing structure-speciation-reactivity relationships for uranium species and linked processes over a hierarchy of scales from molecular to field, we are developing new conceptual paradigms and quantifying rate/stability parameters that provide the basis for improved geochemical models. Field-based research currently focused on the Rifle IFRC site is providing information relevant to other contaminated sites.

Within this context, the SLAC-SFA is using advanced synchrotron-based, microbiological, aqueous geochemical, and field techniques in a tightly integrated collaborative framework to explore the following overarching priority research questions: (1) What are the fundamental compositional/structural controls over the stability of biogenic nanouraninite? This work is focused on the impact of groundwater solutes (anions, trace metal cations) on uraninite stability and on the rates and mechanisms of biogenic uraninite corrosion under groundwater conditions; (2) What other forms of U(IV), such as molecular complexes, occur in reduced sediments? What biogeochemical factors control their stability? What factors lead to the production of molecular U(IV) instead of uraninite? and (3) What is the impact of (bio)geochemical iron oxide structure, composition, and redox cycling on uranium attenuation? What is the fate of U(VI) sorbed to Fe(III) (oxyhydr-)oxides during Fe(III) reduction? What are the mechanisms of uranium sequestration by reduced Fe oxides, and what are their rates?

Major products of this program include an enhanced quantitative understanding of reactions controlling biogenic uraninite stability in aquifers, elucidation of other forms of U(IV) in aquifer sediments and factors controlling their production and structures of natural ferrihydrite, and the fate of sorbed uranium following ferrihydrite reduction. This knowledge informs our understanding of natural attenuation processes and engineered uranium remediation approaches. It is helping to provide the basis for improved cleanup and management of contaminated sites, and public and regulatory acceptance of site management practices.

## Biogeochemical Factors Governing the Formation and Subsurface Stability of Reduced Molecular Uranium Species

SLAC SFA (Laboratory Research Manager: John R. Bargar)

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Subsurface reduction of U(VI) can be mediated by microorganisms and their redox-active mineral byproducts, yielding relatively insoluble U(IV) species. Although uraninite is generally assumed to be the dominant product of biological U(VI) reduction, recent work by the SLAC SFA and others provide evidence that other forms of U(IV), such as molecular complexes, may also be produced. The microbially mediated *in situ* reduction of U(VI) is touted as a potential approach for the remediation of uranium-contaminated U.S. DOE sites. The success of such a strategy is contingent upon the enduring stability of the biologically reduced uranium. The goal of this work is to elucidate and quantify the fundamental structure-composition-stability relationships of these species as part of the SLAC SFA focus on molecular-scale processes of key importance to uranium behavior in the subsurface at DOE sites. We report here key insights to factors controlling the formation of molecular U(IV) product(s) in pure culture and sediment columns. These species lack the U-U EXAFS shell characteristic of uraninite. We carried out lactate-stimulated uranium reduction experiments in columns packed with Rifle IFRC sediment augmented with *Shewanella oneidensis* MR-1 cells. Major findings of the work included a shift in the microbial community composition over time away from *S. oneidensis* in low G+C Gram-positive bacteria, as well as the formation of a molecular tetravalent uranium species lacking intermediate-range structure, as demonstrated by EXAFS.

In addition, several bacterial species, including *Desulfotomaculum reducens* MI-1, *Clostridium acetobutylicum* and *S. oneidensis*, were found to be capable of producing molecular U(IV) as a product of U(VI) reduction in pure culture under specific geochemical conditions. In fact, geochemical conditions appear to exert significant control over the product of microbial U(VI) reduction as *S. oneidensis* produces uraninite under some geochemical conditions and molecular U(IV) under others.

Finally, the reduction of U(VI) by redox-active biogenic Fe(II) phases also generates several distinct U(IV) products depending on the geochemical matrix. In some cases, EXAFS indicated the formation of a molecular U(IV) species, whereas in other instances, uraninite was formed. These findings reinforce the conclusion that uraninite is not the only product of direct and indirect biological U(VI) reduction, but that molecular tetravalent uranium species must also be considered when modeling uranium mobility in the subsurface. Our present research efforts are focused on evaluating the reactivity of molecular tetravalent uranium, as well as the conditions leading to the formation of such species as a result of biological U(VI) reduction. Identification of key factors controlling the formation of molecular U(IV) and biogenic uraninite and knowledge of their reactivity is leading to new geochemical models for uranium cycling in reducing sediments and to a deeper and more defensible scientific basis for *in situ* reductive remediation technologies.

## Compositional and Structural Controls over the Reactivity of Biogenic Nano-Uraninite

SLAC SFA (Laboratory Research Manager: John R. Bargar)

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Uraninite is abundant in sedimentary ore deposits formed at low temperature, is precipitated by U(VI)-reducing microorganisms, is believed to be the most thermodynamically stable form of U(IV), and hence, is of potentially great significance to naturally and artificially bioremediated sediments. The stability of this material is brought into sharp focus by its very fine particle size, typically 2-5 nm, when precipitated biologically. Previous investigators have suggested that the nano size of biogenic uraninite would intrinsically and substantially compromise its ability to resist dissolution and oxidation. Since other forms of U(IV) are thought to be *more* prone to reoxidation than uraninite, the stability of this reduced solid phase in oxic and suboxic ground water can be viewed as a limiting case, a first-order scientific question likely to impact the success of stimulated bioremediation. We are investigating the fundamental mechanisms by which biogenic uraninite structure, composition, and the geochemistry of the aqueous solution govern its reactivity at subsurface conditions.

Dissolution rates have been quantified and mechanisms elucidated as a function of the aqueous geochemistry. Previously, we demonstrated that bio-uraninite dissolution rates increased with dissolved oxygen and decreasing pH. Intriguingly, dissolved inorganic carbon accelerates dissolution at all conditions. More recently, the impacts of other groundwater solutes on biogenic nano-uraninite dissolution have been examined in buffered media and artificial groundwater. The presence of dissolved  $\text{Mn}^{2+}$ , a common groundwater solute and analog for other divalent transition metals, including  $\text{Fe}^{2+}$ , was found to decrease dissolution rates by several orders of magnitude; its impact was most dramatic under oxic solutions. Subsequent experiments examined the impact of  $\text{Ca}^{2+}$  on dissolution rates. These results suggest that adsorbing cations can profoundly impact bio-uraninite surface chemistry and strongly moderate its stability in groundwater. We have subsequently initiated crystal truncation rod (CTR) x-ray diffraction measurements to characterize the structure of the uraninite-water interface and assess the fundamental molecular-scale mechanisms by which adsorbates confer stability. We will present results of initial measurements using the  $\text{UO}_2$  (111) surface under dry and hydrated conditions.

We have also investigated the effects of bulk structure and composition on the stability and reactivity of uraninite. EXAFS, synchrotron-based powder diffraction, and TEM show that biogenic uraninite produced in the presence of  $\text{Mn}^{2+}$  structurally incorporates this solute, resulting in significantly diminished particle sizes (1.5 nm). Remarkably, this product was substantially less soluble and less susceptible to oxidative dissolution than pure biogenic uraninite. *Impact:* This work indicates that cation solutes can greatly stabilize biogenic uraninite in groundwater, either by incorporation during growth, or by adsorption. These conclusions lead to the prediction that biogenic uraninite formed in the subsurface should be more stable than stoichiometric  $\text{UO}_{2.0}$ .

## Impact of Biogeochemical Iron Redox Cycling on Uranium Attenuation by Iron (Oxyhydr)oxides

SLAC SFA (Laboratory Research Manager: John R. Bargar)

F.M. Michel (PI), J.S. Lezama, J.E. Stubbs, G.E. Brown, S.E. Fendorf, M.S. Massey—*Stanford U.*;  
P.S. Nico, *LBL*; J.R. Bargar, *Stanford U.*

The direct incorporation of uranium into Fe-(oxyhydr)oxides provides a potentially large sink/reservoir for this contaminant in the subsurface. Prior research suggests that incorporation of uranium into Fe-(oxyhydr)oxides begins with the adsorption of U(VI) on surfaces of disordered ferrihydrite (Fh), a commonly occurring, nanosized, poorly ordered Fe-hydroxide. Since both uranium and Fe-(oxyhydr)oxides are redox active, the fate of adsorbed uranium under varying redox conditions is of particular interest. Bioremediation, either natural or artificially stimulated, drives strong Fe-reduction, and hence likely drives the redistribution of uranium into Fe-(oxyhydr)oxides.

The objectives of this project are to develop a detailed mechanistic understanding of uranium sequestration processes during the reductive transformation of Fe-(oxyhydr)oxides. Emphasis is placed on reactions involving Fh because it tends to precipitate first and to subsequently transform into more stable phases. Resolving the relevant transformation pathways requires fundamental knowledge of the basic crystal structures and physicochemical properties of all the phases involved. A robust understanding of the structures of Fh under environmental conditions does not exist. This and the lack of understanding of transformation pathways represent major knowledge gaps.

*Structure of environmental Ferrihydrite.* Using a combination of synchrotron-based PDF and laboratory techniques, we have developed a new single-phase structure model for Fh, which predicts a composition of  $\text{Fe}_{8.2}\text{O}_{8.5}(\text{OH})_{7.4}$ , contains 10-12% tetrahedrally coordinated Fe(III), and indicates the presence of ~18% Fe(III) vacancies in the structure. Cation vacancies are typically reactive sites of cation binding and redox reactions. The presence of tetrahedral Fe(III) is expected to affect both the stability and reactivity of ferrihydrite. We postulate that both of these types of sites play catalytic roles in Fh reductive transformation pathways.

*Fate of adsorbed U(VI) during abiotic Fe-oxide reductive transformation.* We have studied the fate of U(VI) during Fe(II)-driven reductive transformation of Fh at variable initial U(VI) concentrations to assess how Fe-(oxyhydr)oxides structurally accommodate uranium. X-ray powder diffraction shows systematic changes in unit cell dimensions of magnetite (Mt) and goethite (Gt) products with increasing uranium content following Fe(II)-driven Fh reduction. X-ray absorption spectroscopy shows that the U-O distances for uranium in the reduced samples are consistent with distorted octahedral coordination. Both sets of observations support the conclusion that uranium is structurally incorporated in Mt and Gt. Furthermore, Fe-oxides forming in natural aquifers are expected to incorporate significant structural impurities such as silicate and aluminum. This will modify stability, effect transformation pathways, and could enhance (or inhibit) incorporation of U. Experiments exploring the individual effects of Si and Al on the transformations of synthetic analogues are ongoing and will be coupled to future field-scale experiments. *Impact:* These results are contributing to conceptual models of Fe-(oxyhydr)oxides redox reaction networks, factors governing the dominant pathways, and their impact on uranium fate and stability.

## ***Student Abstracts***



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

Student Abstract

D.L. Cologgi, G. Reguera (lead PI)—*Michigan State U.*

During biofilm growth, several developmental stages occur (monolayer, immature microcolonies and mature microcolonies). Our group hypothesized that since each stage represents a unique set of transcribed genes and physiological characteristics, each is likely to contribute differently to contaminant transformation. To test this hypothesis, I grew biofilms to various stages of development and challenged them with uranium. These samples have been analyzed three times using x-ray absorption spectroscopy (in collaboration with Dr. Shelly Kelly, EXAFS Analysis), allowing the percentage of U(VI)/U(IV) bound to the cells to be determined. U(VI) was the predominant form of uranium in monolayered and immature biofilms, while U(IV) was most abundant in mature biofilms. From these results, we determined that uranium is transformed first via sorption, then a reductive mechanism.

One cellular component that is known to be expressed only at the later stages of biofilm development is the electrically conductive pilus nanowires, which are responsible for transferring electrons to insoluble metals such as iron oxides. To further investigate whether this appendage might account for the observed differences in uranium reduction, we challenged a pilin-deficient mutant (interrupted at the microcolony stage of development) with uranium. We found that it was able to adsorb U(VI) at rates comparable to that of the wild-type at a similar developmental stage, but was unable to reduce the majority of it to U(IV). This indicated a possible role for the nanowires in the binding and reduction of uranium.

To evaluate the contribution of the nanowires to U(VI) reduction, we developed a different assay in which planktonic cells were grown under conditions that induced pili expression. This causes the cells to aggregate, thereby creating biofilm-like microenvironments with cells and nanowires, but no biofilm matrix. Controls included cells grown under conditions that did not induce pili, and a pilin-deficient mutant. U(VI) removal was significantly higher in cells that expressed pili, either naturally or by genetic complementation, consistent with a role for *G. sulfurreducens* conductive pili in U(VI) reduction. Transmission electron microscopy showed that uranium precipitated on the nanowires or in the periplasmic space, depending on whether the cells were or were not expressing pili, respectively. This suggested to us that the nanowires may also have a protective cellular role during U(VI) reduction, which I confirmed in a fluorescence viability assay. My research thus far provides evidence for a previously unrecognized role for *Geobacter* biofilms in uranium transformations. It also shows that stage-specific biofilm components, such as the nanowires, modulate uranium transformations by the biofilms. These stage-specific biofilm components could therefore serve as molecular markers to assess the physiological status of *Geobacter* bacteria during *in situ* bioremediation and the potential of biofilms as tools for the bioremediation of uranium-contaminated sites.



## Quantitative Imaging of Biofilm in Opaque Porous Media: Assessing the Impact of Fluid Flow Rate and Microbial Species on Biofilm Formation and Architecture

### *Student Abstract*

G. Iltis, D. Wildenschild (lead PI)—*Oregon State U.*

Current understanding of subsurface microbial biofilm formation and the impact on fluid hydrodynamics associated with biofilm growth is limited by our ability to observe the *in situ* pore-scale geometry of developed biofilms. Biomass distribution in porous media has been observed primarily in two-dimensional systems to date; currently, no high-resolution three-dimensional structural data sets exist for opaque porous media that provide sufficient information about biomass distribution, such that the impact on flow and solute transport at the pore-scale can be directly assessed. Previous work conducted by our research group focused on the validation of a new method for resolving high-resolution three-dimensional tomographic images of biofilms in porous media using synchrotron-based x-ray microtomography. This method utilizes silver-coated, neutrally buoyant microspheres as the contrast agent to delineate the surface of the developed biofilm within porous media. With the completion of the two-dimensional method validation, our research has progressed to focus on biofilm imaging within three-dimensional opaque porous media consisting of glass bead-packed columns. Most recently, a set of experiments was conducted at the Advanced Photon Source, Argonne National Laboratory, to (1) evaluate the effectiveness of the silver doping method to characterize biofilm growth formed by various species of bacteria within packed bead columns, and (2) to assess variation in biofilm characteristics such as density and distribution within the pore space as a function of growth media flow rate. Preliminary results from these recent experiments will be presented.

## Isolation and Characterization of Acid-Tolerant Denitrifying Bacteria and Fungi from the Terrestrial Subsurface

*Student Abstract*

P. Jasrotia, O. Prakash, Andy K. Canion, Stefan J. Green, and Joel E. Kostka (lead PI)—  
*Florida State U.*

Nitrate is often a co-contaminant with uranium (U[VI]) in the subsurface of U.S. DOE sites, due to the use of nitric acid in the processing of U(VI) and U(VI)-bearing waste. Because the U and N cycles are intimately linked in the subsurface, an improved understanding of denitrifying microbial communities is required to predict and control uranium as well as nitrate attenuation mechanisms for the remediation of contaminated groundwaters at U.S. DOE sites. Few cultivated representatives are available with which to verify the metabolism of organisms that catalyze denitrification, and existing molecular biology methods do not detect many known denitrifiers. Thus, the overall goal of this research is to apply a combination of cultivation, genomic and metagenomic approaches to elucidate the microbial groups mediating nitrate attenuation at the Oak Ridge Integrated Field Research Challenge (OR-IFRC; Oak Ridge, TN). Bacteria and fungi were isolated from the acidic (pH 4-5) and highly contaminated source zone (Area 3) from enrichment cultures under denitrifying conditions or by spread plating under aerobic conditions. Structural gene (16S rRNA and 18S rRNA) analysis revealed thirteen species of bacteria from four phyla and ten species of fungi from two phyla. Bacterial isolates belonged to the genera *Rhodanobacter*, *Streptomyces*, *Bacillus*, *Afipia*, *Janthinobacterium*, *Castellaniella* and *Variovorax* and *Sphingobacterium*. All bacterial isolates were screened for growth under varying pH conditions ranging from pH 3 to 11, growth with nitrate as the sole electron acceptor, and the production of gaseous nitrogen ( $N_2O$ ,  $N_2$ ). Results show a growth optimum at pH 6 for a majority of the bacterial cultures. Five isolates gave positive results for both  $N_2O$  and  $N_2$  production at pH 4, and belong to genera *Rhodanobacter*, *Bacteroidetes*, and *Pusillimonas*. In particular, several representatives of *Rhodanobacter* spp. demonstrated the capacity for complete denitrification and growth under acidic (pH=4) conditions, and sequences retrieved from sediments and groundwater indicate that *Rhodanobacter* spp. is a predominant microbial group in acidic subsurface of the OR-IFRC. Fungal isolates belonged to the genera *Penicillium* and *Aspergillus*, *Neolinocarpon* and *Coniochaeta*, *Rhodosporidium*, *Lachnum*, *Teberdinia* and *Sclerotinia* and *Heterodermia*. Denitrification potential experiments with fungal cultures showed the accumulation of nitrous oxide, which is believed to be the most common end product of fungal denitrification, at pH 4–5. The fungal isolate that showed the greatest denitrification potential (highest nitrous oxide production and increase in biomass) aligns close to *Teberdinia hygrophila* (99% seq. similarity). Isolation of a broad range of acid tolerant denitrifying bacteria and fungi from the OR-IFRC subsurface indicates adaptation to acidic and high contaminant load conditions, in concurrence with the accumulation of large amounts of nitrous oxide in wells sampled close to the contaminant source zone (Area 3). Very little information is available on fungi from terrestrial subsurface, and these results suggest that fungi might play an important role in the transformation of subsurface contaminants, possibly catalyzing denitrification under low pH conditions.

## Abiotic Reduction of Uranium by Fe(II) in Soil

### *Student Abstract*

D.E. Latta, *U. of Iowa*; E. J. O'Loughlin, K.M. Kemner, M. Boyanov—ANL;  
M.M. Scherer (lead PI), *U. of Iowa*

The current DOE strategy for treatment of radionuclide and heavy metal contamination in the subsurface relies heavily on *in situ* immobilization and stabilization. It is well-established that hexavalent uranium (U(VI)) can be enzymatically reduced by a variety of microbial species, as well as abiotically reduced by many chemically synthesized Fe(II) containing Fe minerals. Despite the prevalence of Fe(II) under conditions where U(VI) is reduced, most studies to date have found little evidence for significant abiotic reduction of U(VI) by Fe(II) in pasteurized or sterilized natural materials. Here, we present evidence for U(VI) immobilization and reduction by Fe(II) present in a pasteurized, redoximorphic Iowa soil. We use data from U LIII-edge x-ray absorption spectroscopy (XAS) to confirm U(VI) reduction and <sup>57</sup>Fe-Mössbauer spectroscopy to confirm oxidation of the soil Fe(II). Soil characterization based on results from chemical extractions, Fe K-edge XAS, and Mössbauer spectroscopy indicate that the reactive Fe(II) phase is structural in nature and may be a green-rust like phase. Our work highlights the importance that abiotic reduction of U(VI) by structural Fe(II) may play in anaerobic environments.

## The Potential Role of Microbes on Iodine-129 Mobility in Groundwater Relevant to Long-Term Stewardship of DOE Sites

### *Student Abstract*

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Iodine-129 is a major byproduct of nuclear fission and among the top three risk drivers for waste disposal at the Yucca Mt., Hanford, and Savannah River Sites (SRS). It is of major concern because of its perceived mobility in the environment, excessive inventory, toxicity, and long half-life (~16 million yrs). The various isotopes of iodine can be strongly bound to macromolecular organic matter, which can significantly decrease or increase its transport, bioavailability, and transfer to man, depending on the molecular weight and physico-chemical properties of the resulting iodine-organic matter species. Microbial activity (most likely oxidases, perhydrolases, and/or peroxidases) has been implicated in the halogenation of soil organic matter, but very little is known about the proposed process. We are investigating the role of naturally occurring bacteria in the speciation and mobility of <sup>129</sup>I in SRS aquifers.

Approximately 350 bacteria were isolated from F-Area sediments at the SRS and identified with 16S rRNA gene sequencing. A total of 7 phyla (54 genera) are represented, mainly dominated by beta-*Proteobacteria* (29%), *Firmicutes* (15%), and *Actinobacteria* (12%). All isolates were screened for iodide oxidizing activity on agar medium plates containing iodide and starch, and none of the strains exhibited a positive phenotype. However, yellow discoloration was noted in 2 of the 24 enrichment cultures containing slurries of SRS sediment and iodide (1 mM) after 22 weeks incubation, indicating that iodide was oxidized to iodine in these tubes. SRS strains are currently undergoing testing for their ability to accumulate iodine under different environmental conditions such as varied ambient iodine concentration (0.01~10 µM) and pH. Of the 138 isolates screened thus far, 11, representing 4 phyla and 6 genera, have demonstrated iodine accumulation (ranging from 0.25 to 1.2% uptake). Addition of H<sub>2</sub>O<sub>2</sub> was found to stimulate this uptake indicating that peroxidases may be involved in this process. We are currently evaluating the cellular location of the accumulated iodine and its speciation.

## Understanding the Adsorption Discrepancies Between Batch and Column Experiments

### *Student Abstract*

V.A. Loganathan, M.O. Barnett (lead PI), and T. P. Clement—*Auburn U.*

One of the most important processes that govern the subsurface transport of contaminants is the adsorption onto geomedia. Typically, the adsorption is quantified using batch and/or column experiments. One of the most perplexing, yet-unresolved problems is the discrepancy observed between batch-derived and column-derived adsorption capacities. Several studies indicate the existence of the above phenomena, especially when U(VI) has been used as an adsorbate. In this study, we attempt to understand the causes of the above problem using both natural heterogeneous geomedia and a homogeneous synthetic adsorbent. The uncontaminated natural geomedia was obtained from the Oak Ridge Reservation (OR) and the synthetic media, iron oxide-coated sand (IOCS), was meticulously prepared in the laboratory.

The OR soil and IOCS were used to perform adsorption experiments with U(VI) as a solute in both batch and column modes. The batch experiments were aimed at understanding the effect of solid-to-solution ratio on U(VI) adsorption. Moreover, a multistep column experiment was conducted to obtain adsorption isotherm that could be juxtaposed with the batch adsorption isotherm. Efforts were made to maintain similar experimental conditions in both batch and column scenarios. Particularly to eliminate CO<sub>2</sub> contamination, all the experiments were conducted at a pH < 5.

The results obtained from the batch and column experiments indicated a huge discrepancy (>100%) in the adsorption capacity of U(VI) onto OR soil—whereas in the case of IOCS, the batch-derived and column-derived isotherm indicated similar adsorption capacity for U(VI). Moreover, adsorption of U(VI) on to IOCS was found to be independent of soil to solution ratio. These results suggest that the frequently observed adsorption discrepancy between batch and column setting exists in systems containing highly heterogeneous adsorbent, since in the case of heterogeneous adsorbent, a multitude of sorption surfaces could prevail that could result in adsorption being dependent on the solid-to-solution ratio. When adsorption is dependent on solid-to-solution ratio, multiple adsorption isotherms could be obtained. This could lead to the observed batch and column adsorption discrepancy. Our current studies include the identification of the dominant sorptive phases present in the OR soil. Moreover, efforts are being made to simulate the experimental results using surface complexation models. Although our study involved U(VI) as a solute, the results are applicable to other DOE-relevant actinides as well, e.g., Np(V), that has properties similar to U(VI).

## **Inverse Modeling Method to Characterize a Heterogeneous Field of Hydrogeological Properties by Assimilating Various Types of Data**

*Student Abstract*

H. Murakami, Y. Rubin (lead PI)—*U. of California-Berkeley*

Within the Integrated Field Research Challenge Project at the Hanford 300 Area, we have been developing an inverse modeling method to characterize a heterogeneous field of hydrogeological properties by assimilating various types of data at the site. For the past year, we have applied our method to characterize a hydraulic conductivity field at the site, by assimilating electromagnetic borehole flowmeter tests and constant-rate injection tests. As a part of this effort, I was in charge of implementing our scheme on the supercomputer Franklin at NERSC to simulate the injection tests on multiple processors.

In addition, our group has provided multiple realizations of conductivity fields based on our inversion to the numerical modelers in the IFRC team for simulating flow and transport at the site. I was in charge of developing a new random field generation code for generating three-dimensional hydraulic conductivity fields based on our inversion method. The new feature that I have added is to include volume-averaged or depth averaged data to condition the fields, in addition to point conductivity values. Now our group is in the process of integrating tracer test data to further improve our characterization. This new random field generation code is one of the core parts of this process.

This year, we have started to integrate other types of data at the site, such as lithology, electric resistivity tomography, and borehole log data. We have found that several-feet-diameter low-conductivity boulders have a significant impact on the field data and possibly on the flow-and-transport processes. We are attempting to incorporate this boulder effect within our hydrogeological model by taking account of their spatial/size distributions.

## Competition Between U(VI) Bioreduction and Biomineralization in a Contaminated Sediment

### *Student Abstract*

K.R. Salome, *Georgia Tech*; M.J. Beazley, *U. of Oklahoma*; S. Green, *Florida State U.*;  
R. J. Martinez, *U. of Alabama*; J. Kostka, *Florida State U.*; P.A. Sobecky (lead PI), *U. of Alabama*;  
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The natural phosphatase activities of microbes isolated from Oak Ridge, TN Field Research Center (ORFRC) soils release sufficient inorganic phosphate during growth at pH 5.5 and pH 7.0, under both aerobic and anaerobic conditions, to precipitate 73–95% of U(VI) as insoluble U(VI)-phosphate minerals. Although microbial phosphatase activity has been demonstrated with bacterial isolates, the ability of natural microbial communities to liberate inorganic phosphate, particularly under reducing conditions, has yet to be investigated. In this study, U(VI)-phosphate biomineralization in ORFRC soils was investigated under anaerobic conditions at pH 5.5 and pH 7.0 in the presence of glycerol-2-phosphate (G2P) as the sole phosphorus source, and sulfate or nitrate as terminal electron acceptors. Nitrate-reducing bacteria were stimulated by G2P, as evidenced by the accumulation of nitrite, even in the presence of elevated concentrations of  $\text{SO}_4^{2-}$ , suggesting they are the dominant microbial community in ORFRC soils. U(VI) was rapidly removed from the solution in all treatments but not reduced, as determined by x-ray absorption near edge structure (XANES) spectroscopy. Simultaneously, extended x-ray absorption fine structure (EXAFS) spectroscopy revealed that adsorption of U(VI) was not significant in the G2P treatments. The rapid removal of dissolved U(VI) coupled with the large production of  $\text{PO}_4^{3-}$  and the absence of U(IV) or adsorbed U(VI) suggest that U(VI) was precipitated as U(VI)-phosphate minerals in the soils amended with G2P. Thus, even under reducing conditions, biomineralization of uranium minerals was favored over bioreduction of U(VI). Future work will include microbial community analysis of G2P-stimulated incubations. In addition, the competition between adsorption, biomineralization, and bioreduction will be investigated using different organophosphate sources.

## Sampling Methods and Uranium Desorption from Passive Flux Meters in Contaminated Groundwaters

### *Student Abstract*

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Passive flux meters (PFMs) have previously been developed to measure uranium groundwater flux at the old mill site in Rifle, CO. These PFMs are composed of an outer layer of Lewatit S 6328 A resin used to sorb uranium and release an organic tracer trapped on an inner-activated carbon core. At this point, two sampling trips have been completed. The most recent PFMs were installed for a period of three weeks in wells previously used for biostimulation, after which they were removed and subsampled. One PFM was sampled anoxically for future analysis of uranium and arsenic speciation. Elevated arsenic concentrations have been seen in this bioreduced area, and PFMs may be able to provide insight into the source and fate of arsenic.

Uranium is desorbed from the resin using nitric acid. Multiple extractions using ~4 grams of resin and 40 mL of a 1% acid yielded the most consistent results with minimal waste. Resins having low sorbed uranium concentrations had typical desorption patterns, in that uranium concentrations decreased in each subsequent extraction. For resins with higher concentrations of uranium, due to higher groundwater fluxes, different desorption patterns are observed. The second uranium extraction yields the highest concentrations. Reasons for this observation are being investigated and may involve competition for exchangeable anions. With higher groundwater flux, higher masses of all contaminants and anions are sorbing to the PFM. Sulfate, a potentially competing ion for desorption, is present at high concentrations in these wells. It is preferentially desorbed over uranium in the first extraction. These desorption studies are necessary to successfully measure uranium fluxes using PFMs at field sites.



## Microbial Fe(III)-Phyllosilicate Reduction in Subsurface Sediments

*Student Abstract*

T. Wu, E. Shelobolina, H. Xu, and E. Roden (lead PI)—*U. of Wisconsin-Madison*;  
R. Kukkadapu, *PNNL*

Recent studies indicate that Fe(III)-bearing phyllosilicate minerals (e.g., smectite, illite) can be important electron acceptors for dissimilatory metal-reducing bacteria in subsurface sediments where stimulation of microbial metal reduction is employed to retard the mobility of radionuclide contaminants. The goal of this research is to compare and quantify experimentally the kinetics of Fe(III)-bearing phyllosilicate versus Fe(III) oxide reduction in DOE-relevant subsurface sediments. A key first step in pursuit of this goal was to separate phyllosilicate and Fe(III) oxide phases to permit experimentation with phyllosilicates in isolation. Physical separation through density gradient centrifugation only partially separated phyllosilicate and oxide phases in sediment from Area 2 at the Field Research Center at Oak Ridge National Laboratory (ORFRC). Hence, we examined the ability of chemical extraction methods to remove Fe(III) oxides phases without altering the properties of the phyllosilicates. Ammonium oxalate in the presence of a small amount of Fe(II) was used to extract both amorphous and crystalline Fe(III) oxides without changing the redox state of phyllosilicates. XRD analysis revealed, however, that both oxalate alone and oxalate with Fe(II) altered the structure of Fe(III)-bearing smectite in the Area 2 sediment. In contrast, citrate-dithionite-bicarbonate (CDB) extraction followed by reoxidation with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) led to minimal alteration of smectite structures. Acetate/fumarate-grown *Geobacter sulfurreducens* cells were used to evaluate the microbial reducibility of Area 2 sediments extracted by different procedures. Ammonium oxalate+Fe(II) extracted sediments were reduced more than CDB extracted,  $\text{H}_2\text{O}_2$  reoxidized solids, consistent with the apparent destabilization of smectite phases during oxalate-promoted dissolution revealed by XRD. CDB extraction was adopted to isolate Fe(III)-bearing phyllosilicates for microbial reduction experiments with ORFRC Area 2 and Atlantic coastal plain sediments from Oyster, VA. The extent of Fe(III)-bearing phyllosilicate reduction detected by chemical extractions (0.5M HCl and HF) was comparable to values observed in Mössbauer studies of phyllosilicate and Fe(III) oxide reduction in ORFRC sediments (Kukkadapu et al., *Geochim. Cosmochim. Acta*, 2006). The extent of phyllosilicate Fe(III) reduction observed in these experiments is being compared to that determined by Mössbauer through an EMSL user facility collaboration between E. Roden and R. Kukkadapu at PNNL. In related research, microbial reduction experiments were conducted with different size fractions (<2  $\mu\text{m}$ , 2–50  $\mu\text{m}$ , and >50  $\mu\text{m}$ ) of ORFRC Area 2 and Oyster, VA sediment in which the relative abundance of phyllosilicate vs. Fe(III) oxide content was determined by CDB and HF extraction. We observed a positive correlation between extent of reduction and the abundance of phyllosilicate-Fe(III) compared to total Fe(III); the Fe(III)-bearing phyllosilicates rich silt-size fraction showed the highest degree of reduction (25%). These results suggest a central role for phyllosilicates in overall microbial Fe(III) reduction potential in this material.

## Investigation of Low-Frequency Dielectric Spectroscopy of Sulfate-Reducing Bacteria (*Desulfovibrio vulgaris*) Cell Suspensions

*Student Abstract*

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I am utilizing dielectric spectroscopy measurements to quantify microbe-mineral transformations in order to improve the interpretation of geophysical signatures recorded in *in situ* bioremediation experiments (e.g., RIFLE-IFRC).

Electrical measurements have repeatedly been shown to be sensitive to microbial growth and microbial induced alterations to geologic material, indicating that electrical methods may serve as a non-invasive tool for bioremediation monitoring. However, the inherent dielectric properties of microbes themselves and how they might directly contribute to the electrical responses observed during bioremediation process are poorly understood. Commonly used in bioremediation, sulfate-reducing bacteria (SRB) are anaerobic bacteria and good candidates for toxic metal removal. As a first step towards improving the understanding of role of SRB in generating electrical signals from microbial-mineral transformations in porous media, I am studying the low-frequency dielectric properties of sulfate-reducing bacteria (*Desulfovibrio vulgaris*) in cellular suspensions during biomineral

To date, I have acquired precise dielectric dispersion curves of *D. vulgaris* cell suspensions over the frequency range 0.1 Hz to 1M Hz using dielectric spectroscopy. *D. vulgaris* cells were grown at 30°C under anaerobic conditions for 48 hours, and then suspended in 5 mM HEPES buffer. Different concentrations of *D. vulgaris* cell suspensions were placed between two parallel stainless steel electrodes that are enclosed in a cylindrical glass tube. The distance between the electrodes was 3 mm, and the electric field was 0.15 V. The complex impedance of the sample was measured relative to a known resistor. Electrode polarization impedance arising at the interface between electrodes and ionic solutions at low frequencies (< 1000 Hz) has traditionally complicated interpretation of this technique. I have adopted a simple and robust strategy to measure, analyze, and remove this polarization impedance, and the feasibility of this polarization removal technique has been tested on water-saturated silica beads. I have found that the broadband dielectric response of *D. vulgaris* cell suspensions can be precisely determined using this approach, with the increase of cell concentration being proportional to the increase in dielectric permittivity at low frequencies. I have modeled these measurements assuming a dilute suspension of polarizable particles embedded in a nonpolarizing medium, with the polarization attributed to the surface charge on the cell walls. My results suggest that quantitative prediction of pore- and nano- (cell wall) scale microbe-mineral transformations may be possible from electrical data, and provide insights into the likely contribution of the cells themselves to electrical signals previously observed during biomineralization processes. My future work will concentrate on measuring and modeling varying thicknesses of FeS mineral growth in/on the *D. vulgaris* cell membrane.