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## University-Led Research Abstracts

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# University-Led Research

## Induced Polarization Signature of Biofilms in Porous Media: From Laboratory Experiments to Theoretical Developments and Validation

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Our newly funded grant is focused on characterizing the major components and processes within bacterial biofilms contributing to detectable biogeophysical signals, spectral induced polarization (SIP) signatures in particular. Although the SIP technique has emerged as the technique most sensitive to the presence of microbial cells and biofilms in porous media, mechanistic aspects of the relationships between biological interactions with geologic media and the ensuing SIP response remain poorly understood largely because of competing effects of a myriad of contributing factors. Thus it is often difficult to unambiguously distinguish the impact of multiple and often competing processes that occur during *in situ* biostimulation activities on the SIP signatures. Through geophysical laboratory column experiments, coupled with time-lapsed synchrotron based X-ray microtomography (CMT) and Scanning Confocal Laser Microscope (SCLM) analysis of biofilms we propose to: (i) evaluate the contribution of biofilm components to SIP signatures using field relevant organisms, (ii) determine the contribution of nanoparticulate biogenic minerals in biofilms to SIP signatures, (iii) determine if the SIP signatures can be used to quantify the rates of biofilm formation and biogenic mineral accumulation in subsurface media, (iv) develop a fundamental understanding of potential underlying polarization mechanisms at low frequencies (<40 kHz) resulting from the presence of microbial cells and biofilms, and (v) evaluate the use of reactive transport models to predict the geophysical response associated with the development of biofilms in field conditions.

Although we are still in the very early stages of our project, so far we have started by expanding the POLARIS model initially developed to model the induced polarization response of silicate materials to the modeling bacteria growth in porous media. Our model results suggest that the polarization of the electrical double layer coating the surface of bacteria increases the quadrature conductivity of the porous material. Thus the change in quadrature conductivity can be directly related to changes in the specific surface area of the bacteria and to the increase in cell density/population due to bacterial growth. We tested this new model using the experimental dataset of Davis et al. (2006) and found that the model is able to reproduce the magnitude of the polarization in the experimental dataset. We conclude that this new model allows us to quantitatively assess SIP signatures of bacterial growth in complex environments.

The next phase in our project is to evaluate the contribution of biofilm components to SIP signatures using field relevant organisms. We will begin by examining the effects of exopolymeric substances (EPS) and assess how the latter differs from the effect of high cell density.

## **Integrated Microscopic and Metagenomic Analysis of Subsurface Microorganisms that Contribute to Carbon and Metal Cycling**

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Our research is conducted at the Department of Energy’s (DOE) Rifle Integrated Field Research Challenge (IFRC) site in Rifle, Colorado, USA. Microscopic analyses have advanced our understanding of well-studied bacteria that proliferate during initial subsurface acetate biostimulation and provided insight into the novel diversity that appears during secondary stimulation.

*Geobacter* species are important planktonic Fe-reducing bacteria (FeRB) abundant during primary stimulation (2010). We applied a new sample preparation method in which samples were cryo-plunged directly on site immediately after sampling (Comolli *et al.* 2011). Cells and cell-associated minerals were analyzed using 2D and 3D cryo-TEM, high resolution TEM (HRTEM), energy dispersive spectroscopy (EDS) and scanning transmission X-ray microscopy (STXM). Confocal laser scanning microscopy (CLSM) performed on cells labeled with *Geobacter*-specific fluorescence *in situ* hybridization (FISH) probes confirmed cell identities. This approach revealed that *Geobacter* accumulate remarkable aggregates of Fe-oxyhydroxide nanoparticles capable of providing sufficient electron acceptor to support planktonic growth and cell motility. To characterize relevant physiological states and expressed metabolic pathways more completely, correlated cryo-TEM and proteomic profiles associated with different growth stages and electron acceptors for *Geobacter* grown in laboratory experiments are under way. The development of antibody-based high-resolution image labeling is under way using *c*-type cytochromes detected in outer-membrane and extracellular protein fractions.

An experiment was designed partly to test the hypothesis that novel “Candidate Division” (CD) bacteria are active during secondary acetate biostimulation (2011). Clone library analysis of filtrate that passed through a 0.2  $\mu\text{m}$  filter but is retained on 0.1  $\mu\text{m}$  filter revealed that CDs OD1 and OP11 were dominant members of the community. Flash-frozen cryogenic groundwater samples (09/03/2011) were characterized via 2D and 3D cryo-TEM. We have identified and characterized highly unusual, ultra-small cells (200 - 250 nm in diameter), and are currently elucidating the 3D ultrastructure and associations. These data show median cell sizes below previously estimated lower size limits for life (NRC 1999). Evidence of unique spatial optimization strategies are evident in their 3D architecture, such as: a very compact packaged genome, distribution of ribosomes and polysomes, and cell wall-attached ribosomes. The cell wall has a novel architecture, with a remarkable and distinct surface layer, and several appendages. Higher resolution structure determination of the macromolecular components is under way. While uncultured, both OD1 and OP11 cells are inferred from Rifle community genomics data to have small genomes, lack a Gram-negative cell envelope, and encode pili, consistent with these observations (Wrighton *et al.*, submitted). Work is ongoing to perfect fluorescence *in situ* hybridization probes that would correlate cell structure with identity. Metagenomic analysis suggests that both OD1 and OP11 are obligate fermentative bacteria, and likely play a carbon cycling role in the Rifle subsurface.

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

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

The sorption of Np(V), the dominant oxidation state of Np in the environment, onto sand from the SRS does not occur under any environmentally relevant conditions. In contrast, Np(V) is sorbed onto clay substrate under slightly basic conditions. XPS and EDS spectroscopy were used to probe the specific minerals that the Np(V) is associated with, and invariably the Np(V) is found associated with Fe minerals. In addition, the transport of Np(V) has been shown to be mitigated by the precipitation of U(VI) minerals that act as carriers for Np(V).

Th(IV) [as an analogue for Pu(IV)] transport experiments are being conducted using columns packed with SRS geomeedia. At low pH (pH=4) and high ionic strength (I=0.1 M) conditions, Th(IV) transport is strongly retarded by the soil. However, a decrease in ionic strength to I=0.001 M caused colloids to be released from the geomeedia. In addition to colloids, high concentrations of Th(IV) were also detected in the effluent samples immediately after the change in solution chemistry. However, after measuring dissolved and total Th(IV) concentrations, it was determined that colloid-facilitated transport was not the predominant cause for peaks in Th(IV) concentration up to ten times that of the influent solution. Instead, a drop in pH caused by the change in solution chemistry was responsible for the peaks in Th(IV) concentration. Depending on the surface charge of the geomeedia, fluctuations in ionic strength can cause pH fronts to occur, producing dramatic effects on the transport of strongly actinides that can cause effects more pronounced than conventional colloid-facilitated transport.

The Advanced Light Source-Molecular Environmental Sciences (ALS-MES) Beamline scanning transmission X-ray microscope (STXM) successfully imaged and conducted near-edge X-ray absorption fine structure (XAFS) spectroscopy studies on sets of particulates derived and prepared from several sources within the project. One of the most interesting results supported by the STXM experiments, in addition to laboratory studies, was that U interactions with the non-hydrophobic components of the humic acids studied were more important than anticipated. Additional STXM experiments on particulates obtained from column experiments provided information about elemental associations and the presence of C on the particles. Upcoming STXM experiments will continue these investigations and will initiate new experiments as beamtime permits. Hard X-ray XAFS studies were also conducted on representative U, Np, and Pu materials obtained from selected materials from the sorption experiments.

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

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The U.S. Department of Energy Hanford 300 Area (300A) site experiences periodic hydrologic influences from the nearby Columbia River as a result of changing river stage, which causes changes in groundwater elevation, flow direction and water chemistry. An important question is the extent to which the mixing of Columbia River water and groundwater impacts the speciation and mobility of uranium (U). This year, we designed experiments to mimic interactions among U, oxic groundwater or Columbia River water, and 300A sediments in the subsurface environment of Hanford 300A. The results revealed that U was immobilized by 300A sediments predominantly through reduction (80–85%) when the column reactor was fed with oxic, organic-amended synthetic groundwater (OA-SGW). The reduced U in the 300A sediments fed with OA-SGW was relatively resistant to remobilization by oxic Columbia River water. Oxic Columbia River water resulted in U remobilization (~7%) through desorption, and most of the U that remained in the 300A sediments fed with OA-SGW (~93%) was in the form of uraninite nanoparticles. These results revealed that: 1) the reductive immobilization of U through OA-SGW stimulation of indigenous 300A sediment microorganisms may be viable in the relatively oxic Hanford 300A subsurface environments and 2) with the intrusion of Columbia River water, desorption may be the primary process resulting in U remobilization from OA-SGW-stimulated 300A sediments at the subsurface of the Hanford 300A site.

In continuation of our work related to understanding how heavy metals interact with subsurface biofilms at the microscale, noninvasive nuclear magnetic resonance imaging (MRI) and spectroscopy (MRS) approaches were used to monitor spatiotemporal responses of live *S. oneidensis* MR-1 biofilms to U(VI) and Cr(VI). MRI and spatial mapping of diffusion in the biofilms revealed that, although the overall biomass distribution was not significantly altered upon exposure to U(VI) or Cr(VI), microenvironments in the biofilm matrix changed as indicated by localized changes (~ 20 microns) of water diffusivity in the biofilms, suggesting potentially important contaminant-induced changes in structural or hydrodynamic properties of biofilm matrix upon exposure to the metals. We also quantitatively demonstrate that the responses of cellular metabolism in biofilms interacting with environmental contaminants are spatially stratified, implying the possibility that the strategies for detoxification or adaptation utilized by cells in biofilms may be altered in response to the local microenvironments. Lastly, we developed a 2-dimensional mathematical model to predict substrate utilization and metabolite production rates in *Shewanella oneidensis* MR-1 biofilms as well as U immobilization by considering reduction and adsorption processes in the cells and in the extracellular polymeric substances (EPS). The model included the production of EPS using the experimental data we generated last year. The EPS bound to the cell surface and distributed in the biofilm were considered as bound EPS (bEPS) and loosely-associated EPS (laEPS), respectively. COMSOL® Multiphysics finite element analysis software was used to solve the model numerically. We used a custom designed biofilm reactor placed inside a nuclear magnetic resonance (NMR) micro-imaging and spectroscopy system, and monitored substrate utilization and metabolite production rates to compare model with experimental data.

## Isotopic Characterization of Biogeochemical Pools of Mercury and Determination of Reaction Pathways for Mercury Methylation

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The project objective is to use stable Hg isotope measurements to reveal locations of Hg methylation in the Poplar Creek (PC) watershed, TN and place new constraints on the processes that lead to methyl mercury (MeHg) production, transport and degradation. We seek to better understand the decoupling of inorganic Hg and MeHg concentrations in the East Fork PC (EFPC) ecosystem. We are using a two-pronged approach that involves 1) an ecosystem level study of natural samples, and 2) experimental studies of Hg isotope fractionation. Natural samples are being collected from wetland sediments, stream sediments and waters, stream bottom periphyton and, as a monitor of the MeHg in the ecosystem, young-of-year herbivore/ detritivore stoneroller minnows and omnivore/piscivores redbreast sunfish. During the first 6 months of this new project we: 1) completed a sampling campaign for water, sediment and periphyton from EFPC, Hinds Creek and adjacent wetlands, 2) obtained archived samples of fish tissues from the ORNL Ecol. Assess. Group, 3) obtained splits of samples of Clinch River, PC, and EFPC sediments from TN Dept. of Envir. and Conser., 4) completed Hg isotopic analyses of sediments, suspended particulates and periphyton, and 5) conducted preliminary experiments on Hg(0) oxidation by natural organic matter (NOM) to investigate Hg isotope fractionation.

Sediment samples from the Clinch River upstream of the confluence with PC (and from Hinds Creek) establish very low background Hg concentrations [Hg] of 11-23 ng/g. The background mass dependent isotopic composition displays a narrow range ( $\delta^{202}\text{Hg}_{\text{NIST-3133}} = -1.43$  to  $-1.46\text{‰}$ ) and there is a deficit of the odd mass isotopes ( $\Delta^{199}\text{Hg} = -0.24$  to  $-0.29\text{‰}$ ). Two samples from the Clinch River downstream of PC have elevated [Hg] of 242 and 763 ng/g, with Hg isotopic compositions ( $\delta^{202}\text{Hg} = -0.26$  and  $-0.31\text{‰}$ ;  $\Delta^{199}\text{Hg} = -0.07$  and  $-0.08\text{‰}$ ) that are highly contrasting from background values. Sediments from PC below EFPC have even more highly elevated [Hg] (2150 to 3870 ng/g) and isotopic compositions close to the downstream Clinch River samples ( $\delta^{202}\text{Hg} = -0.13$  to  $0.07\text{‰}$ ;  $\Delta^{199}\text{Hg} = -0.02$  to  $-0.13\text{‰}$ ). Suspended sediment in EFPC collected near the Y-12 outflow pipe has  $\delta^{202}\text{Hg} = -0.63\text{‰}$  and  $\Delta^{199}\text{Hg} = 0.03\text{‰}$ . Isotopic values of suspended sediment and periphyton change systematically with distance downstream reaching values of  $\delta^{202}\text{Hg} = 0.07\text{‰}$  and  $\Delta^{199}\text{Hg} = -0.12\text{‰}$  after 19 km. Patterns in Hg isotopic variation in stream sediments around the PC watershed can be summarized as follows. 1) Background sediment [Hg] is very low with a narrow range of Hg isotopic composition. 2) Sediments in EFPC near Oak Ridge have much higher [Hg] and have  $\delta^{202}\text{Hg}$  and  $\Delta^{199}\text{Hg}$  that are highly contrasting from background values. 3)  $\delta^{202}\text{Hg}$  and  $\Delta^{199}\text{Hg}$  in EFPC sediments vary with distance downstream likely due to fractionation associated with chemical transformations of Hg. 4) When PC mixes into the Clinch River the [Hg] and Hg isotopic compositions of sediments shifts dramatically; consistent with the addition of ~10% sediment from PC.

Preliminary experiments were conducted to investigate Hg isotope fractionation during dark and photochemical oxidation of Hg(0) by NOM. Results from dark oxidation of Hg(0) by reduced soil humic acid suggest that both mass dependent and mass independent isotope fractionation occur, and that the oxidation enriches heavy isotopes in the product Hg(II), which is opposite to the Hg(II) reduction processes. Results of photo-oxidation experiments indicate that the presence of NOM can substantially enhance the oxidation rate of Hg(0) particularly in EFPC water.

## Reactivity of Iron-Bearing Phyllosilicates with Uranium and Chromium Through Redox Transition Zones

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Operational extractions for the measurement of valence state-specific concentrations of Fe, U and Cr need to minimize secondary reactions that might occur in extract solutions. Because the reduction potentials for Fe and U species are similar, the thermodynamic favorability of one particular redox transformation (e.g., U(IV) O<sub>2</sub> oxidation by Fe(III)) may increase during the extraction phase but may not have been important under the geochemical conditions in which the sample was collected. In this scenario, the measured concentrations of U(IV/VI) and Fe(II/III) would not reflect the speciation in the sample but instead the speciation in the extract solution. To avoid these analytical problems when working iron(II/III)-bearing phyllosilicates, U(IV/VI), and Cr(III)/Cr(VI), we have developed a sequential extraction method as an alternative method for the conventional parallel extractions for (e.g., HF-H<sub>2</sub>SO<sub>4</sub>-phenanthroline for clay-Fe(II), and NaHCO<sub>3</sub> for U(VI)). We demonstrate that any extraction artifacts can be eliminated by using a sequential extraction procedure where U is first solubilized into H<sub>3</sub>PO<sub>4</sub> and physically separated from the clay by centrifugation, and then the clay-Fe(II) is measured by HF-H<sub>2</sub>SO<sub>4</sub>-phenanthroline. A similar procedure is developed for Cr measurement. Using this this new procedure, we demonstrate excellent stoichiometric agreement.

Using the new procedure, we have performed Cr reduction experiments using clay-Fe(II). Nontronite (iron-rich smectite), smectite, chlorite, and Hanford site sediments were first bioreduced with *Geobacter sulfurreducens*. After pasteurization and removal of aqueous Fe<sup>2+</sup>, clay-Fe(II) was used to reduce Cr(VI) at 10°, 20°, and 30°C to mimic natural conditions at DOE contaminant sites and to obtain activation energy of the reaction. The clay reactivity was strongly dependent on temperature. At 10°C, nontronite was most reactive and chlorite was not reactive at all, despite a large amount of Fe(II) in its structure. The reactivity of the Hanford site sediments was between these two end members. At the end of Cr(VI) reduction, there were various amounts of Fe(II) remaining in the clays. In order to estimate the capacity of clay-Fe(II), multiple spikes of Cr(VI) were added to the system. The rate of Cr(VI) reduction slowed with each spike. At the end of several spikes, there was still 20% structural Fe(II) in nontronite remaining that was considered not reactive. At 30°C, the rates of Cr(VI) reduction by clay-Fe(II) were several times higher than at 10°C. Nontronite was still most reactive and its structural Fe(II) was fully consumed upon two spikes of Cr(VI). At this temperature, even chlorite became reactive, but about 50% structural Fe(II) was still remaining after several spikes of Cr(VI).

Scanning and transmission electron microscope (SEM and TEM) observations revealed that reduced Cr was present in micro-structural associations with the clay minerals. Focused ion beam (FIB)-SEM and TEM observations and electron energy loss spectroscopy (EELS) revealed that reduced Cr was likely in the form of Cr(OH)<sub>3</sub>. The association of reduced Cr with low-permeability clay minerals would minimize any chance of Cr reoxidation and remobilization. The clay minerals are currently undergoing redox cycling to assess any change of clay properties and their reactivity toward Cr under redox-fluctuating conditions.

## Inorganic Controls on Neptunium-237 Mobility in the Subsurface

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### Objective

Neptunium-237 has a long half-life (2.14 million years) and is highly soluble in near-surface groundwater in the pentavalent oxidation state. It is identified as a subsurface contaminant of concern by the DOE. As co-precipitation of Np into minerals may be important in determining the long-term behavior of this radionuclide in the subsurface, we aim to develop a detailed understanding of the factors, including structural constraints, that impact co-precipitation of Np(V) into minerals. To achieve this aim, we have the following working objectives:

1. To develop syntheses that show incorporation of Np(V) by rock-forming minerals, and
2. To investigate the structural mechanisms through which Np(V) is incorporated
3. To facilitate a comparative investigation of the inorganic factors valid to understanding the environmental fate of Np(V)

### Results/Plans

The synthesis of a variety of mineral phases has allowed us to make preliminary contaminant uptake comparisons based on the steric constraints of the mineral structures. As of March 2012, fourteen mineral phases have been synthesized for this study. These include carbonates, sulfates, nitrates, phosphates, and a borate. Stage 1, the optimization of these mineral phases, is complete.

In the second stage, the mineral phases are synthesized in the presence of actinides—uranium and neptunium, separately. As of March 2012, eight mineral phases have been synthesized in the presence of uranium and neptunium for this study. These include carbonates, sulfates, and a borate.

Methods of synthesis, including a new method for the synthesis of calcite, and characterization of the phases by ICP-MS in solid and solutions modes will be presented and discussed. We will present hypothetical models for the incorporation of Np(V) into the crystalline structures of carbonates and sulfates along with a side-by-side comparison of the neptunium uptake potential for a variety of mineral phases.



## ***In Situ* Generation of Iron–Chromium Precipitates for Long-Term Immobilization of Chromium at the Hanford Site**

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*In situ* Cr immobilization involves either microbial or abiotic reduction of Cr(VI), followed by precipitation of the much more insoluble Cr(III), typically in the form of a Fe(III)–Cr(III) (Fe–Cr) precipitate when Fe minerals are present. Numerous geochemical variables can impact the solubility of such Fe–Cr precipitates, including the rates of Cr(VI) reduction and Fe–Cr precipitation, particle size, and the Fe:Cr ratio. Poorly crystalline minerals, typically formed upon rapid precipitation, tend to be more soluble than highly crystalline minerals that are precipitated more slowly. Reoxidation of Cr(III) to Cr(VI) by manganese (Mn) oxides is well known, with the reaction taking place between dissolved Cr(III) and the solid Mn(III/IV) oxide surface. This means that many factors that influence the solubility of Fe–Cr precipitates also likely influence the rate of Cr(III) oxidation by Mn oxides.

The overall objective of this research is to determine the factors that most strongly influence the solubility of Fe–Cr precipitates formed in microbial and abiotic systems. Specific objectives are to: (1) measure the rates of Cr(VI) reduction by Fe(II) minerals and microorganisms (in separate microcosms) and measure the equilibrium solubility of the resulting Fe–Cr precipitates; (2) characterize the size, crystal structure, and composition of selected Fe–Cr precipitates that vary in solubility; and (3) measure oxidation rates of dissolved Cr(III) in equilibrium with Fe–Cr precipitates by birnessite. Abiotic systems will contain FeS or dithionite-reduced iron-rich nontronite. Microbial systems will contain one of three pure cultures of Cr(VI)-reducing bacteria isolated from the Hanford site, along with small quantities of hematite, Al-substituted goethite, or iron-rich nontronite as a source of Fe. Work to date includes preliminary experiments with pure cultures of Cr(VI) reducing bacteria and preparation/purification of Fe(III) oxides and clay minerals.

We hope that this research will lead to a more systematic understanding of how specific methods for *in situ* Cr immobilization in the presence of Fe (e.g., microbial reduction in the presence of Fe(III) oxides or abiotic reduction by Fe(II) minerals) affect the structure and solubility of the resulting Fe–Cr precipitate, and, in turn, how these properties affect the rate of reoxidation of Fe–Cr precipitates to Cr(VI) by birnessite. This knowledge will lead to a better understanding of how to generate Fe–Cr precipitates *in situ* that will be oxidized to Cr(VI) to the smallest extent over long time periods.

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

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Our proposition targets a critical element of the Environmental Remediation Science Program (ERSP) research portfolio, which is the development of enabling scientific tools for characterizing the spatial and temporal evolution of complex subsurface systems. We are developing a field-portable, on-site sensing device for real-time speciation of elemental and inorganic mercury in surface stream waters, groundwater, and sediment samples. The sensing device relies on the combination of two well-known phenomena, i.e. the amalgamation of metallic mercury (Hg(0)) and gold (Au) and the Surface Plasmon Resonance (SPR) of gold nanorods (Au NR). When Au NR are exposed to the presence of Hg(0), its amalgamation to Au causes a reduction of the effective aspect ratio of NR and a blue shift of the maximum absorption wavelength of the longitudinal mode band. The linear correlation that exists between the aspect ratio of Au NR and the position of the maximum wavelength of the longitudinal mode of the SPR makes quantitative analysis possible. Efforts have been primarily directed towards the following: (a) Development of an immobilization procedure for Au NRs to produce Hg (0) detection substrates; (b) Analytical figures of merit for Hg (0) detection with various types of Au NRs when in solution and immobilized on the detection substrate under static conditions; (c) Interference studies with synthetic interference mixtures; (d) Evaluation a flow injection analysis (FIA) system for Au nanoparticles detection; and (e) Development of Hg (II) and Hg (I) reduction techniques and speciation strategy.

Scanning electron microscopy (SEM) and absorption spectroscopy confirm a well-dispersed deposition of NRs on the surface of the solid substrates. Calibration curves with NRs in aqueous solutions were built for six NRs samples with different maximum absorption wavelengths (610, 615, 630, 690, 730 and 740). The 630nm NRs exhibited the greatest slope and were the most sensitive to the concentration of Hg (0). Their limit of detection was approximately  $10^{-20}$  M/NR. The immobilized NRs showed a limit of detection ( $10^{-19}$  M/NR) approximately one order of magnitude better than their counterparts suspended in liquid solutions. Exposure of Au NRs substrates to several inorganic ions showed no interference. A one-line and a two-line FIA system were implemented in our lab to monitor Au nanoparticles in water flows. On-going studies are focusing on the following: (a) extending FIA measurements to monitor Au NRs substrates exposed to ORNL contaminated water flows; (b) reducing mercury species via a cyclic voltammetry (CV) speciation procedure; and (c) combining CV with SPR-Au NRs spectroscopy for the detection of mercury species.

## Dominant Mechanisms of Uranium-Phosphate Reactions in Subsurface Sediments

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Widespread subsurface contamination at DOE sites has resulted from past mining, processing, and waste disposal processes. *In situ* remediation technologies can sequester uranium on site by transforming uranium to stable, low solubility species. While substantial recent research has investigated biogeochemical U(VI) reduction, this approach may have limited long-term sustainability because of the potential for reoxidation to occur. Phosphate addition is an alternative approach that can enhance the sequestration of uranium without requiring sustained reducing conditions. However, the geochemical factors that determine the dominant immobilization mechanisms are insufficiently understood to design efficient remediation strategies or accurately predict uranium transport in treated systems.

The overall objective of our project is to determine the dominant mechanisms of U(VI)-phosphate reactions in subsurface environments. Specific objectives are to: (1) resolve uncertainty regarding the specific U(VI) phosphate solids that form in homogeneous solutions; (2) determine the molecular mechanisms controlling U(VI) speciation in heterogeneous phosphate-bearing systems and the conditions where specific mechanisms dominate; (3) characterize how the interaction of competitive and cooperative reactions controls uranium speciation in sediments; (4) identify chemical divides that separate regimes where specific mechanisms dominate U(VI) speciation in subsurface sediments. In the first six months of this project our team has focused on meeting objectives 1 and 2.

For objective 1 we have investigated homogeneous precipitation of U(VI) phosphates to determine the conditions where specific phases form. Studies to date have focused on systems lacking simple inorganic electrolyte cations in order to characterize the relative stability and occurrence of chernikovite [ $\text{H}_3\text{OUO}_2\text{PO}_4 \cdot 3\text{H}_2\text{O}$ ] and uranyl orthophosphate [ $(\text{UO}_2)_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ ]. Precipitation is initially rapid upon supersaturation and equilibrium is approached within 4 days. Chernikovite forms at all conditions studied; it may co-form with uranyl orthophosphate at pH 4 and high phosphate conditions. Solid characterization by XRD, SEM, EXAFS, and TRLFS is in progress. We plan to explore the effects of electrolyte cations (Na, Ca) and competition with calcium phosphate precipitation over the next six months.

For objective 2 we have investigated U(VI) and phosphate co-sorption on smectite to determine if substantial ternary complexation occurs and to characterize the transition between adsorption and precipitation. Wet chemistry studies show that phosphate has little effect on U(VI) adsorption at pH 4, 6, and 8 prior to the onset of U(VI) phosphate precipitation. Phosphate adsorption is low and these observations suggest that, unlike for iron oxide minerals, little U(VI)-phosphate ternary complexation occurs on smectite. The effect of phosphate on U(VI) adsorption mechanisms and U(VI) phosphate nucleation on smectite is currently being investigated by an integrated series of EXAFS and TRLFS measurements. Over the next six months we plan to determine the effects of Ca on U(VI) adsorption to smectite in the presence of phosphate and extend this work to goethite. Our efforts will then transition to characterizing U(VI)-phosphate reactions in sediments obtained from the Rifle and Hanford IFRCs.

## Isolation of Fe-Oxidizing Microorganisms from the Rifle IFRC Site: Toward Understanding Post-Biostimulation Permeability Reduction and Oxidative Processes

Clara Chan (PI), Kevin Cabaniss, Chaofeng Lin—*U. of Delaware*; Ken Williams, *LBNL*

In order to optimize bioremediation of subsurface uranium contamination, we need to understand effects on aquifer permeability, as well as biogeochemical processes that occur after biostimulation ceases. At the Rifle IFRC, injection of organic carbon stimulates the production of reduced Fe and S chemical species and also results in significant permeability decreases especially within injection wells. After biostimulation ceases, reduced Fe and S species may be oxidized by microbes that produce Fe oxides, elemental S(0), and biomass that can clog pores. Additionally, Fe oxides may sorb uranium, thereby influencing its mobility. The overall goal of our exploratory project is to evaluate whether Fe and S oxidizers are indeed present, active, and contributing to post-biostimulation element cycling and pore-scale modification at the Rifle site.

Our first step involved demonstrating the presence of Fe- and S-oxidizing microorganisms (FeOM and SOM) in the Rifle aquifer. We use culturing methods because (1) we are still discovering the diversity of FeOM, so gene (e.g. 16S) similarity will not necessarily reveal FeOM presence and (2) because there are no well-characterized Fe oxidation genes to definitely show presence and activity. In order to sample active FeOM, we periodically monitored wells with a downhole video camera and sampled soon after the appearance of Fe floc. In December 2011, we sampled 3 wells: CU01, a background well; CD03, a well downgradient from injection wells, exposed to acetate in 2010 and 2011; and P101, a well that experienced acetate biostimulation in 2008 and 2009. We also sampled background sediment, which had not been exposed to acetate amendment. We enriched for aerobic Fe and S-oxidizers in gradient tubes, using a mineral medium and FeS or FeCO<sub>3</sub> substrate. Only the biostimulated wells CD03 and P101 showed S-oxidizers. All groundwater and sediment samples yielded FeOM growth, confirming the presence of FeOM in both amended and unamended areas of the Rifle aquifer.

Next, we are in the process of isolating model FeOM for biomineral characterization and microslide chamber growth experiments. Initial DGGE results indicate that cultures are highly enriched, possibly pure; purity will be checked by 16S rRNA sequencing and analysis. Transmission electron microscopy and selected area electron diffraction show that CD03 cultures contain curved rod cells and lepidocrocite. Uninoculated controls also formed lepidocrocite, suggesting that the observed mineralogy is a function of aqueous chemistry.

Our future work includes laser-scanning microscopy (LSM)-monitored microslide chamber experiments with isolates and enrichments; these will allow us to visualize pore-scale processes in real time and to test hypotheses relevant to permeability alteration. We will also perform field incubations of sediments in wells during and after planned biostimulation field experiments. Field and lab samples will be analyzed by LSM, SEM, TEM, and energy dispersive X-ray spectroscopy to identify newly formed material, and quantify biomass and porosity changes. Comparison of field and lab results will give insight on how to apply experimental results to the field. The project outcomes will help achieve the Rifle and SFA goals of relating pore-scale phenomena to intermediate- and field-scale processes. The results will contribute not only to improvement of biostimulation approaches, but also towards understanding of contaminant fate post-amendment.

## Uranium and Strontium Fate in Waste-Weathered Sediments: Scaling of Molecular Processes to Predict Reactive Transport

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The need for better prediction of contaminant transport motivates multi-faceted lines of inquiry to build a strong bridge between molecular- and field-scale information. By focusing multiple lines and scales of observation on a common experimental design, our collaborative team seeks to reveal non-linear and emergent behavior in contaminated weathering systems. In prior work, we have successfully coupled weathering, adsorption and transport in geochemical models of sediments impacted by *hyperalkaline* waste streams. A goal of the current project is to expand our modeling capabilities to include *acidic* weathering reactions that, as described above, are expected to result in profoundly different products that will nonetheless undergo analogous silicate and non-silicate transformation, ripening and aging processes. We predict that these weathering reactions will vary with waste stimulant chemistry in ways that can be directly incorporated into the model structure.

Objectives of the project: (1) Determine process coupling between mineral transformation and uranium speciation change in acid waste-weathered Hanford sediments; (2) Establish linkages between molecular-scale contaminant speciation and meso-scale contaminant lability, release and reactive transport; (3) Make conjunctive use of molecular- to field-scale data to constrain the development of a reactive transport model that includes contaminant sorption-desorption and mineral transformation reactions.

Hypotheses: (1) Uranium speciation in legacy sediments from the U-8 and U-12 Crib sites can be reproduced in bench-scale weathering experiments conducted on unimpacted Hanford sediments from the same formations; (2) Reactive transport modeling of future U releases from the vadose zone of acid-waste weathered sediments can be constrained by combining molecular-scale information on contaminant bonding environment with grain-scale information on contaminant phase partitioning, and meso-scale kinetic data on contaminant release from the waste-weathered porous media; (3) Although field contamination and laboratory experiments differ in their diagenetic time scales (decades for field vs. months to years for lab), sediment dissolution, neophase nucleation, and crystal growth reactions that occur during the initial disequilibrium induced by waste-sediment interaction leave a strong imprint that persists with memory effects over subsequent longer-term equilibration time scales.

Research approach: We are developing an iterative measure-model approach that is applicable to elucidate mechanistic underpinnings of reactive contaminant transport in weathering geomedia.

Experimental design: Hypotheses are being tested by comparing the geochemical transformations and transport behaviors that occur in bench-scale studies of waste-sediment interaction with parallel solid-phase analyses of core sample extractions (scheduled for 2012–2013) from the acid uranium waste impacted U-8 and U-12 Crib sites at Hanford. Crib waste aqueous simulants are being reacted with Hanford sediments in batch and column systems. Coupling of contaminant uptake to mineral weathering is being monitored using a suite of methods both during waste-sediment interaction, and after, when waste-weathered sediments are subjected to infusion with circumneutral background pore water solutions.

Results: We are currently in year 1 of the project, and have initiated a set of bench-scale batch weathering experiments where we are reacting Hanford Sediment with acidic, uranium bearing synthetic crib waste (SCW) solutions comprising a range in pH, uranium and phosphate concentrations. Parallel homogeneous nucleation experiments are being conducted to assess precipitation of reference uranium-bearing phases from supersaturated aqueous solution. Geochemical modeling is being conducted to assess aqueous speciation and the solubility envelopes of uranium-bearing solids as a function of system composition. These initial data streams guide follow-on experimental design and reactive transport modeling. These initial data streams guide follow-on experimental design and reactive transport modeling to understand the fate and transport of U in the Hanford subsurface.

## Sequestration of Uranium in Iron-Rich Sediments under Sequential Reduction-Oxidation Conditions

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Integrated field and laboratory studies indicate that iron species play a critical role in the sequestration of uranium at DOE sites with the relatively high iron content of sediments (up to 5-6% as w/w). In a long-term study conducted in Area 3 of the ORIFRC site, intermittent addition of ethanol stimulated sulfate and Fe(III) reduction with a concomitant decrease in soluble U to low levels. Re-oxidation with oxygen and nitrate led to a rebound in soluble U. Solid phase analyses revealed the formation of Fe(III)-U(IV) solids.

Well-defined solutions of dissolved Fe(II) reduced soluble U(VI) (50 mg/L) to U(IV) precipitates when pH was increased to values that were favorable for U(VI) reduction (pH > 5.5 for these systems). When solutions containing U(IV) were then exposed to oxygen, U(IV) species were oxidized, as confirmed by XANES analysis, but almost all the oxidized U(IV) was bound to Fe(III) solids. Aqueous phase U(VI) levels were low (<0.01 mg/L) at pH > 5.5. This result establishes that U can be sequestered in the presence of Fe(III) solids. A long-term (1210-day) microcosm experiment was performed to monitor the effects of dissolved oxygen (DO) on the sequestration of bioreduced U. Two bottles containing sediment from the bioreduced zone (1404 mgU/kg) were allowed to oxidize through long-term exposure to headspace air. As oxygen penetrated the sediment, aqueous uranium initially increased from <0.03 mg/L to a peak concentration of 0.1 mg/L at 400 days, but then decreased to <0.03 mg/L. Aqueous U concentrations remained at < 0.03 mg/L, at pH 7.0-7.2, despite the presence of 5-6 mg DO/L, 0.5 mM Ca and 2 mM HCO<sub>3</sub>. This indicates that the uranium was strongly attached to the solid phase. To evaluate the impact of nitrate on U sequestration, nitrate (1.2 mM) was added to one of these bottles. A change in sediment color from black to brown indicated that the sediment was oxidized, but aqueous U concentrations did not increase. The U remained sequestered under the conditions of this incubation. Long-term field testing at ORIFRC Area 3 (over 1,000 days) has continued to examine the effects of exposure of bioreduced sediments to nitrate. Nitrate-contaminated groundwater was allowed to invade the bioreduced area via natural groundwater inflow. The geochemical response was variable depending on the proximity to the influx of contaminated groundwater from the highly contaminated source zone located up gradient. In general, the nitrate concentrations in the previously bioreduced area increased gradually from near zero to ~50-300 mM and then stabilized. The pH declined from post manipulation levels of 6.2-6.7 to below 5.0 as the site was recontaminated. Uranium concentrations rebounded in all monitoring wells but were well below upgradient contaminated groundwater levels for two years or more after active bioreduction stopped. In several monitoring wells uranium levels rebounded, declined, then a slowly rebounded as contaminated groundwater reentered the bioreduced zone. The results indicates significant sequestration of U when nitrate was allowed to re-enter the bioreduced zone. The change in microbial communities in the subsurface was monitored in relation to the reduction and oxidation processes and functional microorganisms for iron and U reduction and oxidation were identified. The results suggest that reduction followed by re-oxidation could be an effective *in situ* U sequestration strategy for iron-rich sediments.

## Subsurface Conditions Controlling Uranium Incorporation in Iron Oxides: A Redox Stable Sink

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Although large quantities of uranium can be potentially immobilized through microbially mediated U(VI) reduction, the potential for re-oxidative mobilization causes long-term stabilization to be technically challenging. Within natural environments, however, uranium often correlates with iron rather than existing as a discrete uranium oxide phase; uranium incorporation into iron oxides is a mechanism that can explain the co-occurrence of these two elements and may represent a natural attenuation pathway for uranium. Further, incorporated U(VI/V) within Fe (hydr)oxides appears stable with respect to oxidative dissolution, representing a potential means for long-term sequestration of uranium in the subsurface. Within this project we are examining the molecular mechanisms by which uranium is retained by iron oxides, conditions optimal for retention, and the long-term stability of sequestered uranium.

Uranium incorporation into an iron (hydr)oxide lattice is dependent on Fe(II) propagated transformation of ferrihydrite. However, Fe(II) can also induce reduction of U(VI) to U(IV), with subsequent precipitation of UO<sub>2</sub>, and thus represents a competing retention process for uranium. Deciphering (bio)geochemical factors influencing the predominance of each pathway is therefore critical to determine uranium fate within the subsurface. To elucidate the factors controlling uranium retention, we first conducted batch incubations where ferrihydrite slurries (100 mL of ~180 mg L<sup>-1</sup>) were reacted with U(VI) and Fe(II) under varying water compositions (varying U(VI), Fe(II), and Ca concentrations, with total carbonate at 3.8 mM and pH buffered at 7.0). Using high-resolution synchrotron X-ray powder diffraction and extended X-ray absorption fine structure (EXAFS) spectroscopy, we identified the reaction products and thus the retention pathway. Incorporation of U into goethite is the dominant sequestration pathway at < 10 μM aqueous U concentration, with lesser amounts of UO<sub>2</sub> and U(VI) adsorption contributing to uranium retention. Similarly, low Fe(II) concentrations (<0.3 mM) promote incorporation over reductive precipitation of UO<sub>2</sub>. Importantly, conditions conducive to the formation of uranyl-calcium-carbonate complexes were critical for incorporation, retarding reduction of U(VI). However, at high Fe(II) concentrations (3 mM), reduction was the dominant sequestration pathway independent of uranyl speciation or concentration. We further investigated the impact of mineralogical impurities common to the subsurface and found that although Al can inhibit ferrihydrite transformation, U(VI/V) incorporation occurred even at Al contents as high as 20% in Al-ferrihydrite. The combination of Ca-UO<sub>2</sub>-CO<sub>3</sub> aqueous complexes and structural Al in ferrihydrite, however, resulted in limited incorporation or reduction. Our results demonstrate that U incorporation can be a major U sequestration pathway across a variety of aqueous phase and mineralogical conditions. However, multiple retention processes of uranium will occur in the subsurface and it is critical to appreciate the dominant pathway when considering the long-term fate of this hazardous element.

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

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The objectives of our project are to: (1) Determine if the trajectories of microbial community structure, composition, and function following organic carbon (OC) amendment can be related to, and ultimately predicted through evaluation of key determinants. (2) Assess the relative importance of the characteristics of the indigenous microbial community, sediment, groundwater, and OC supply rate as major determinants of microbial community functional response and bioremediation capacity. We are using sediments from three DOE sites: Oak Ridge, TN; Rifle, CO; and Ringold formation, Hanford, WA; in a full-factorial reciprocal transplant experiment that includes sterile controls. Each sterilized sediment/inoculum combination receives OC supplied as sodium-acetate at two concentrations in synthetic groundwater that approximates the *in situ* conditions at each site. Sediments from the three sites were sieved, homogenized and a portion sterilized by gamma-irradiation. Sterilized sediments were inoculated with a small mass (2.0 g) of “live” sediment, thoroughly mixed and packed into flow-cells. The 2.0 g mass of inocula was selected following a gene-sampling-effort relationship experiment using PhyloChip and GeoChip arrays to determine the minimum mass required to provide inocula with reproducible phylogenetic content and functional capacity.

The 174 flow-cells constructed for this experiment are operating in an anaerobic chamber with synthetic anaerobic groundwater. All cells received groundwater without OC for approximately 6 weeks. The first destructive sampling of flow-cells (T0) occurred prior to OC addition to establish a baseline for the subsequent trajectory analysis. The remaining flow-cells are supplied with two OC supply rates: 0.1 and 1.0 mmol OC/kg/day. The OC supply rates were chosen based on prior experiments using Oak Ridge, TN sediment. Early results suggest inoculum and sediment-specific functional responses. Trajectory analysis will be performed as communities evolve over several months of biostimulation.

Remediation of subsurface metal contaminants at DOE sites involves microbial mechanisms of oxidation/reduction or complexation; which is 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.



## Combined Effects of Hydrology, Geochemistry, and Biology on the Remediation of U(VI) in Microfluidic Experimental Systems (Micro Models)

Kevin T. Finneran (PI), Timothy Strathmann (co-PI), Charles Werth (co-PI)—*Clemson U.*

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

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

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

In addition, the abiotic precipitation of uranium (U(VI)) was evaluated in a microfluidic pore network (i.e. micromodel) to assess the efficacy of using a phosphate amendment to immobilize uranium in groundwater and mitigate the risk of this contaminant to potential down-gradient receptor sites. U(VI) was mixed transverse to the direction of flow with hydrogen phosphate ( $\text{HPO}_4^{2-}$ ), in the presence or absence of calcium ( $\text{Ca}^{2+}$ ) or sulfate ( $\text{SO}_4^{2-}$ ), in order to identify precipitation rates, the morphology and types of minerals formed, and the effects of mineral precipitates on pore blockage. Each reactant was initially at 100 mM. Precipitation occurred over the time scale of hours to days, and rates varied with influent conditions. Relative to when only U(VI) and  $\text{HPO}_4^{2-}$  were present, precipitation rates were 2.3 times slower when  $\text{SO}_4^{2-}$  was present, and 1.4 times faster when  $\text{Ca}^{2+}$  was present; larger crystals formed in the presence of  $\text{SO}_4^{2-}$ . Raman backscattering spectroscopy and micro X-ray diffraction ( $\mu\text{-XRD}$ ) results both showed that the only mineral precipitated was chernikovite, also known as hydrogen uranyl phosphate;  $\text{UO}_2\text{HPO}_4$ .

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

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We have continued our work on microscopic characterizations and quantifications of colloid detachment by moving air-water interfaces. Experimental and theoretical results show that the advancing air-water interface was significantly more effective in detaching colloids from a glass surface than the receding interface. For hydrophilic colloids, the advancing interface movement generally exerts a stronger detachment force than the receding, except when the hysteresis of the colloid-air-water contact angle is small and that of the solid-air-water contact angle is large. To generalize these results, we expanded our work from spherical colloids to colloids of different shapes (barrels, ellipsoids, disks). Direct measurements of capillary forces acting on natural sediment particles from Hanford showed that particle shape (and surface roughness) is an important factor controlling the magnitude of capillary forces. We experimentally distinguished between the maximum capillary force and the snap-off force when the air-water interface detaches from the particle.

We continued the monitoring of colloid transport in the field lysimeters at the Hanford 300N Lysimeter Site. The facility consists of six lysimeters, each 7.6 m deep. Fiberglass wicks were installed at 1, 2, 4, and 7 ft below surface to collect vadose zone water. We applied Eu-hydroxycarbonate colloids to the lysimeters. Small amounts of Eu colloids were detected in the deepest wick sampler (7-foot depth) only 2.5 months after application and cumulative precipitation of only 20 mm. Large water infiltration, mimicking Chinook snowmelt events in late winter/early spring, caused peaks of Eu in the wick outflow. A main peak of Eu outflow was detected in 4- and 7-foot depth in December 2011 and January 2012 (2.5 years after colloid application). Our results indicate rapid transport of Eu colloids under natural precipitation and artificial irrigation, i.e., the leading edge of the Eu colloids moved at a velocity of at least 3 cm/day within the first two months after application. The main peak of Eu colloids, however, moved at a rate consistent with long-term recharge estimates.

To elucidate the combined effects of water content and flow rate on colloid transport in unsaturated porous media, we conducted colloid transport experiments under different water contents and flow rates. To independently control flow rates and water contents, we used the geocentrifuge at the Idaho National Laboratory. This unique experimental setup allowed us to run a series of colloid transport experiments at different water contents (effective saturation of 1.0, 0.6, 0.32, 0.19) but identical pore water velocity (10.6 cm/min). In general, decreasing water content led to increased colloid retention inside the columns. A portion of the retained colloids could be released by changing the solution chemistry, indicating that colloids had been retained in the secondary energy minimum. A DLVO analysis supports this assumption. We attribute unrecovered colloids in the outflow to the presence of flow stagnation zones.

## **Ecophysiology and Extracellular Electron Transfer by the Metal-Reducing Bacterium *Geobacter daltonii* FRC32<sup>T</sup>**

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*Geobacter daltonii* FRC32<sup>T</sup> is an iron(III) and uranium(VI)-reducing bacterium isolated from uranium-contaminated subsurface sediments at the DOE ORFRC. Structural and functional gene analysis has confirmed that strain FRC32<sup>T</sup> is abundant and active in those sediments. *G. daltonii* was cultivated in bioreactors using a chemically defined medium with acetate as the electron donor and fumarate as the electron acceptor. Under electron acceptor limited conditions, FRC32<sup>T</sup> produced branched extracellular appendages that are morphologically similar to conductive nanowires those produced by *Geobacter sulfurreducens* strain PCA. *G. daltonii* also grew in chemostat cultures with oxygen as the sole terminal electron acceptor, but only when provided at growth-limiting concentrations. Amending the medium with amino acids to support the production of protein increased the production of extracellular filaments under electron acceptor limitation and increased the ability of this organism to reduce and transform solid phase iron oxide and dissolved forms of oxidized uranium.

This presentation provides an update on the molecular and electronic properties of extracellular filaments produced by *G. daltonii*. Novel cultivation methods will be described that can be used to more fully evaluate the enzymatic transformation of heavy metals and radionuclides across defined redox gradients. Information gained from this research will be used to better understand the biogeochemical processes that influence the fate and transport of uranium and other contaminants at the Oak Ridge Integrated Field Research Center. Understanding the components and mechanisms of charge transfer to extracellular electron acceptors by this and other environmentally-relevant organisms is an important step in realizing their full potential as tools for remediation of contaminated subsurface systems.

## A Universal Framework for Predicting Deposition and Transport Behavior of Microorganisms in Subsurface Environment

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**Objectives:** This project aims to explore and develop new AFM-enabled techniques for micro-/ nano-scale characterization and quantification of microbe cell surface properties and cell-surface interactions with the aim to correlate the conventional macroscopic deposition-transport measurement with the microscopic single cell characterization. Nano-scale cellular surface properties that govern the macro-scale cell-cell aggregation and cell-surface attachment tendencies for a number of IFRC-relevant microorganisms are obtained and, new theoretical and mathematical framework for microbe transport model in subsurface environments is proposed.

**Material and Methods:** In addition to comprehensive surface characterization of a number of DOE relevant microorganisms, the nature, magnitude and range of intersurface forces and surface potential at cell-cell and cell-substrate interfaces are quantified using Atomic Force Microscopy (AFM) combined with developed computation methods. Recognizing the complexity of factors dictating the cell-surface interactions and subsequent cell attachment behavior, an integrated and dimensionless parameter, Tabor value,  $\mu$ , was proposed by us with the aim to parameterize the micro-scale cell-surface interactions in order to predict macro-scale cell deposition behavior. An improved theoretical model based on the DLVO theory but incorporating microorganism-specific features is constructed, aiming to generalize the transport behavior of a wide spectrum of microbes in water-saturated porous media. The microbial transport model will ultimately be extended to include other factors influencing microbe deposition to allow the prediction of cell deposition under different fluid condition, ionic strength and relevant sub-surface environments.

**Results and Discussion:** The sample microorganisms exhibit distinctive aggregation tendency and attachment efficiency via transport through porous media, indicating microbial bio-originated factors that influence the microbe movement. The attachment efficiency ( $\alpha$ ) ranges widely from 0.93 for strong microbe-substrate attachment to 0.06 for weak attachment, depending on the bacteria strain as well as the ionic strength of the aqueous environment. The electrolyte concentration is found to have significant influence on the cell stiffness and adhesion-detachment kinetics. No consistent correlation is found between  $\alpha$  and any individual conventional micro-scale physicochemical properties such as electrophoretic mobility, hydrophobicity and extracellular polymeric substance. Mathematical calculation of interaction energy based on current DLVO formula failed to predict the deposition rates observed, indicating that the current DLVO model is not sufficient for predicting microbial deposition. AFM analysis allowed for quantification of the range and magnitude of the repulsive/attractive surface forces, including both short-ranges forces such as van der Waal and electrostatic and long-range forces such as those due to the presence of cellular surface substances (CSS) with varying thickness and density for each strain. A modified Tabor parameter based on classical colloidal and adhesion science is proposed, which collectively quantify the microscopic cell behavior and interfacial properties. Tabor values determined for the organism studied correlated well with the macroscopic attachment efficiencies measured by flow-through column experiments. This indicates that the rudimentary solid-mechanics model is promising to reliably predict the deposition-transport behavior by the Tabor parameter. For microorganism that have much larger  $Ka$  (reflect relative particle size to electrostatic layer thickness and density) value than colloids and with soft shell nature, the inevitable elastic deformation couples caused by the presence of both repulsive energy barrier and an attractive secondary minimum in the DLVO surface potential drastically alters the adhesion-detachment mechanism. We derive the theoretical description of distribution of 1st and 2nd minimum force associated to the detachment of a spherical / cylindrical shell of bacteria from an adhering substrate. An experimental verifiable grand theory of microbial deposition will ultimately be constructed to account for static-dynamic behavior of strains in porous media.

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

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This UM/ASU research seeks to identify the potential for reduced iron sulfide minerals to inhibit the rate of oxidation of reduced U solid phases formed by sulfate reducing bacteria (SRB). Sulfate-reducing bacteria (SRB) utilize sulfate as a terminal electron acceptor and produce sulfide. When iron is also present, iron sulfide solids are produced. At DOE sites contaminated with U, aqueous phase U concentrations can be effectively lowered by reducing dissolved U(VI) species to insoluble U(IV) solids such as uraninite (UO<sub>2</sub>(s)). SRB can accomplish this reduction step either directly by enzymatic electron transfer processes, or indirectly, through chemical reduction by the sulfides species produced. *The working hypothesis of this study is that iron sulfides are preferentially oxidized over U(IV) solids when oxidants such as oxygen, denitrification products, or Fe(III) are introduced present.*

Batch studies were performed to continue the examination of the mechanism and kinetics of FeS protection against UO<sub>2</sub> oxidation under typical groundwater conditions. Experimental results show that mackinawite serves as an effective oxygen scavenger to inhibit the fast oxidation of chemogenic uraninite under simulated groundwater conditions. The kinetic profiles of dissolved uranium indicate that 5 g/L mackinawite inhibits UO<sub>2</sub> oxidative dissolution for about 60 hr under pH = 7, P<sub>O<sub>2</sub></sub> = 0.02 atm, and P<sub>CO<sub>2</sub></sub> = 0.05 atm. During the lag time, oxidation of structural Fe(II) and S(-II) of mackinawite control the DO levels, leading to the formation of iron hydroxides and elemental sulfur, respectively. After FeS is depleted, UO<sub>2</sub> oxidative dissolution occurs at a faster initial rate relative to the control experiments where mackinawite is absent. The kinetic data, along with XRD, Mössbauer, and XAS characterization of reaction products, suggest that the rapid uptake of U(VI) by FeS oxidation products during the initial stages of UO<sub>2</sub> oxidation is responsible for the accelerated rate of UO<sub>2</sub> oxidative dissolution compared to the control in absence of FeS. Ongoing research is testing groundwater conditions (including pH, pCO<sub>2</sub>, pO<sub>2</sub>) to understand the effects of these geochemical constraints in uranium reoxidation.

Sterile column experiments were performed to observe abiotic UO<sub>2</sub>(s) oxidation by oxygen and denitrification products in natural sediments from Rifle CO. Both oxidation studies started with two RABS sediment columns supplemented with (1) chemogenic FeS (0.58g FeS(s)/kg) and UO<sub>2</sub>(s) (0.5g UO<sub>2</sub>(s)/kg) or (2) UO<sub>2</sub>(s) alone (0.5g UO<sub>2</sub>(s)/kg), sterilized with 5 Mrad gamma irradiation and by a 0.2 µm cartridge filter. For the oxygen columns, dissolved oxygen was passed through the columns, and for the denitrification products columns, nitrite, nitrous oxide, and dissolved oxygen were passed through sequentially. Effluent concentrations of uranium, sulfate, hydrogen sulfide, in combination with solid phase extractions on uranium, iron, and elemental sulfur show that under abiotic flow-through conditions: (a) iron sulfides protect uraninite from oxidation by dissolved oxygen and oxidizing minerals in RABS, (b) the terminal oxidation state for sulfide is elemental sulfur, (c) nitrite and nitrous oxide are not effective uranium or iron sulfide oxidants, and (d) oxidized RABS has a significant oxidizing capacity for uraninite. Upcoming experiments will further test the oxidizing potential of denitrification products, and will use sterilized bio-reduced sediment columns to better replicate natural systems undergoing oxidative U remobilization.

Biogenic iron sulfide minerals were produced by *Desulfovibrio vulgaris* supplied with various iron sources. Biogenic mackinawite production was demonstrated using either soluble iron or Fe (III) (hydr)oxide minerals in presence of *D. vulgaris*. The biogenic mackinawite derived from soluble Fe<sup>3+</sup> was less crystallized than the mackinawite derived from soluble Fe<sup>2+</sup>. By reducing Fe(III) (hydr)oxide solids and sulfate, *D. vulgaris* produced poorly crystalline mackinawite. XRD data further suggested that, in addition to mackinawite, residual Fe(III) (hydr)oxides, elemental sulfur, and vivianite [Fe<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>·8(H<sub>2</sub>O)] were formed. Soluble iron, however, resulted in spectra containing only mackinawite patterns. The electron donor (lactate or pyruvate) did not have great impact on crystallization of mackinawite, except for soluble Fe<sup>3+</sup>, where pyruvate led to more crystalline mackinawite. Residual Fe(III) (hydr)oxides and vivianite were present in the final solid products when pyruvate was the electron donor. These results illuminate the impact of the iron source coupled with the electron donor on the biogenic formation of iron sulfide solids that can be used to protect reduced uraninite from reoxidation in natural settings.

## Biotic Controls on Uranium Sequestration and Release by Framboidal Pyrite in Bioreduced Sediments

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Uranium-containing framboidal pyrite, recently discovered in both IFRC/SFA sites at Rifle and Hanford, may play a significant role in controlling the fate and transport of subsurface contaminant U(VI). Biofilm-associated bacteria present on the framboidal pyrite surfaces could potentially influence the fate of uranium through enzymatic redox processes in the subsurface. Alternatively, framboidal pyrite-associated microbes could also play an indirect role in governing U(VI) bioreduction by catalyzing the initial formation of framboidal pyrite via iron-monosulfide precursors that can subsequently engage in non-enzymatic redox processes leading to U reduction and immobilization. Identifying framboidal pyrite-associated microbes and their distribution in biofilms may provide key insight into electron transfer processes driving U uptake and/or release.

An initial batch microcosm study was conducted to examine the relationship between biostimulants (lactate and acetate), pyrite (non-framboidal) amendment, and the microbial community dynamics using Rifle background sediment that was initially devoid of pyrite. A subsequent study will be conducted using synthetic-framboidal pyrite to amend the batch microcosms and compared with naturally bioreduced sediments to gain insight into the broader indigenous microbial community associated with framboidal pyrite. Several chemical synthesis methods involving aging of amorphous iron monosulfide are being employed to reproducibly generate substantial yields of pure framboidal pyrite for additional microcosm and parallel column studies. A key challenge in most synthesis methods involves scaling-up the production and ensuring phase purity. Flow-through column studies are being designed to evaluate uranium uptake and release by framboidal pyrite and compare uranium sorption and desorption behavior to the field site. Multivariate and statistical approaches applied to analyze microbial communities observed in the Rifle sediment and column studies will identify key groups of microbes associated with framboidal pyrite and help explain uranium removal under different test conditions. The biofilms formed on the surface of framboidal pyrite will also be visualized using fluorescence *in situ* hybridization (FISH) and confocal microscopy. Additionally, advanced microscopy and surface-sensitive spectroscopic techniques will be applied to probe molecular-scale redox interactions between framboidal pyrite and uranium. This exploratory research effort will help gain an understanding of interfacial biogeochemical processes impacting framboidal pyrite, the microbial population and activity, and U interactions in heterogeneous natural systems. This new insight in turn will aid in the development of molecular models to predict the rate and extent of U attenuation, as well as its long-term mobility, in bioreduced sediments. Further, the ability to stimulate these interactions may offer a new remediation strategy for DOE sites.

## Assessment of the Bioavailability and Methylation Potential of Mercury Sulfides with Sediment Slurry Experiments

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In the natural aquatic environment, neurotoxic methylmercury (MeHg) mainly originates from anaerobic microbes that methylate inorganic forms of mercury in sediments. MeHg production rates can vary widely across aquatic ecosystems, sometimes by orders of magnitude. Accurate prediction of mercury methylation potential in the environment has remained elusive, partly because the mechanisms by which anaerobic microbes take up and methylate mercury are not well known. The overall goal of our research is to establish a quantitative relationship between the geochemical speciation of mercury in sediment pore water and the bioavailability of mercury to the methylating bacteria. In our previous work with pure cultures of sulfate reducing bacteria (SRB), we have demonstrated that the availability of mercury for methylation decreased during aging of mercury in sediments, a process in which mercury is expected to transform from dissolved Hg-sulfides to nanoparticulate and microparticulate forms of HgS.

To better capture the complexity of real sediment systems, we performed sediment slurry microcosm experiments to investigate the aging effect on the availability of Hg for methylation. For our slurries, we selected sediments and water at two locations in the San Francisco Bay-Delta estuary (California, U.S.A.) to represent sediments in freshwater and saline settings. The slurries were amended with one of the three forms of mercury: dissolved  $\text{Hg}(\text{NO}_3)_2$  freshly mixed with  $\text{Na}_2\text{S}$ , HgS nanoparticles (<30 nm), and HgS microparticles (>1000 nm). In these HgS treatments, we compared the net MeHg production and other water quality parameters relative for Hg speciation. Our results showed that SRB were predominantly responsible for mercury methylation and their activity and subsequent MeHg production could be limited by the supply of sulfate and/or labile organic carbon. In the presence of abundant sulfate and carbon source, the bioavailability of Hg was the limiting factor for microbial methylation. The net MeHg production in the slurries amended with  $\text{Hg}(\text{NO}_3)_2 + \text{Na}_2\text{S}$  was found to be the largest of the three types of Hg treatments, similar to our previous experiments with SRB in pure culture. Likewise, the methylation potential of nano-HgS was higher than that of micro-HgS. Nevertheless, the chemistry in natural sediments is more complex than that found in pure cultures of bacteria. Partitioning of Hg to bulk-scale mineral particles and colloids (especially FeS) may considerably influence the speciation of mercury and MeHg production. As a result, dissolved Hg in the bulk porewater (i.e., Hg remained in the supernatant after ultracentrifugation) in sediment pore water may not accurately represent the available fraction of Hg for methylation. Additional approaches targeting the Hg species that are likely taken up by the methylating bacteria (e.g., Hg-thiol complexes) are needed to assess the Hg availability in natural samples.

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

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The Hanford 300 Area sediments are mainly river cobble and gravel, with sands and fines (silt + clay) variably filling the voids in between. Fines comprise only 1.78% of the total sediment mass, but hold a disproportionately large fraction of the total U. However, the coarser sediments (e.g., >2 mm), with their larger mass fraction, may serve as a long-term U release pool despite their lower U concentration. We hypothesized that slow U release at the 300 Area is partly due to low connectivity of intra-granular pores. The objectives of this project are to evaluate the U distribution in 2-8 mm size sediments, to determine whether low pore connectivity affects U distribution and release from Hanford 300 Area sediments, and if so, to assess its implications for long-term release. Our approach integrates laboratory experiments (ICP-MS instrumentation to measure multiple elemental concentrations in liquid samples, laser ablation (LA)-ICP-MS for elemental mapping of 2-8 mm sized basaltic clasts) and pore-scale network modeling.

Over the last year we performed the following tasks with associated results:

1. Conducted complementary laboratory tests (stirred flow cell, saturated column, and batch reactor) to study U release behavior from each of five size fractions (<75  $\mu\text{m}$ , 75-500  $\mu\text{m}$ , 500-2000  $\mu\text{m}$ , 2-8 mm, and a <2 mm composite). The stirred flow cell results indicate that 8.7% of the total U is in the 2-8 mm fraction, significantly reducing uncertainty in the U inventory. In addition, released U concentration shows a power-law relationship to elution volume (i.e., elution time) for all size fractions (published in *J. Environ. Radioact.*). Numerical simulations are being developed to recreate both stirred cell and saturated column experiments to better characterize U release rate constant distributions, and evaluate rate constant uncertainty for different size fractions.
2. Assessed concentration distributions of U and other elements in a 2-8 mm basaltic clast as a function of distance from the grain's surface. A second 2-8 mm basaltic clast was mapped and compared to the first mapped clast, examining fewer layers but to deeper depths. U concentration was power-law distributed with distance from the grain surface (published in *Environ. Sci. Technol.*).
3. Tested a pore-scale network model to examine pore connectivity issues in contaminant retention and release. The model supports our hypotheses about accessible porosity and anomalous patterns of contaminant release, and predicts U concentration distributions consistent with those observed in (2). A finite-difference model closely matches the network model results, indicating that we correctly captured how porosity and diffusion change with distance (published in *Water Resour. Res.*).
4. Carried out 3-D elemental mapping of a 2-8 mm basaltic clast, after vacuum saturation with a suite of nonsorbing and sorbing tracers, to better assess the edge-accessible porosity distribution; data processing and analysis are underway.
5. Performed unsaturated column transport experiments for 2-8 mm size fraction (at water saturations of 77% and 14%) and <2 mm size fraction (at 88% water saturation). Uranium release from 14% saturation is greater than that from 77% saturation under otherwise identical conditions, probably because at lower saturation, flow paths have more contact and interaction with the grains.



## Fate of Uranium During Transport Across the Groundwater–Surface Water Interface

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Discharge of contaminated groundwater to surface waters is of concern at many DOE facilities. Essentially all surface or subsurface contaminants will move vertically through a vadose zone to an underlying aquifer and then laterally until it reaches some riparian zone or wetland. For example, at F-Area and TNX-Area on the Savannah River Site, contaminated groundwater, including uranium, is already discharging into natural wetlands. It is at this interface where contaminants come into contact with the biosphere. These wetlands exist in humid as well as arid regions. Furthermore, the numerous sharp biogeochemical transitions occurring in wetlands have profound effects on the ultimate fate of redox-sensitive trace metals and radionuclides, including uranium.

The goal of this research is to provide new insights on how plant-induced alterations to the sediment biogeochemical processes affect the key uranium reducing microorganisms, the uranium reduction, its spatial distribution, the speciation of the immobilized uranium, and its long-term stability. For this purpose we have formulated the following three **hypotheses**. (1) U(VI) discharged from ground- to surface-waters can be immobilized effectively as U(IV) in the sediments at the groundwater-surface water interface. The electron donor required to stimulate the microorganisms capable of reducing U(VI) is provided by wetland plants via their root exudates and root turnover. (2) Oxygen released into the sediments by plants reoxidizes Fe(II), forming iron oxy(hydroxi)des, which provide the bioavailable Fe(III) for long-term bacterial iron-reducing activity, which is key for a sustained biological uranium reduction. (3) Because wetland sediments are anaerobic and wetlands are usually nitrogen limiting, uranium immobilized as U(IV), which is readily oxidized in the presence of nitrates, will remain stable in the sediments for extended time periods.

To test these hypotheses we initiated a series of greenhouse **mesocosm studies** in which we will simulate the discharge of uranium contaminated groundwater into surface water through vegetated and non-vegetated sediments. These mesocosm studies, which will give information regarding macroscopic-scale processes and kinetics, will be augmented with laboratory batch studies to isolate the effect of specific variables. This characterization will be to determine the role the root-induced biogeochemical dynamics have on Fe speciation (to be determined via micro-XRD and Mössbauer analyses), microbiologic activities ( $^{13}\text{C}$  stable isotope pulse chase experiments coupled with  $^{13}\text{C}$  phospholipid fatty acid analysis and pyrosequencing), and their role in controlling U speciation/oxidation states (via X-ray Absorption and X-ray fluorescence Spectroscopy) and U partitioning between the solid and aqueous phases. Spatial distribution of these characterizations and processes from the root surface to the bulk sediment will be quantified to gain detailed new insights into these biogeochemical processes and their overall impact onto the dynamics of uranium at the groundwater-surface water interface.

The project has just been initiated and we will show initial results of the microcosm operation, specifically the microbial community differences, including *Geobacter sp.* numbers as we move away from the root surface into the rhizosphere and into sediments where root growth has been excluded. Characterization of three U-contaminated wetland sediments from the SRS have shown that the U is only slightly more preferentially associated (1.5x) with the fine particle fraction (<2-  $\mu\text{m}$  or <0.5- $\mu\text{m}$ ), whereas Th(IV) was strongly concentrated (29x) in the finer particle size fraction. A sequential extraction of these sediments revealed that ~50% of the U in these heavily contaminated sediments was organically bound (sodium pyrophosphate extractable), 7% was either associated with amorphous or crystalline Fe-oxides (extractable with ammonium oxalate or dithionite extractable), and another ~25% was exchangeable (acetic acid exchangeable). Uranium extractions were not similar to Th(IV) extractions, suggesting that some of the U may exist in the +6 oxidation state. Together these data indicates that: 1) the soil organic carbon is responsible for most of the sequestration of uranium in the wetland, and 2) a vast majority of the uranium in these wetland sediments may be in a labile state associated with organic matter, exchangeable fraction, and iron oxides in this dynamic chemical system.

## Impact of Biostimulation on Permeability and C-13 Stable Isotope Probing of Biostimulation Experiments to Identify Acetate Utilizers

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Here we are reporting results of two separate experiments. First we are conducting column experiments to study the link between biostimulation and changes in permeability. For this purpose seven columns were built that allow for dissolved species sampling and pressure change measurements along their vertical axis, and the experiment has been operating now for approximately one year. One column is equipped with electrodes to determine the induced polarization (IP) and link this to changes in permeability and to IP measurements that have been conducted at Rifle during previous biostimulation experiments. Results of this ongoing experiment show that there was a small and gradual (seemingly linear) increase in pressure over time during the first 200 days of biostimulation. Then, after about 220 days of biostimulation, we see a significant change in pressure along the longitudinal axis of the columns. This corresponds to approximately 50 days after sulfate is completely reduced. Effluent analyses show that by this time, methane is near solubility. A column that was sacrificed after ca. 90 days of biostimulation revealed that methanogen counts were the highest among microbial community members sampled. Although Rifle field experiments, focusing on dissolved groundwater samples have not reported significant presence of Archaea, these results, that focused on sediment analyses, seem to indicate that Archaea, in this case methanogens, might be a key organism competing for electron donors during biostimulation. In terms of permeability changes in response to biostimulation, we conclude that Under Rifle flow conditions, and with acetate as the electron donor, permeability changes are expected to be negligible, at least until separate-phase methane is building up.

A separate experiment was conducted to determine which microorganisms take up acetate during biostimulation and how the uptake of acetate by specific organisms, especially *Geobacter* species, changes over time, a 120-day column biostimulation experiment was performed. Eight columns were loaded with Rifle sediments and operated under continuous flow conditions using Rifle groundwater, amended with 3 mM C-12 acetate. At regular time intervals, C-12 acetate flow into a specific column was switched to C-13 acetate. That column was then operated under C-13 acetate amendment for 36 hours before it was sacrificed for detailed geochemical and microbiological analyses. Column operation started under iron reduction (based on the measured Fe(II) in the column effluent), while sulfate reduction (based on removal of sulfate between influent and outflow), was noted at about 25 days of operation. The microbial characterization consisted of phospholipid fatty acid analysis (PLFA) and stable isotope probing (SIP), while differentiating between the C-12 and C-13 incorporation into the biomass. Results showed that there was a differentiation between the community that was taking up acetate actively throughout the 120 days of operation and the overall microbial community. Of interest was that the fraction of *Geobacter* population remained fairly constant throughout the duration of the experiment, as well as its acetate uptake. Based on previous results conducted strictly during iron reduction, we estimate that of the acetate incorporated into the overall biomass, about 20% or less, was incorporated into *Geobacter* biomass. These results are key for the proper numerical simulations of biostimulation via acetate amendment and the biostimulation of *Geobacter*.

## **Integrated Geophysical Measurements for Bioremediation Monitoring: Combining NMR, Magnetic Methods and SIP**

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Our research aims to develop borehole measurement techniques to monitor subsurface processes, such as changes in pore geometry and iron/sulfur geochemistry, associated with remediation of heavy metals and radionuclides. Previous work has begun to identify methods that are capable of surveying the subsurface environment. One such method, spectral induced polarization (SIP), has been used to monitor the progress of subsurface contaminant remediation; however, its interpretation is of limited value in isolation. In our research we aim to combine measurements from multiple geophysical methods, i.e. nuclear magnetic resonance (NMR), and magnetic susceptibility (MS), with SIP, to allow us to reduce or overcome the limitations associated with using one measurement alone. The integration of measurements from multiple geophysical methods, each sensitive to mineral form and/or mineral–fluid interfaces, will provide better constraints on subsurface biogeochemical processes and evolution of pore geometries and significantly improve our understanding of processes impacting contaminant remediation.

In the first year of the research project, NMR and MS borehole logging measurements were collected at the Rifle Integrated Field Research Challenge (IFRC) site. The Rifle IFRC site is located at a former uranium ore–processing facility in Rifle, Colorado. Although removed from the site by 1996, leachate from spent mill tailings has resulted in residual uranium contamination of both groundwater and sediments within the local aquifer. Since 2002, research at the site has primarily focused on quantifying uranium mobility associated with stimulated biogeochemical processes. Ongoing studies at the site include an acetate amendment strategy, in which stimulation of native microbial populations by introduction of a carbon source serves to alter local redox conditions and immobilization of uranium in insoluble forms. NMR and MS logging measurements were taken before, during, and after acetate amendment. Changes in these signals were expected to correlate with changes in redox conditions and iron speciation. Experimental data were collected from two wells upstream of the acetate amendment, used as controls, and from three downstream wells.

The MS measurements revealed vertically stratified magnetic mineralization, likely the result of a detrital magnetic fraction within the bulk alluvium. Data were highly replicable over the monitoring interval, with little to no change observed in the MS measurements, suggesting negligible production of magnetic phases (e.g. magnetite, pyrrhotite) as a result of sulfidogenesis. NMR measurements had high levels of noise contamination requiring significant signal processing, and ongoing analysis suggests that any changes due to stimulated microbial activity may be difficult to differentiate from simultaneous changes in water content.

In the second year of the project we will collect laboratory SIP, NMR, and MS measurements on columns packed with sediments from the Rifle IFRC site as the columns are amended with acetate; changes in the geochemistry and pore geometry inferred from these measurements will be verified by standard, independent laboratory measurements. We will integrate the field and laboratory results to develop a strategy for the interpretation of coupled SIP, NMR and MS measurements during biostimulation.

## Translation of Geophysical Log Responses to Estimate Subsurface Hydrogeologic Properties at the Hanford 300 Area

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The geology and chemistry of the subsurface environment are fundamental factors controlling contaminant fate and transport, and thus play a critical role in remediation efforts at DOE sites. However, the subsurface is often heterogeneous and not well characterized. Petrophysical models that relate borehole neutron and gamma ray data to reservoir properties such as clay content, matrix density, porosity, and permeability are critical in the formation of meaningful reactive transport models.

The objectives of our research are to: 1) Analyze core and/or outcrop samples from representative facies for a variety of mineralogical, chemical and physical properties, 2) Predict the response of a variety of neutron and gamma logging tools based on these measurements, and 3) Develop algorithms to translate log responses into formation properties such as matrix density, lithology, porosity, and permeability, which are useful for input in flow and reactive transport models.

Our analysis of selected core samples from Hanford 300 Area boreholes reveals significant correlations between K, Th, and U concentrations and both matrix density and total clay, as determined by pycnometry and Dual Range Fourier Transform Infrared spectroscopy (DR-FTIR), respectively. Based on these relationships, we developed algorithms to predict total clay and matrix density from existing spectral gamma logs. The availability of laboratory bulk density data allows us to compute porosity estimates for some of our samples. The comparison of clay content and porosity reveals a relationship that is consistent with the relationship observed by Marion et al. (1992) and others, suggesting the existence of both clay-supported and framework (or grain) supported domains and the ability to estimate porosity from clay content. Further, the availability of total clay, matrix density, and porosity permit application of the k-Lambda model as a means to estimate permeability. We will present new data from wells distributed within 300 Area IFRC 399-3-31 (C6214), 399-2-30 (C6217), and 399-2-31 (C6218) and deeper wells from the surrounding Hanford\_300 Area for which both geophysical logs and bulk density measurements are available - 399-1-23 (C5000), 399-3-19 (C5001), and 399-3-20 (C5002). Based on these new data, we will present refined algorithms for matrix density, clay content, porosity and permeability.

In addition, we have developed new environmental correction algorithms based on least-squares regression techniques that include uncertainty in the data, and propagation of errors with respect to the predicted correction factors. Goodness of fit evaluation of the new algorithms yields reduced chi-square that are lower than the Hanford algorithms and acceptable chi-square probabilities ( $>0.001$ ). When these new algorithms are applied to existing log data along with an empirical detector based efficiency correction, a significant improvement in the accuracy and precision of the SGLS derived K, U, and Th concentrations are achieved as indicated by the reduction in uncertainties and good agreement between core and log data.

## Development of Surface Complexation Models of Cr(VI) Adsorption on Soils, Sediments, and Model Mixtures of Kaolinite, Montmorillonite, $\gamma$ -Alumina, Hydrous Manganese and Ferric Oxides, and Goethite

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Cr(VI) is a toxic contaminant that has been introduced into aquifers and shallow sediments and soils via many anthropogenic activities. Cr(VI) contamination is a problem or potential problem in the shallow subsurface at several DOE sites, including Hanford, Idaho National Laboratory, Los Alamos National Laboratory and the Oak Ridge Reservation (DOE, 2008). To accurately quantify the fate and transport of Cr(VI) at DOE and other contaminated sites, robust geochemical models, capable of predicting changes in chromium speciation resulting from sorption, dissolution, precipitation and redox reactions, are required. The objectives of this study are to: (1) measure Cr(VI) adsorption kaolinite, montmorillonite, hydrous manganese oxide,  $\gamma$ -alumina as a function of ionic strength, pH,  $p\text{CO}_2$  and sorbate/sorbent ratio, (2) develop surface complexation model descriptions of Cr(VI) sorption for these systems, (3) test component additivity predictions for Cr(VI) adsorption on mixtures of six well-characterized sorbents, (4) measure Cr(VI) adsorption on four bulk natural soils and sediments before and after four step-wise sequential extractions, and (5) develop methods for extending the component additivity approach to natural soils and sediments.

Significant adsorption of Cr(VI) occurs on  $\gamma$ -alumina (5 g/L solid;  $10^{-4}$  or  $10^{-5}$  M Cr) at low pH, with 50% of the Cr(VI) adsorbed between pH 6.5 and 8. Adsorption is suppressed with increasing ionic strength, especially at high  $p\text{CO}_2$ . Surface complexation models provide adequate fits to individual adsorption edges, but generally fail to reproduce the full range of observed ionic strength and sorbate/sorbent ratio dependence of sorption. Significantly less Cr(VI) occurs on hydrous manganese oxide, although 100% of  $10^{-5}$  M Cr(VI) is sorbed on 20 g/L HMO at low pH. Increasing ionic strength (0.001 to 0.1 M  $\text{NaNO}_3$ ) significantly decreases Cr(VI) sorption. Cr(VI) sorption is slow, failing to reach steady state after 2 weeks, and nearly irreversible with pH changes from 3 to 10 on untreated kaolinite. Pretreatment with 0.5 M HCl, 0.4 M hydroxylamine HCl or 30%  $\text{H}_2\text{O}_2$  greatly increase the rate of adsorption, as well as the total quantity of sorbed Cr(VI). Cr(VI) is similarly irreversible on untreated montmorillonite, but is much more rapid than on kaolinite. Pretreatment with 30%  $\text{H}_2\text{O}_2$  dramatically decreases the quantity of Cr(VI) sorbed on montmorillonite. These data suggest that Fe(II) in the lattice of these clay minerals is responsible for the irreversible sorption of Cr(VI), presumably by promoting reduction to Cr(III). Adsorption edges were measured on an organic-rich soil from Kleinstuck Marsh, MI, before and after stepwise removal of four target fractions (exchangeable, carbonate, reducible and oxidizable) for periods of 24 hrs to 2 weeks. For all sediments except those with the oxidizable portion removed, Cr(VI) sorption increases with time below pH 7, reaching a maximum of 100% sorbed at pH 3 and decreasing with increasing pH. Below pH 7, all Cr remaining in solution is Cr(VI), but above pH 7, dissolved Cr(III) increases with time, with the largest increases occurring at the highest pH, demonstrating reduction of Cr(VI) in contact with sediments. Cr(VI) sorption is greatly diminished after removal of the oxidizable fraction, suggested that the bulk of the Cr(VI) binds to organic matter or Fe(II)-bearing clays.

## Chromate Reduction in Heterogeneous Porous Media

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Chromate is a common groundwater contaminant at the U.S. Department of Energy (DOE) Hanford Site and poses a long-term threat to the water quality because of its high toxicity and aqueous solubility. Reduction of Cr(VI) to Cr(III), followed by the precipitation of Cr(III) hydroxide and / or mixed Fe/Cr (oxy) hydroxides, is one of the promising strategies to attenuate dissolved Cr(VI). Both aqueous and solid Fe(II) are abundant in Hanford sediments (biotite, clinocllore, magnetite, and ilmenite) and can serve as effective reductant for Cr(VI).

Under well-mixed laboratory conditions, the reduction of Chromate by aqueous Fe(II) often occur at a time scale of minutes. In natural systems, however, high Fe content sediments are typically unevenly distributed, with its majority in clay-rich low permeability zones. This can have a large impact on Fe(II) availability for the reduction of Cr(VI) and therefore the reduction rate of Cr(VI). The objective of this work is to investigate how and to what extent spatial distribution of Fe(II)-rich clay determine the rate of Cr(VI) reduction.

Column experiments with different mineral distribution patterns will be conducted, with quartz and illite representing the major solid components at the Hanford site. Illite will be pre-sorbed with Fe(II). Three columns will be set up, with one of the columns having Fe-saturated illite evenly distributed within the quartz matrix across the whole column (mixed column), while the other two having Fe-saturated illite distributed as one or three layers embedded within the sand matrix (1-layer column and 3-layer column). The total mass ratios of illite and quartz in the three columns will be kept constant. To investigate the effects of spatial distribution on the rate of Fe (II) desorption and Cr(VI) reduction under different geochemical conditions, flows at different pH conditions will be used. As the Fe(II) desorption and Cr(VI) reduction reactions are highly pH dependent and are sensitive to local geochemical conditions, a series of batch experiments is being conducted to determine 1) the suitable pH conditions for the Cr(VI) reduction in the column experiments, 2) the difference between Cr(VI) reduction rates with Fe(II) and with Fe(II)-sorbed illite. Understanding from batch reactor experiments will be used later to examine the difference between rates at the laboratory scale and the rates at the column scale. Reactive transport modeling will be used to quantify the rates under different conditions, to understand the role of heterogeneity, and to upscale laboratory rates to the field scale.

## Monitoring Microbial Uranium Reduction at the Oxic-Anoxic Interface

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This collaborative, multi-investigator research project aims to develop innovative molecular and biogeochemical tools to enhance understanding of the microbiology controlling the redox state of metals and radionuclides in subsurface environments and near oxic-anoxic interfaces. Diverse groups of microorganisms affect the oxidation state of metals and, therefore, affect many biogeochemical processes including carbon turnover and the mobility of toxic radionuclides in subsurface environments. Relevant to uranium (U) transport are bacteria that can reduce the relatively water-soluble and mobile hexavalent U(VI) to the less-soluble U(IV) redox state.

We have applied physical, chemical and biological tools to analyze the fate of U under various geochemical conditions. Using EXAFS analysis we found that phosphate concentrations and the specific metal-reducing bacterial population can affect the form of reduced U(IV), indicating that a diversity of mononuclear U(IV) forms may be commonly formed during bioreduction. Using high precision mass spectrometry, we have also shown that significant U isotopic fractionation occurs during U(VI) reduction. During microbial reduction, the U(VI) remaining in solution becomes isotopically lighter; however, different U(VI)-reducing species resulted in different fractionation extents, and a new *Shewanella* isolate reduced U(VI) efficiently but without fractionation. These results indicate that different U reduction pathways/mechanisms operate that yield different products and can lead to varying amounts of isotopic fractionation. *c*-type cytochromes have been implicated in electron transfer to oxidized metal species, and using metaproteomic analysis we identified specific proteins expressed in cultures of *Anaeromyxobacter dehalogenans* strain 2CP-C, *Geobacter daltonii* strain FRC-32, and *Shewanella oneidensis* strain MR-1. Cytochromes expressed in response to the presence of specific electron acceptors provide information about the physiological state of the microbe, thus making predictions of metal-reducing activity possible.

To better investigate the spatial relationship associated with flow in a groundwater system we have completed the design of a rectangular Plexiglass flow-through column that will allow the simultaneous measurements of nucleic acid and protein biomarkers, U isotopes, and *in situ* EXAFS and XANES in a zone of controlled redox transition changes (i.e., the oxic-anoxic interface). The combined application of these tools will monitor the responses of metal-reducing bacteria and advance our understanding of the relevant microbiology and processes that determine the fate of electron donors (e.g., carbon) and electron acceptors (e.g., oxidized metals and radionuclides, oxygen) near oxic-anoxic transition zones.

Application of the new tools to DOE IFRC or relevant field sites will demonstrate their value to assess, monitor, and predict *in situ* reductive processes. The comprehensive understanding of the mechanisms and pathways affecting metal speciation, in particular U precipitation and mobilization, will contribute to the development of models that predict the long-term fate of metals in redox transition zones.

## Multi-Scale Modeling Framework for Optimizing Uranium Bioremediation

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Reductive immobilization of hexavalent uranium to uraninite by stimulation of indigenous bacteria has been extensively investigated as an efficient remediation strategy for subsurface U(VI) contamination. The challenge is how to achieve a sustainable, cost-effective and efficient U(VI) immobilization under specific and various sedimentary conditions. The overall objective of the project is to address this complex and systemic challenge by developing a multi-level computational framework capable of predicting uranium mobility for a long period of time under different conditions.

We have made progress in six parts that complement each other for achieving the project goal. These six parts cover every detailed aspect of the study of bioremediation process through computational modeling approaches, i.e. from very small test environment to field sites, from individual microorganism to microbial community, from regular biostimulation to optimal control of chemical amendments, and from single computer processor to grid engine. These six parts can be summarized as follows: 1) We have built a more complex mechanistic model that incorporates the latest research findings on both biotic and abiotic processes in subsurface sediments, providing insights into uranium immobilization that cannot be obtained by experiments alone. We have shown the value of model by validating its predictions under dramatically different environmental conditions; 2) We have performed a preliminary construction and analysis of the *Anaeromyxobacter dehalogenans* model at the genome scale and we are planning to incorporate this organism into our computational framework due to its diverse and wide-ranged metabolic capabilities relevant to the reduction of iron oxides, selenium, nitrate, chlorophenols and uranium for growth; 3) We have investigated the interaction of *Geobacter* and sulfate-reducing bacteria both in sediment incubations and with *in silico* metabolic modeling, revealing that Fe(III) availability, rather than competition with sulfate-reducing bacteria, is the key factor limiting the activity of *Geobacter* during *in situ* uranium bioremediation; 4) We have developed an optimization framework based on optimal control theory to maintain the uranium concentration below the environmental safety standard while minimizing the cost of the acetate and Fe(III) addition rates as well as the difference between the predicted and target uranium concentration; 5) We have succeeded in optimizing and parallelizing our preliminary 3D research codes containing different kinds of kinetic and equilibrium equations. We then adapted the model to be executed on Grid Infrastructure through OpenMP implementation, achieving good speedups that are close to the theoretical maximum speedups; and 6) We have incorporated a genome-scale metabolic model of *Geobacter sulfurreducens* into a pore-scale simulation of reactive transport and microbial growth to assess the manifestation of pore-scale variability (microenvironments) in terms of apparent Darcy-scale microbial reaction rates. We are trying to ultimately integrate all of these individual components to build a multilevel model framework for assisting the design of an effective and efficient bioremediation strategy.



## Molecular Mechanisms Underlying the Metallic-Like Conductivity of *Geobacter* Pili

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The discovery of long-range electron transport along the pili of *Geobacter sulfurreducens* via metallic-like conductivity is a paradigm shift in biological electron transfer and materials science with important implications in bioremediation and bioenergy. The metallic-like conductivity of the *Geobacter* pili differs significantly from previously described biological electron transport in which electrons hop or tunnel between discrete redox-active molecules, such as cytochromes. This finding also contradicts the long-standing belief that biological proteins act as insulators and can not be conductive. In the last year we have made significant progress in understanding the mechanisms for the metallic-like conductivity of *Geobacter* pili and the role of pili in extracellular electron transfer.

Additional evidence for metallic-like conductivity along pili was the finding that decreasing the pH of the pili preparations enhanced conductivity by as much as 100 fold. This response is characteristic of materials with metallic-like conductivity, but inconsistent with the alternative model of electron hopping/tunneling via cytochromes. In fact, the highest conductivity was observed at pH 2, a pH at which cytochromes are denatured.

Structural studies of *Geobacter sulfurreducens* pili using X-ray diffraction revealed overlapping pi orbitals. This is significant because pi orbital stacking is the basis for the metallic-like conductivity observed in synthetic conducting polymers, such as polyaniline. This finding led to the hypothesis that aromatic amino acids exposed on the outer surface of the pili play a key role in establishing the metallic-like conductivity of the pili.

In order to investigate this possibility, we genetically constructed mutant strains in which alanine was substituted for individual aromatic amino acids located at the carboxyl terminus of PilA, the structural pilin protein. Through these studies we identified an aromatic amino acid moiety that is required for the conductivity of pili sheared from the cells and for long-range electron transfer via pili attached to viable cells. The strain with this single amino acid substitution continued to produce pili that were functional in other aspects, such as promoting attachment to surfaces. These results are consistent with the hypothesis of metallic-like conductivity, but inconsistent with the alternative model of cytochromes mediating electron transport along pili.

Although *c*-type cytochromes can not account for conduction along pili, the cytochromes OmcS and OmcE are positioned outside the cell and are involved in electron transfer to Fe(III) oxide. Previous studies have demonstrated that OmcS is localized on the pili and is thought to facilitate electron transfer from pili to Fe(III) oxide. In order to localize OmcE, whole cells were treated with antibodies raised to OmcE, followed by secondary gold-labeled antibodies. Examination with transmission electron microscopy revealed that, unlike OmcS, OmcE is associated with the outer cell surface rather than the pili. These results suggest that a potential role of OmcE is to facilitate electron transfer to the pili. However, the ability of cells to adapt to deletion of the gene for OmcE indicates that there may be multiple proteins capable of this electron transfer step, a possibility that will be evaluated with adaptive evolution studies.

## Influence of Protozoa on Anaerobic Groundwater Bioremediation

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The importance of bacteria in the anaerobic bioremediation of groundwater polluted with organic and/or metal contaminants is well recognized and in some instances so well understood that modeling of the *in situ* metabolic activity of the relevant subsurface microorganisms in response to changes in subsurface geochemistry is feasible. However, a potentially significant impact on bacterial growth and activity in the subsurface that has not been adequately addressed is protozoan predation of the microorganisms responsible for bioremediation. In order to evaluate the potential role of protozoa, anaerobic bioremediation studies were conducted at a uranium-contaminated aquifer located in Rifle, CO in which microbial reductive precipitation of uranium was stimulated with the addition of acetate to the groundwater. As observed in previous field experiments, acetate amendments initially promoted the growth of metal-reducing *Geobacter* species followed by the growth of sulfate-reducers. Analysis of 18S rRNA gene sequences revealed a broad diversity of sequences closely related to known bacterivorous protozoa in the groundwater prior to the addition of acetate. In 2010, 41 different protozoan species were detected in the untreated groundwater and 37 species were detected in 2011. The bloom of *Geobacter* species associated with added acetate was accompanied by a specific enrichment of protozoa most closely related to the amoeboid flagellate, *Breviata anathema*, which accounted for over 80% of the recovered protozoan sequences. The abundance of *Geobacter* species declined following the rapid emergence of *B. ananthema*. The subsequent growth of sulfate reducers in the acetate-amended groundwater was accompanied by another specific enrichment of protozoa, but with sequences most similar to the diplomonad flagellate, *Hexamita inflata*, which accounted for over 80 % of the protozoan sequences recovered during this phase of the bioremediation. These results suggest a prey–predator response during uranium bioremediation with specific protozoa responding to increased availability of preferred prey bacteria. Thus, quantifying the influence of protozoan predation on the growth, activity, and composition of the subsurface bacterial community is essential for predictive modeling of bioremediation strategies. It is expected that protozoa may play an important role in controlling the activity of microorganisms in a diversity of anaerobic soils and sediments.

## Electrode-Based Approaches for Monitoring Microbial Activity and Carbon Cycling in Soils and Sediments

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There is a compelling need for strategies to simply and inexpensively monitor the activity of microorganisms in soils and sediments. This is true not only for the microbial monitoring that is required during subsurface bioremediation, but also for assessing changes in microbial activity due to environmental perturbations, such as climate change. Current methods typically involve invasive sampling of the environment, which can alter microbial activities, and require the addition of tracers or indicators, adding complexity and expense. Furthermore, these traditional methods are not amenable to real-time monitoring of *in situ* activity.

Our previous studies at the Integrated Field Research Challenge (IFRC) site in Rifle, CO demonstrated that microbially generated current was a good predictor of the amount of acetate reaching downgradient monitoring wells when acetate was added to the subsurface to promote uranium reduction. This monitoring system worked well even though the graphite anodes deployed in monitoring wells were connected to cathodes located 5 m away at the surface. Low levels of current were detected with anodes deployed in monitoring wells in control zones not amended with acetate, presumably due to microorganisms producing current from acetate, and possibly other electron donors, steadily produced over time from the natural degradation of complex organic matter in the subsurface.

Therefore, we hypothesized that it might be possible to monitor microbial activity in a diversity of anaerobic soils and sediments in real time based on the amount of current generated from anodes deployed in the environments of interest. Our primary goal for this project is to monitor microbial activity in unamended sites at Rifle to evaluate the relationship between indigenous rates of anaerobic microbial activity and the degree of uranium removal via the natural attenuation of microbial U(VI) reduction. Field experiments will be validated by laboratory studies using Rifle-derived microflora and natural organic matter concentrated from a variety of locations across the Rifle floodplain. However, as an initial proof-of-concept we evaluated current production versus rates of methane production in freshwater methanogenic sediments, because monitoring methane production in these sediments provided a simple independent means of estimating rates of carbon turnover under different conditions.

There was a strong correlation between rates of methane production in sediments and rates of current produced by electrodes emplaced in the sediments. Although the sediments were methanogenic, microorganisms in the family *Geobacteraceae* accounted for 69 % of the 16S rRNA gene sequences recovered from the anode surface. It is likely that the *Geobacteraceae* living on the electrode surface were utilizing acetate, and possibly other intermediates of anaerobic metabolism in the methanogenic sediments with electron transfer to the electrode. Thus, these results suggest that current production is likely to respond to changes in rates of carbon turnover in the same manner as natural terminal electron accepting processes. Therefore, it is expected that electrodes can provide an inexpensive noninvasive method for monitoring microbial activity and carbon cycling not only in groundwater, but also in a diversity of soils and sediments.

## Catalytic DNA Biosensors for Radionuclides and Metal Ions

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

In the past year, we have made significant progresses in two areas. In the first area of developing novel catalytic DNA sensors for radionuclides and metal ions, we have demonstrated that functional DNA-linked gold nanoparticles can detect and quantify mercury ion in aqueous solution, with high sensitivity and selectivity. This system has been converted into a dipstick test using lateral-flow devices, making it even more practical for on-site and real-time detection. Furthermore, to lower the cost of quantitative detection, we have taken advantage of the wide availability and low cost of the pocket-sized personal glucose meter and demonstrated a method to use such meters to quantify radionuclides such as uranium with 9.1 nM detection limit. The method is based on the target-induced release of invertase from a functional-DNA–invertase conjugate. The released invertase converts sucrose into glucose, which is detectable using the meter. The approach should be easily applicable to the detection of many other radionuclides and metal ions. In the second area of fundamental understanding of metal ion selectivity in order to design more selective sensors, we have studied metal-ion-dependent folding of a uranyl-specific DNAzyme and obtained insight into the selectivity and function from fluorescence resonance energy transfer studies. In addition, we have also discovered the importance of peripheral sequences in determining the metal selectivity of an *in vitro*-selected  $\text{Co}^{2+}$ -selective DNAzyme.

Detecting and quantifying radionuclides and metal contaminants onsite and in real-time in a simple and cost-effective way will impact many other areas of research under the SBR program. For example, it will enhance geochemistry/biogeochemistry research by lowering the costs of characterization and by providing more accurate information of the radionuclides and metal contaminants at the DOE sites. This information will strengthen the correlations between results obtained from microbial ecology and community dynamic analyses and DOE site properties, improve the understanding of the mechanisms of biotransformation, and provide deeper insight into biomolecular science and engineering. Practical applications of these sensors will not only help assess the effectiveness of science-based solutions for remediation performed by researchers and engineers, but will also contribute to the long-term monitoring of DOE contaminated sites by DOE staff members, state and local regulation agents, and concerned citizens around the sites.

## Development of U Isotope Fractionation as an Indicator of U(VI) Reduction in U Plumes

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We are evaluating U isotope ratios ( $\delta^{238}\text{U}$ ) as a tool for monitoring U(VI) reduction in the controlled field setting at the Rifle, CO IFRC site. The increased understanding of the biogeochemical behavior of U can guide remedial strategies and long-term stewardship activities at DOE sites. Several key questions are under investigation: (1) Are there confounding processes that complicate efforts to use  $^{238}\text{U}/^{235}\text{U}$  as an indicator of U(VI) reduction (e.g., adsorption, mixing of distinct U from different flow paths)? (2) Is the U isotopic fractionation factor ( $\epsilon = (\alpha - 1) \times 10^3$ ;  $\alpha = ^{238}\text{U}/^{235}\text{U}_{\text{product}}/^{238}\text{U}/^{235}\text{U}_{\text{reactant}}$ ) for U(VI) reduction constant? (3) Does the fractionation factor for U(VI) reduction differ between natural and stimulated conditions? (4) How do effective (field experiments) and intrinsic (laboratory experiments) fractionation factors compare? (5) Can reoxidation be detected using  $^{238}\text{U}/^{235}\text{U}$ ? (6) Can biotic and abiotic reduction be distinguished by  $^{238}\text{U}/^{235}\text{U}$ ?

$\delta^{238}\text{U}$  was measured in groundwater samples from the 2010–11 experiment to evaluate the degree of isotopic fractionation due to (1) bicarbonate induced U(VI) desorption and (2) acetate amended U(VI) reduction (occurring with or without bicarbonate amendment). Samples from a well impacted only by bicarbonate amendment reveal no significant U isotopic fractionation resulting from adsorption/desorption of U(VI) from aquifer solids. Samples from a well impacted by only acetate amendment show a similar shift in  $\delta^{238}\text{U}$  to that observed for the 2007 biostimulation experiment [1] ( $\Delta^{238}\text{U} = 1.35\text{‰}$  and  $1.05\text{‰}$ , respectively), where  $^{238}\text{U}$  is preferentially removed as reduced U(IV). In addition, the 2010–11 results give us, for the first time,  $\delta^{238}\text{U}$  measurements during rebound of U(VI) concentrations after acetate amendment is terminated. In a well impacted only by acetate amendment, groundwater U(VI) concentration and  $\delta^{238}\text{U}$  return to preinjection values within 128 days. Lack of an increase of  $\delta^{238}\text{U}$  above preinjection values implies the primary source of U is advection of U(VI) rather than reoxidation of U(IV). This is particularly important, as the long-term success of this remediation technique depends on the stability of sequestered U(IV). Notably, the recovery of U(VI) concentration and  $\delta^{238}\text{U}$  is much slower for a well impacted by both bicarbonate and acetate amendments; by 201 days after acetate injection stopped, U(VI) concentrations returned to preinjection values, yet  $\delta^{238}\text{U}$  remained significantly lower ( $0.43\text{‰}$ ) than initial conditions, possibly reflecting resorption of upgradient U.

Rifle floodplain samples exhibit limited but significant variation in U concentration and  $\delta^{238}\text{U}$ . The three surface waters collected north of the site are characterized by a mean U concentration and  $\delta^{238}\text{U}$  of 36.9 ppb and  $0.12\text{‰}$ , respectively. Similarly, the background well LR01 (not impacted by mill operations) is characterized by a U concentration and  $\delta^{238}\text{U}$  of 44.5 ppb and  $0.19\text{‰}$ , respectively. The remaining site floodplain samples exhibited significantly higher U concentrations ranging from 111 to 327 ppb and  $\delta^{238}\text{U}$  from  $0.05$  to  $0.24\text{‰}$ . The total variation,  $0.43\text{‰}$ , is greater than the long-term uncertainty,  $0.09\text{‰}$  ( $2 \times$  root mean square difference between measurements of 16 full sample duplicates). Among floodplain samples, as the U concentration increases, a corresponding increase is observed in the  $\delta^{238}\text{U}$  value. This relationship may be due to mixing between background U coming into the site with contaminant U remaining from historical U mill operations.

[1] Bopp, C.J. et al. (2010) *Environ. Sci. Technol.* 44, 5927–5933.

## Linking As, Se, V, and Mn Behavior to Natural and Biostimulated Uranium Cycling

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Biogeochemical redox cycles in groundwater systems frequently mobilize metals and metalloids that directly impact human health. These same biogeochemical cycles can also alter uranium speciation and mobility thus directly impacting groundwater quality at DOE legacy sites. Interestingly, the reducing conditions that immobilize uranium may be optimal for mobilizing metals such as As, Mn, Se, and V. These processes are occurring at the Department of Energy's Field Research Challenge (IFRC) site near Rifle, Colorado and offer a unique opportunity to study biogeochemical redox cycles. At well U01 near the Colorado river, natural redox fluctuations occur with changing river stages. When the river stage is high the aquifer appears oxidized. When the river stage is low the aquifer becomes reducing and Mn, As, and sometimes Fe becomes mobilized. Interestingly, little change in U concentrations are observed. During biostimulation at the IFRC As concentrations dramatically increase in the groundwater. During Fe(III) reduction, As levels increase to concentrations similar to levels observed in well U01 (~1.0  $\mu\text{M}$ ). However under sulfate reducing conditions As concentrations can be over ten times higher. Initial speciation work by IC-ICP-MS from samples collected during the "Super 8" experiment in 2011 indicated that arsenite was the dominant species during Fe(III) reduction but thioarsenates, in particular trithioarsenate, become dominant during sulfate reduction. No thioarsenites were observed. Speciation results were in close agreement with geochemical modeling. Future work will focus on understanding the sorption properties of thioarsenates along with the observed speciation during natural redox fluctuations. The goal is to better constrain the mobility of thioarsenate species and the species present and extent of As transport under natural and stimulated redox conditions.

## Colloids, Deposits, and Clogging in Groundwater Remediation

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This poster describes ongoing research (DE-SC0006962) aimed at linking colloidal phenomena and clogging in groundwater remediation. Colloids—particles between 1 nm and 10  $\mu\text{m}$ —include bacteria, precipitates, and clay minerals that are ubiquitous in natural porous media. Soil science and filtration engineering indicate that colloidal phenomena (*i.e.*, colloid deposit morphology) can cause clogging that is detrimental for groundwater remediation. Can we avoid or manage clogging in groundwater remediation through better understanding of deposit morphology? To address this question, the current research comprises three components:

First, a series of laboratory experiments is being performed to simultaneously measure colloid accumulation, clogging, and deposit morphology. Here we report the first ever measurements of deposit morphology in flow cells, using a novel application of static light scattering in refractive index matched porous media (*i.e.*, Nafion) that quantifies deposit morphology as a fractal dimension. Details are given in the companion poster by Roth, Mont-Eton, and Mays. Ongoing experiments will measure how colloid accumulation, clogging, and deposit morphology vary over a range of physical (*i.e.*, fluid velocity) and chemical (*i.e.*, ionic strength) conditions.

Second, we have developed the first iteration of a clogging model that quantitatively accounts for the effect of deposit morphology. This clogging model is adapted from the literature on water treatment, in which a basic process is removal of colloid aggregates by settling, which provides a link between permeability and aggregate fractal dimension: Settling velocity depends on aggregate permeability, which in turn depends on fractal dimension. This new clogging model provides a framework for interpretation of the laboratory work above.

Third, we have planned a suite of measurements that will link the laboratory and modeling efforts with other research sponsored by the Department of Energy. Specifically, we plan to measure the fractal dimension of colloid suspensions taken from (1) laboratory experiments and (2) the Old Rifle field site. Task (1) links this work to ongoing research on chemical precipitation, x-ray computed microtomography, and nanogeochemistry. Task (2) links this work to conditions at the Old Rifle Integrated Field Research Challenge (IFRC) in Colorado.

## Radiochemically-Supported Microbial Communities: A Potential Mechanism for Biocolloid Production of Importance to Actinide Transport

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This work focuses on the hypothesis that radiogenic substrates such as  $H_2$  at DOE sites may support the growth of microorganisms, and thus indirectly influence the mobility of redox-sensitive radionuclides. Consistent with this hypothesis are early project results indicating the presence of  $H_2$  and SSU rRNA genes, closely related (up to 99% sequence identity) to candidatus *Desulforudis audaxviator*, in fluids from underground nuclear test cavities (U12N.10 tunnel and ER-EC-11) at the Nevada National Security Site (NNSS). In deep South African mines, *D. audaxviator* is believed to utilize  $H_2$  and  $SO_4^{2-}$  from radiochemical reactions; and until recently, this region was thought to define the geographic limit of the genus. Thus, the detection of *D. audaxviator* in radioactive subsurface water at the NNSS supports a radiochemical lifestyle for *D. audaxviator*. The current project builds upon these observations with the sampling of new sites, detailed microbial community and chemistry assessments, and radiochemical modeling.

In 2011, groundwater was obtained from six new wells from the NNSS and Nevada Test and Training Range: ER-20-5 #1 and #3 (4/26/11), ER-20-8 (6/27/11), ER-20-4 (9/21/11), U12N vent hole #2 (10/5/2011), and ER-EC-12 (11/28/11). These samples are currently being analyzed for dissolved ions, gases, and radiologic components. All samples contained sulfate (3 mg/L to ~140 mg/L) but only the U12N sample contained measurable  $H_2S$  (~6  $\mu M$ ). Four of six sulfate samples have been analyzed for sulfur isotopes, with  $\delta^{34}S$  values ranging from +4.43‰ to +17.44‰.  $H_2S$  fractionation values are pending. Corresponding DNA extracts are currently undergoing molecular community assessments including T-RFLP, SSU rRNA gene libraries and 454 pyrotag analysis. Various physiotypes were also cultivated using defined media based on NNSS groundwater. Aerobic heterotrophs were present at all sites analyzed, with densities ranging from  $10^4$ - $10^6$  cells  $mL^{-1}$ . Sulfate-reducing bacteria were also present (~ $10^3$ - $10^4$  cells  $mL^{-1}$ ) at sites ER-EC-11 and U12N. Enrichments from U12N grew equally well in the presence or absence of  $^{239}Pu$ , suggesting a community adapted to a high radiation environment.

To better constrain the abundance of *D. audaxviator* in the U12N.10 tunnel and ER-EC-11 samples, DNA extracts were subjected to pyrotag analysis (~20,000 reads, M. Sogin Lab, MBL). Sequences from the Actinobacteria, Deinococcus-Thermus, Firmicutes, and Proteobacteria phyla were detected in both ER-EC-11 and U12N.10; the U12N.10 tunnel sample also contained Bacteroidetes, Chlorobi, and Nitrospirae. Over 40 genera of *Clostridia* were present, with *Symbiobacterium* and *Dethiobacter* dominating the ER-EC-11 and U12N.10 libraries, respectively. *Clostridia* comprised 25% of the U12N.10 library, and this sample also produced many sequences affiliated with *D. audaxviator* (93-99% similar). Flow cytometric cell sorting was performed (six 384 well plates, Bigelow Lab. R. Stepanauskas) on a cellular retentate washed free of radioactivity from U12N, with subsequent multiple displacement amplification (MDA) yielding ~60 useable sequences. Full genome sequencing will follow if a single amplified genome (SAG) from a North American *D. audaxviator* is obtained.



## Electrical Responses of Grain Surfaces Measured by Spectral Induced Polarization and Atomic Force Microscopy

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Quantitatively describing the spectral induced polarization (SIP) response (a.k.a complex conductivity) of the subsurface is a problem that spans nanometer to meter length scales. We are combining surface-sensitive, micron-scale polarization and atomic force microscope (AFM) techniques with column scale SIP observations to bridge these scales. Recently we have been evaluating whether complex conductivity measurements of grain surfaces can be obtained using AFM. Initial AFM calibration measurements were performed on glass slides with different geometries to confirm that complex surface conductivity values could be achieved. After accounting for geometry, we found consistent patterns in the real conductivity change with frequency for different slide geometries and a very strong capacitive response; the order of the magnitude for the low frequency real conductivity was  $10^{-11}$  S/m which is comparable with the theoretical values for glass ( $10^{-11}$  to  $10^{-15}$  S/m). We then performed SIP experiments and AFM measurements using glass beads under four different experimental conditions: (1) unaltered, (2) etched with hydrofluoric acid, (3) calcite coated, and (4) iron oxide coated. The calcite coated beads showed a decrease in real conductivity of 10% compared to the plain beads for the SIP measurements and a decrease of approximately two orders of magnitude for the AFM measurements. This observation is consistent with the fact that surface charge maps obtained with the AFM also showed a decrease in surface charge density for the calcite coated beads. In contrast, both the etched and iron coated beads showed an increase in real conductivity relative to the plain beads, though the magnitude of increase was inconsistent between the SIP and AFM measurements. The iron oxide coated and etched beads increased in real conductivity by 34% and 12%, respectively, for the SIP measurements. For the AFM measurements the real conductivity of the iron coated beads doubled, whereas it increased by approximately an order of magnitude for the etched beads. The difference between the SIP and AFM response could be an indication that even though the etching created a larger impact on individual grain surfaces, the iron oxides bridge grains to create a continuous conductive surface pathway through the porous medium. The SIP measurements showed that the peak response of the imaginary conductivity shifted from approximately 0.03Hz for the plain beads to approximately 0.1Hz for the etched beads and 0.5Hz for calcite coated beads without a significant change in magnitude between any of these samples. In contrast, the imaginary conductivity of the iron oxide coated beads exhibited a shift in peak response to 0.05Hz, but doubled in magnitude relative to the other treated beads. Consistent phase measurements for the beads have not yet been obtained using the AFM.

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

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Bacteria are ubiquitous in a wide-range of low temperature aqueous systems, and can strongly affect the distribution and transport of metals and radionuclides in the environment. However, the role of metal adsorption onto bacteria, via the reactive cell wall functional groups, has been largely overlooked. Previous macroscale metal sorption, and XAS studies have shown that carboxyl and phosphoryl functional groups to be 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.

We examined the sorption and structure of Hg complexes on the cell membranes of *Bacillus subtilis*, *Shewanella oneidensis*, and *Geobacter sulfurreducens* as a function of pH, and Hg and  $\text{Cl}^-$  concentration. The concentration of reactive thiols on cell membranes are also characterized using a newly developed fluorophore technique. Mercury adsorbs strongly, and exhibits similar sorption trends for all bacterial species; however, our XAS and fluorescence spectroscopy studies indicate that Hg speciation is significantly different on methylating and non-methylating organisms. In the case of *S. oneidensis*, Hg forms  $\text{HgS}_3$  (S= organic thiol) complex at nanomolar Hg concentration, and  $\text{HgS}_2$  and  $\text{HgS}$  (S = cysteine) with increasing Hg concentrations in the sub-micromolar range. Mercury binds to carboxyls with further increases in Hg concentration, and this transition occurred in the range of 20-25 mM of Hg per gram of cells. Our fluorescence spectroscopy studies indicate that the concentration of reactive thiols is also in the same range on cell membranes of *S. oneidensis*, indicating saturation of all available thiol sites by Hg before binding to carboxylates. Whereas *G. sulfurreducens* exhibit  $\text{HgS}_2$  and  $\text{HgS}$  complexes although it has more reactive thiols on its cell envelope when compared to *S. oneidensis*. *G. sulfurreducens* may exhibit  $\text{HgS}_3$  complexes at much lower Hg concentration range, which is difficult to access using XAS. The presence of  $\text{Cl}^-$  reduced Hg adsorption on cells significantly because of the formation of Hg-chloro aqueous complexes. The XAS studies indicate that the structure of the Hg surface complex on the cell membranes is not affected by the presence of  $\text{Cl}^-$ .

Our current studies are in progress- i) to evaluate the site density of thiols on cell envelope, and how they vary between different organisms under different environmental conditions, and ii) the role of DOM on Hg interactions with different bacteria under different pH and NOM concentrations. 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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Mercury released to the environment at the U.S. Department of Energy's Oak Ridge Y-12 Site during the Cold War is now distributed downstream in the floodplain of East Fork Poplar Creek as Hg(II) bound to natural organic matter, mercury sulfide minerals, and methyl-mercury. Strong binding to reduced sulfur in soil or dissolved natural organic matter, organic matter coatings on mineral surfaces, or sulfide minerals may immobilize mercury(II) and/or inhibit methylation reactions. This project is focused on identifying and quantifying binding and release mechanisms of Hg to organic matter, clay minerals, and soils characteristic of the floodplain. We hypothesize that (1) Hg(II) release from reduced sulfur sites requires biogeochemical redox-active agents, whereas release from oxygen sites does not; (2) Mercury bonded to organic matter on clay mineral surfaces is immobilized to a greater extent 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.

We are investigating adsorption and release of Hg(II) in controlled laboratory experiments using single minerals and natural organic matter. Synopses of sub-projects and results in progress are as follows. (1) Mercury(II) uptake on vermiculite, which comprises 12 to 16% of all minerals in the floodplain soil (N 35°57.959; W 84°21.570), shows an adsorption edge between approximately pH 7.5 and 9.5 in experiments using ~11 mg of solid in 30 mL of an initial 100 ppb Hg(II) solution. Downward shifts of pH by around one pH unit across the edge do not result in mercury(II) release. Work is underway to measure Hg(II) uptake in the presence and absence of well-characterized dissolved organic matter similar to that leached during simulated flooding and drying experiments from floodplain soils (see below). (2) Mercury(II) release from cinnabar under reducing conditions is somewhat higher in the presence of simple quinones, is not pH-dependent over the range of 4.0 to 7.0, and decreases with increasing mass. (3) Using Hg-L<sub>3</sub> edge EXAFS spectroscopy we have characterized the kinetics of mercury(II) binding to dissolved organic matter. (4) To characterize Hg binding to the EFPC soils, we have reassessed how to quantify sulfur speciation in natural organic matter using S-K edge XANES spectra. We refined the Gaussian curve fitting method commonly applied and also used a new linear combination fitting approach to determine sulfur speciation.

We are characterizing mercury release from soil cores collected from the top few tens of cm at a stream bank and floodplain site along the East Fork Poplar Creek downstream from Oak Ridge in simulated flooding and drying experiments (Poulin et al., this volume). Mercury concentration, mercury physiochemical speciation via selective sequential extraction, organic matter content, elemental composition, cation exchange capacity, and soil mineralogy were determined in undisturbed, intact soil cores. Pore fluids during flooding periods were analyzed for total dissolved mercury, elemental indicators of redox state, dissolved sulfate and sulfide, dissolved organic carbon, and colloids. Distinct differences in mercury release kinetics and release mechanisms were observed between O and A horizons. Work is in progress on identifying Hg binding using S-K edge XANES and Hg-L<sub>3</sub> edge EXAFS spectroscopy.

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

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In this project, we are combining molecular genetic, spectroscopic, and microscopic techniques with kinetic and reactive transport studies to describe and quantify biotic and abiotic mechanisms underlying anaerobic, nitrate-dependent U(IV) and Fe(II) oxidation, which influences the long-term efficacy of *in situ* reductive immobilization of uranium at DOE sites. In these studies, *Thiobacillus denitrificans*, an autotrophic bacterium that catalyzes anaerobic U(IV) and Fe(II) oxidation, is used to examine coupled oxidation-reduction processes under either biotic (enzymatic) or abiotic conditions in batch and column experiments with biogenically produced  $U^{IV}O_2(s)$ . Research is focused on identifying the primary redox proteins that catalyze metal oxidation, environmental factors that influence protein expression, and molecular-scale geochemical factors that control the rates of biotic and abiotic oxidation.

Biogenic  $UO_2(s)$  was synthesized under anaerobic conditions (using *S. oneidensis* strain MR-1) following previously published methods. Mixtures of biogenic  $UO_2(s)$  and quartz were packed into 1 mL or 5 mL polypropylene columns. For experiments in the presence of *T. denitrificans*, cells were harvested anaerobically, washed and resuspended, mixed with quartz and biogenic  $UO_2(s)$ , and packed into the columns. Oxidation experiments were carried out in the dark at  $25 \pm 2^\circ C$  in an anaerobic glove box (10%  $H_2$ , 90%  $N_2$ ) in buffered solution (MOPS, pH  $\sim 7.2$ ). In abiotic experiments,  $UO_2(s)$  oxidation by nitrate (up to 20 mM) was relatively slow; higher dissolved U concentrations were observed in abiotic experiments with nitrite (up to 20 mM). Reactive transport modeling (using Crunchflow) of abiotic column experiments, which included sorption of U(VI) in the column, simulated steady-state U release. In the presence of *T. denitrificans* and 10 mM nitrate, higher rates of dissolved U release were observed compared with abiotic controls, suggesting that *T. denitrificans* catalyzed the oxidative dissolution of  $UO_2(s)$ . Characterization by synchrotron XAS showed higher fractions of U(VI) compared to U(IV) in post-experiment solids reacted with higher dissolved nitrite concentrations. Structural analysis of biogenic  $UO_2(s)$  by EXAFS suggested variable particle size and/or a variable degree of local structure disorder. Spatially resolved characterization of samples reacted with nitrite and Fe(II) solutions by SXTM showed association of U with C and Fe around edges of  $UO_2(s)$  particles. Experiments examining Fe(II) and Fe(III) in coupled redox reactions are in progress.

Current work with *T. denitrificans* focuses on determining the enzymes responsible for anaerobic, nitrate-dependent Fe(II) oxidation. We previously reported that two *c*-type cytochromes, Tbd\_0187 and Tbd\_0146, were involved in anaerobic nitrate-dependent U(IV) oxidation in *T. denitrificans*. However, we have found that these same mutants were *not* defective in nitrate-dependent Fe(II) oxidation. Here we report on efforts to identify genes involved in anaerobic Fe(II) oxidation in *T. denitrificans* based on screening of its Tn5 transposon mutant library; over 10,000 mutants have been screened to date, with defects in Fe(II) oxidation as great as 40%. One mutant that is 27% defective in Fe(II) oxidation relative to the wild-type has the Tn5 transposon inserted in a putative *c*-type cytochrome. The insertion is downstream of the CXXCH heme-binding motif. A deletion knockout mutant for the disrupted gene is under construction, and more mutants from the library are being screened.

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

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Mineral dissolution and precipitation in porous media alters pore network structure and subsequent flow velocities, creating complex interactions between reaction and transport. This project investigates mineral reactions and alterations in flow permeability in a multi-scale, multi-dimensional study that combines experimentation, modeling and imaging. Primarily, we are targeting the reaction of highly caustic, radioactive waste solutions with subsurface Hanford sediments, and the immobilization of radionuclides.

(1) *Reactive flow experiments.* We are conducting experiments using reactive flow-through columns of several sizes. The most recent experiments performed at PNNL used 7.6-cm-long x 1.9-cm-diameter columns. Separate columns were packed with quartz sand and with Hanford sand to evaluate the influence of mineralogy on transport and reaction rates and changes in pore-structure resulting from contact with a synthetic tank waste liquid (STWL). Another column was flown through using STWL without Al, in which only dissolution is occurring, which results in porosity increases. Experiments have shown porosity changes ranging from 0.04 to 0.11, resulting in calculated permeability reductions on the order of 2 to 3 times. Also observed were changes in pore structure that would affect the unsaturated hydraulic properties of the sediments (moisture retention characteristics and relative permeability).

(2) *Multiscale Imaging and Analysis.* To examine the reaction-induced alterations of pore structure, we are using 3D X-ray computed microtomography, and 2D backscattered electron and energy-dispersive X-ray imagery. In our early work, columns reacted at PNNL were imaged using X-ray computed microtomography at BNL, but recently we are using an XMT system at EMSL (PNNL). The 3D images are analyzed in novel ways to quantify pore structure, mineral distribution, structural changes and fluid-air and fluid-grain interfaces. This informs pore network models for calculating expected changes in permeability and reactive surface area. At Princeton, using the method we have developed for sectioning the columns and using SEM and EDX for 2D imaging, sub-pore scale structural alterations have been observed. For example, secondary cancrinite precipitates were observed in reacted columns as a relatively uniform coating on all grain surfaces. 2D imaging also revealed that Hanford sediments have a large amount of intragranular porosity and that secondary precipitates were observed in intragranular space.

(3) *Multiscale Modeling and Up-Scaling.* This project involves modeling at several spatial scales. The column experiments are being modeled using the STOMP simulator to evaluate ways of representing effective reaction rates and feedback between changes in pore structure and the permeability relations needed for the continuum-scale. The column experiments have also been modeled using a custom reactive transport model to investigate the capacity for intragranular pore space in Hanford sediments to sequester and release radionuclides. Long-term leaching of Cs from intragranular pores is predicted to prolong the period of secondary contamination at the site. Finally, a specific application of our pore-network reactive transport modeling was aimed at understanding how CO<sub>2</sub>-rich brines alter rocks containing fast-reacting minerals such as calcite and dolomite. These simulations of porosity and permeability evolution reveal the importance of considering different flow regimes (advection-controlled vs diffusion-controlled) and inlet fluid mixing scenarios. We have shown that there is not a unique continuum-scale permeability relationship that describes all reactive flow scenarios for even the same porous medium and that the spatial and temporal evolution of the network dictates the unique relationship between porosity and permeability.

## Design and Application of Proteomics Workflows to Monitor and Predict *In Situ* Activity of Metal-Reducing Bacteria

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The expression of *c*-type cytochromes has been correlated with bacterial metal and U(VI) reduction activity. With recent advances in environmental proteomics, *c*-type cytochrome expression may serve as an indicator for microbial activity contributing to metal and U(VI) reduction. Cytochrome expression was studied in *Anaeromyxobacter dehalogenans* strain 2CP-C, *Geobacter daltonii* strain FRC-32, and *Shewanella oneidensis* strain MR-1, which represent bacterial clades commonly detected in U-contaminated soils and sediments. Based on the presence of characteristic heme-binding motifs, 68, 42 and 72 putative *c*-type cytochromes have been predicted for *A. dehalogenans*, *S. oneidensis* and *G. daltonii*, respectively. Biomass of each strain was collected from media amended with different electron acceptors, and global proteome measurements recovered protein datasets that were queried against predicted proteome databases via SEQUEST. Variable levels of *c*-type cytochrome expression were observed for each bacterial species grown with different electron acceptors. In *A. dehalogenans*, 53 out of 68 predicted *c*-type cytochromes were detected across nine growth conditions tested. In *S. oneidensis*, 23 *c*-type cytochromes out of annotated 42 were identified. In *G. daltonii*, the expression of 29 out of 73 *c*-type cytochromes was noted; however, only three growth conditions have been tested so far. Extending this work to field samples, groundwater filters from the emulsified vegetable oil (EVO) demonstration (4 days post amendment) were selected for proteomic analysis. The peptide profiles were searched with two databases: (i) an artificial database of ~ 12 sequenced soil microbes and (ii) the database of three model species. Initial results confirmed that proteins, including *c*-type cytochromes, can be identified and that EVO injection resulted in metaproteome differences. Current efforts aim at the integration of quantitative DNA and RNA measurements with the proteomics datasets to obtain a refined picture of abundance and activity of bacteria relevant for metal and radionuclide reduction. Knowledge gained from these studies will help to better predict the fate of subsurface radionuclides, thus enabling science-based decision making for long-term site management.

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

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This project is focused on developing a fundamental understanding of 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 overarching hypothesis of the project that the strong interactions of actinides with mineral surfaces result from the formation of inner sphere complexes with a limited number of high-energy surface sites, and that displacement of solvating water molecules from the actinide and mineral surface during sorption is energetically favorable and results from a large increase in entropy.

In order to test the above hypothesis without involving the complex redox chemistry of plutonium, Eu(III) and Np(V) were selected as strongly and weakly hydrated, respectively, species. Sorption of Eu(III) to hematite increased strongly with temperature while Np(V) exhibited little change. This is consistent with our hypothesis showing that sorption of Eu(III) is entropically driven as hydrating waters are removed from the mineral surface and the Eu(III) ion upon sorption. A series of unique, high energy k-shell Eu EXAFS measurements were performed and confirmed at the coordination number of Eu(III) decreases upon sorption (consistent with the removal of hydrating waters) and that a bidentate, mononuclear surface species forms. Sorption enthalpy and entropy values were calculated using a surface complexation model and the van't Hoff equation. Consistent with the overarching hypothesis of this work, sorption of Eu(III) was entropically driven. This observation of the dominant entropy term is consistent with the experimental hypothesis that the energetic favorability of actinide surface complex formation is strongly influenced by positive sorption entropies, which are mechanistically driven by displacement of solvating water molecules from the actinide and mineral surface during sorption. These results are currently being compared with quantum mechanical models of Eu(III) sorption as both inner and outer sphere complexes.

Sorption of Pu(IV) and Pu(V) to goethite has exhibited a similar increase with increasing temperature. These experiments as well as many others in the literature, have observed surface mediated reduction of Pu(V) to Pu(IV) although the specific mechanism, including the reductant, is still unclear. Experiments are being performed to examine possible mechanisms. Experiments with multiple Pu isotopes ( $^{238}\text{Pu}$  and  $^{242}\text{Pu}$ ) have shown that alpha particle generated radiolysis does not appear to significantly influence the reduction rate. Attempts to remove trace Fe(II) during hematite nanoparticle synthesis by aeration did not influence the observed reduction rates. Furthermore, the reduction rate on the Pu(V) nanoparticles was significantly influenced by light, indicating that semiconducting properties of the mineral can influence the electron transfer reaction. It is possible that goethite may indirectly participate in Pu(V) reduction by acting as a catalyst or electron shuttling device to transfer charges between coadsorbates, as suggested by recent quantum mechanical modeling of uranyl reduction on goethite in the presence of hydrogen disulfide.

These Pu sorption studies will be used to develop thermochemically based surface complexation models as discussed above for the Eu(III)-hematite system. The surface complexation constants will be used to predict sorption of Pu to sediments from the Savannah River Site and the Hanford Site. Experiments quantifying sorption of Th(IV), Pu(IV), Pu(V), and Np(V) have been completed. Based on differences between systems with initially Np(V) and Pu(V), both sediments appear to facilitate surface mediated reduction of Pu(V) to Pu(IV).

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

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Matrix Diffusion and Adsorption within a rock matrix are important mechanisms for retarding transport of radionuclides in fractured rock. Due to computational limitations and difficulties in characterizing complex subsurface systems, diffusive exchange between a fracture network and surrounding rock matrix is often modeled using simplified conceptual representations. There is significant uncertainty in “effective” parameters used in these models, such as the “effective matrix diffusivity”. Often, these parameters are estimated by fitting sparse breakthrough data, and estimated values fall outside meaningful ranges (e.g. effective matrix diffusivity much greater than free molecular diffusivity), because simplified interpretive models do not consider complex three-dimensional flow. There is also evidence for an apparent scale-dependence in effective matrix diffusivity, also a consequence of using over-simplified interpretive models. These observations raise questions on 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. Due to tremendous advances in computational technology over the last 10 years, DFNM modeling in relatively large domains is now feasible. The overarching objective of our research is to use DFNM modeling to improve fundamental understanding of how effective parameters in conceptual models are related to fracture network structure and matrix properties. An advanced three-dimensional DFNM model is being developed, which combines upscaled particle-tracking algorithms for fracture-matrix interaction and a parallel fracture-network flow simulator. The particle-tracking algorithms 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, multi-scale preferential flow and matrix heterogeneity. 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 (i) the relationship between effective fracture-matrix interaction parameters, network structure and matrix properties and (ii) their scale dependence in different types of fractured rock environments.

We will demonstrate results obtained using “high-resolution” particle tracking algorithms at the single fracture scale and at fracture intersections; and “upscaled” particle-tracking algorithms, which allow use of much larger time steps. The upscaled algorithms have been verified using the “very-high-resolution” simulation results as a benchmark, and hold significant promise as an efficient tool for field-scale simulation. A generalized approach for particle tracking in interfaces has been developed, which captures the complexities of Stokes flow through variable-aperture intersections with excellent accuracy. Flow simulations in fracture networks illustrate the important role of head variations along an intersection in driving flow through slow advective loops in dead-end fractures. Such loops have been postulated as potential mechanisms for producing long-tailed behavior even in the absence of true matrix diffusion, and often confused for matrix diffusion. Ongoing efforts are focused on extending the network flow and transport simulations to very large scales, and including the influence of adsorption.

The final stage of 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. We will explain the differences in behavior observed at these sites using our network model and subsequently simulate radionuclide transport at the site scale and 100+ year time scales.



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

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An insufficient knowledge of the biological mechanisms of contaminant transformation often limits the performance of *in situ* subsurface bioremediation and long-term stewardship strategies. The *in situ* stimulation of Fe(III) oxide reduction by *Geobacter* bacteria, for example, leads to the concomitant precipitation of U(VI) from groundwater. However, the biological mechanism behind this reaction has remained elusive for almost two decades. Because Fe(III) oxide reduction requires the expression of conductive pili in *Geobacter*, we evaluated their contribution to uranium reduction in piliated and non-piliated strains of *Geobacter sulfurreducens*. We observed a direct correlation between the levels of piliation and the extent of U removal and reduction. Furthermore, pili expression also prevented the permeation of U in the cell envelope and its periplasmic mineralization. As a result, pili expression preserved the vital respiratory activities of the cell envelope and the cell's viability. Uranium preferentially precipitated along the pili as a mononuclear U(IV) complexed by carbon containing ligands and, to a lesser extent, on outer membrane redox-active foci. These results demonstrate a previously unrecognized role for *Geobacter* pili in the extracellular reduction of uranium and highlight its essential function as a catalytic and protective cellular mechanism that is of interest for the bioremediation of uranium-contaminated groundwater.

The expression of pili by *Geobacter* also promotes cell aggregation and biofilm formation. Thus, we investigated the contribution of biofilms of *G. sulfurreducens* to U transformations. Multilayered biofilms reduced substantially more U than planktonic cells and for prolonged periods of time. They also tolerated higher concentrations of U, making them an attractive option for the development of permeable biobarriers for U bioremediation. While U was immobilized by both monolayered and multilayered biofilms, only pili-expressing multilayered biofilms reduced the U. Similarly, a pili-deficient mutant, which is interrupted at the monolayer stage, immobilized but did not reduce U. Thus, the pili are also the primary U reductase in the biofilms. To gain more insights into how biofilms transform U, we screened a library of 4,000 transposon-insertion mutants and identified mutants with biofilm defects. After identifying the interrupted genes and their functions, we built a genetic model for biofilm formation that links biofilm development to the mechanisms of U transformations. This study confirmed the role of *Geobacter* pili in the biofilm formation and U reduction and identified molecular markers involved in biofilm electron transport and metabolism that can be used to predict and monitor the physiological state of *Geobacter* bacteria during the *in situ* bioremediation of U.

## Geoelectrical Surveys at the Oak Ridge Field Research Center

*Oak Ridge ORFRC (Principal Investigator: André Revil)*

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We performed a 3D resistivity survey and self-potential mapping at the Oak Ridge Field Research Center (ORFRC) to locate contaminant migration from the former waste disposal S3 ponds. The S3 area historically received liquid waste containing uranium and nitric acid, which has leached into the underlying groundwater. The pH of the contaminated groundwater is less than 4 and the electrical conductivity is very high, exceeding 5 S/m at several locations in the plume. The groundwater composition made it possible to detect zones of contaminant migration using electrical resistivity. A total of 17 resistivity profiles were collected along three sides and downgradient of the former S3 ponds. All but two profiles used an electrode spacing of 2 m with a total profile length of 126 m while the electrode spacing of the two 235 m long profiles was 5 m. The electrical conductivity of water samples collected from monitoring wells and inverted electrical conductivity at the same locations are correlated. The correlation provides evidence that ERT can be used to detect plumes with high total dissolved solids emanating from the former ponds. ERT measurements were also inverted to create a 3D resistivity model. Zones of low resistivity correspond to zones where contamination is observed. Time-lapse electrical resistivity measurements collected along one profile downgradient of the S3 ponds were inverted using a new time-lapse algorithm with an active time constrain approach to investigate the effect of infiltration on plume dilution and attenuation. Decreasing electrical resistivity was observed immediately after heavy rainfall and the greatest electrical resistivity was observed between May and September, which is the driest period during the year. Self-potential profiles were corrected for the presence of anthropogenic noise and provide further evidence for the location of the plumes.

In addition to the field measurements, we also investigated the petrophysical properties of saprolite core samples in the laboratory including complex conductivity and streaming potential coupling coefficients at different salinities and pH. Contaminated saprolite was neutralized in a separate column experiment. The results showed that complex conductivity was related to the pH and highlighted the large acidity accumulated in the sediments. The formation factor of saprolite samples measured in the laboratory was less than the formation factor estimated from field measurements. The difference is likely related to disturbance of the samples during sampling and repacking into columns. The electrical surface conductivity determined in the laboratory, on the other hand, corresponds to what was observed in the field and was related to the relative clay content of the samples. The background sample collected from the greatest depth (26 feet) contained more illite, smectite and chlorite than samples collected from 9 and 16 feet and also exhibited greater surface conductivity.

## Microbial Communities Associated with Anaerobic Metabolism and U(VI) Reduction in ORFRC Area 2 Sediment Amended with Acetate or Ethanol

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This project examined bulk terminal electron accepting processes (TEAPs) and U(VI) reduction in ORFRC Area 2 sediments undergoing biostimulation through ethanol or acetate amendment. The central hypothesis is that patterns of U(VI) reduction will be linked to shifts in the predominant TEAP and to changes in abundance and activity of different types of respiratory microorganisms. Bulk TEAPs, U(VI) reduction, and microbial community composition were monitored in semicontinuous culture reactors (SCRs) (10 day residence time) amended with different amounts acetate or ethanol (either 0, 0.1 or 0.2 mM d<sup>-1</sup>, in duplicate, for a total of 10 reactors) over a ca. 8-month period. Overall TEAP patterns were similar in the acetate and ethanol-amended reactors. Complete consumption of incoming nitrate was observed in all amended reactors. Complete reduction of Fe(III) phases (oxides and clays) took place in sediments receiving the higher levels of electron donor addition, in which sulfate reduction became active after 2–3 months. Roll tube enumerations confirmed major stimulation of Fe(III)- and sulfate-reducing populations. Patterns of U(VI) reduction indicated that the extent of reduction was similar in acetate vs. ethanol-fed reactors, and proportional to the amount of electron donor added. A major increase in the accumulation of residual HNO<sub>3</sub>-extractable U (presumably uraninite) took place during the transition between Fe(III) and sulfate reduction, indicating significant contribution of sulfate reducing bacteria to U(VI) reduction. The microbial community response to electron donor amendment in the SCRs was assessed via quantitative PCR (q-PCR) analysis of taxa-specific 16S rRNA genes and selected functional genes, 16S rRNA gene pyrosequencing, and <sup>13</sup>C stable isotope probing (SIP) of bacterial PLFAs. The q-PCR analyses revealed a ca. 10-fold increase in total 16S rRNA gene copies in the amended reactors. *Betaproteobacterial* 16S rRNA gene and *nosZ* functional genes were most abundant, increasing ca. 5-fold in the amended reactors and indicating a predominance of nitrate-reducing biomass in the reactors. Densities of *Deltaproteobacterial* 16S rRNA genes were lower, but increased more dramatically (ca. 100-fold) in response to electron donor addition. Although a full analysis of the 16S rRNA gene pyrosequencing results (7 time points for each of the 10 reactors) is not yet available, data from ca. 4 months into the experiment revealed communities dominated by known *Betaproteobacterial* nitrate-reducing taxa (e.g. *Dechloromonas*, *Herbaspirillum*, *Azoarcus*). Metal- and sulfate-reducing taxa (e.g. *Anaeromyxobacter*, *Desulfotomaculum*) were present in the libraries, but their relative abundance was generally much lower than putative nitrate-reducers. Bacterial PLFAs showed dramatic shifts with ethanol or acetate amendment. Profiles from unamended sediments were dominated by the fatty acids 16:0 and cy17:0, while amended sediments showed increased importance 16:1w7 and 18:1w7 and a reduction in cy17:0. Short-term (3-day) SIP (<sup>13</sup>C-labeled ethanol or acetate) assays conducted periodically during the experiment showed incorporation of <sup>13</sup>C values into some but not all PLFAs. The most heavily labeled fatty acids in assays conducted at day 150 were associated with metal/sulfate-reducing bacteria and other anaerobic taxa (br17:1a, 10Me16:0, i15:0). The collective geochemical and microbial community analysis results provide the basis for application of a population-based biogeochemical reaction model to the SCR experiments.

## Processes Affecting Iodine-127,129 Speciation and Mobility in Two Contaminated DOE Plumes

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**Background:**  $^{129}\text{I}$  is among the key risk drivers at all DOE nuclear disposal facilities. In our on-going SBR project we developed several highly sensitive iodine speciation techniques and one of our key findings was that sediment bacteria are capable of influencing the chemical behavior of iodide, the most common form of iodine found in groundwater, via accumulation and oxidation to iodate. In turn, sediment bacteria, as well as reduced metals in the aquifer, are capable of reducing iodate to organo-iodine via iodide. Based on this conclusion and others, we proposed the following **hypotheses** and objectives. **H1:** Despite its thermodynamic stability, iodide is readily transformed to organic iodine and iodate, a transformation that is facilitated by biotic (e.g., non-specific oxidative enzymes) and abiotic (e.g., redox reactions by Mn(IV,II), Fe(III,II), or hydroquinone/quinone moieties in humic acids) factors. **H2:** Microbial mediated oxidation of iodine species irreversibly transfers I into natural organic matter, of which aromatically bound iodine is the most stable. **H3:** I mobility is dependent on physico-chemical speciation, which decreases from iodide to iodate to organic iodine, and from low molecular weight to high molecular weight to particulate organic I species. Our **approach** will determine how microbial activity, concentrations and chemical speciation (iodide, iodate, and organo-I) of  $^{129}\text{I}$  and  $^{127}\text{I}$ , as well as redox reactive metals and organic carbon, affect I mobility and isotopic fractionation in groundwater samples from contaminated SRS and Hanford site locations.

**Highlights:** A sensitive and rapid method was developed which enabled us to determine isotopic ratios ( $^{129}\text{I}/^{127}\text{I}$ ) of speciated I via GC-MS [1]. The fact that I occurs in multiple oxidation states leads to complex biogeochemical cycling of I. We demonstrated that the mobility of I species greatly depends on the I concentration used [2], mostly due to covalent binding of I to a limited number of aromatic C moieties of the particle surface and in solution [3–5]. Bacterial accumulation of  $\text{I}^-$  followed by iodination of cellular organic molecules was demonstrated, but likely plays a minor role in the formation of organic I in the subsurface [6]. First-order calculations indicate that the modest increase of 0.7 pH units detected in the study site groundwater over the last 17 years since closure of the basins may be sufficient to produce the observed increased groundwater  $^{129}\text{I}$  concentrations [up to 1 nCi/L; [7]. Removal of I from the groundwater through the formation of high molecular weight organo-I complexes is modified by the release of more mobile organo-I species [8,9]. Extracellular, enzymatic oxidation of  $\text{I}^-$  and organic acid production by bacteria significantly contribute to organo-I formation [10,11].

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## The Role of Natural Organic Matter in Immobilizing or Re-mobilizing Plutonium in the Far Field of the Savannah River Site, USA

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Plutonium (Pu) contamination of soils or groundwater can be a serious problem at DOE sites. Pu is an element with a complex chemistry, exhibiting a number of oxidation states in aquatic environments, predominantly Pu(IV) and Pu(V). In surface and subsurface waters, the prevailing Pu oxidation state depends on the abundance of natural organic matter (NOM) and selected minerals. NOM reducing moieties can significantly modify the mobility of Pu in the environment by producing colloidal organic carriers that strongly bind Pu, mostly as Pu(IV).

**Objectives:** *Objective 1:* Build on our successful characterization of the chemical composition of organo-Pu species in water leachates from the remediated RFETS site by using a similar approach on other soils or aquifer sediments from different Pu-contaminated DOE sites. These Pu-associated ambient colloidal organic leachates will then be compared to those that have formed over time when a Pu tracer was added to the same soils, and will be used for transport studies. *Objective 2:* Determine the biogeochemical factors that give mobility and/or immobility to the colloidal organic carrier molecule. This requires a full molecular level characterization of the chemical composition of strongly Pu-binding biopolymers in NOM extracted from laboratory incubation, leaching and column studies with these soils. *Objective 3:* Determine thermodynamic stability constants of Pu to the well-characterized Pu-carrying NOM compounds isolated from Pu contaminated soils.

**Hypotheses:** *H1:* Mobile organic Pu species from Pu contaminated soils contain biomarker compounds that can be used to predict the future spread of Pu contamination as well as Pu bioavailability. *H2:* Amphiphilic EPS molecules of moderate molecular weight (e.g., 5-50 kDa) containing reducing moieties (e.g., hydroquinones, ferredoxins, or flavodoxins) with clustered ligand groups for Fe(III)-binding are most effective in immobilizing organo-Pu species that sorb to sediment particles, while colloids of  $\leq 105$  kDa molecular mass are potentially mobile in groundwater. *H3:* Binding constants of Pu to well characterized Pu-carrying NOM compounds isolated from Pu contaminated soils depend on applied Pu concentrations due to the well-known surface site heterogeneity effect.

**Experimental Design:** The objectives of this proposal will be accomplished through collaborative research at TAMUG and ODU, as well as researchers from national laboratories. **The potential benefits** of the project to DOE are a better understanding of the role and mobility of different organic bio- and geopolymeric Pu vectors that will greatly advance science in general, and in particular, biogeochemistry of Pu at low, environmental levels.

**Preliminary Results:** In the present study, SRS sediment cores collected from a reservoir lake, which was used as part of a cooling water loop for two production reactors during the period 1961 to 1964, is analyzed for elemental composition and plutonium content. A significant positive correlation is shown between NOM content and Pu concentration. Humic acid obtained from these sediments extracted following the IHSS standard method is prepared in different ways, and Kds of Pu to the humic acid fraction are calculated and compared. Pu bound NOM is further separated and purified using isoelectric focusing method [Xu et al., 2008. ES&T 42, 8211-8217]. Composition of the Pu enriched organic fraction is determined by elemental analysis, FT-IR, NMR, and FT-ICR-MS.

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

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Understanding relationships between physical and chemical processes at different scales is critical to effective prediction of contaminant mobility. This study addresses the molecular-scale response of dissolved uranium to advective chemical gradients, simulating ground water encountering soil and sediment matrices that are representative of subsurface environments at the Oak Ridge Integrated Field Research Challenge (ORIFRC) site.

Column experiments were devised to investigate the role of changing fluid composition on mobility of uranium through a sequence of geologic media. Synthetic ground waters were pumped upwards at 0.05 mL/minute for 21 days through layers of quartz sand alternating with layers of uncontaminated soil, quartz sand mixed with illite, quartz sand coated with iron oxides, and a second soil layer. Increases in pH or concentration of phosphate, bicarbonate, or acetate were imposed on the influent solutions after each 7 pore volumes while uranium (as uranyl) remained constant at 0.1mM. A control column maintained the original synthetic groundwater composition with 0.1mM U. Pore water solutions were extracted to assess U retention and release in relation to the advective ligand or pH gradients. Following the column experiments, subsamples from each layer were characterized using microbeam X-ray absorption spectroscopy (XANES) in conjunction with X-ray fluorescence mapping.

U retention of 55 – 67 mg occurred in phosphate >pH >control >acetate >carbonate columns. The mass of U retained in the first-encountered quartz layer in all columns was highest and increased throughout the experiment. The rate of increase in acetate- and bicarbonate-bearing columns declined after ligand concentrations were raised. U also accumulated in the first soil layer; the pH-varied column retained most, followed by the increasing-bicarbonate column. The mass of U retained in the upper layers was far lower.

A preliminary transport model that incorporates surface complexation, using the code PHREEQC (7, USGS, V.2.18), captures the major features observed in the carbonate and pH gradient experiments but is less successful for the phosphate column. The acetate column was not modeled. In the carbonate column, the model indicates that uranium complexes adsorbed to quartz can account for the observed U uptake and subsequent release. The pH-varied simulation showed increasing pH to lead to U retention, as observed.

Speciation of U, interpreted from microbeam XANES spectra and XRF maps of sediments collected from the columns, varied within and among the materials. Evidence of minor reduction to U(IV) was observed in the first-encountered quartz layer in the phosphate, bicarbonate, and pH columns while only U(VI) was observed in the control and acetate columns. Spectral evidence suggests poorly ordered precipitates in this layer of the phosphate, acetate, and pH columns. In the soil layer, the acetate and bicarbonate columns both indicate minor reduction to U(IV), but U(VI) predominated in all columns. Evidence of precipitates in the soil layer is restricted to the control column. These results will be used to inform and refine the transport models.

## Hg(II) Uptake and Methylation in Iron-Reducing Bacteria

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Mercury uptake and methylation by microorganisms is a key first step in the accumulation of methylmercury in aquatic ecosystems such as the East Fork Poplar Creek (EFPC) in the US DOE Oak Ridge Reservation. Our current model for this process in *Geobacter sulfurreducens* is that Hg(II) enters cells by active transport where it is methylated and rapidly exported as methylmercury. To determine the likely role of a divalent metal transporter in the uptake of Hg(II), we have tested mercury methylation in the presence of a variety of essential heavy metals to act as possible competitive substrates for Hg(II) uptake. Experiments are currently on-going. Further, we have performed knock-outs of a number of putative heavy metal transporters and are now testing these mutants for the ability to take up and methylate Hg(II). Although transport appears to be a critical first step in the methylation of Hg(II), it remains unclear whether methylation occurs in the cytosol or periplasm of these organisms. To clarify this, methylmercury production was monitored in lysed spheroplasts isolated from *G. sulfurreducens*, indicating a cytosolic location for Hg methylation. Thus, mercury must cross both the outer and inner membranes prior to its methylation, and we are actively trying to identify such transporters. In addition, we have observed differences in the type of cellular exudates released during growth of methylating and non-methylating iron-reducing bacteria which have significant impacts on Hg availability. For instance, *G. sulfurreducens* produces cysteine during growth which enhances Hg(II) uptake and methylation; while *Shewanella oneidensis*, a non-methylating species, excretes an unknown compound only during anaerobic growth which renders Hg(II) unavailable to both the parent strain and to *G. sulfurreducens*. Identification of the possible compound(s) by solid-phase extraction and electrospray mass spectrometry is on-going. These results will provide a greater understanding of the biological factors governing methylmercury production in known methylating strains which is critical for controlling its accumulation in the environment.

## Electron Transfer and Atom Exchange between Aqueous Fe(II) and Structural Fe(III) in Clays: Role in U and Hg(II) Transformations

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Recent advances in spectroscopic and microscopic techniques, stable Fe isotope measurements, and theoretical calculations of mineral electronic structures have lead to a new conceptual framework for the reaction of aqueous Fe(II) with Fe oxides. Fundamental processes in the reaction of aqueous Fe(II) at the Fe(III) oxide-water interface include electron transfer between aqueous Fe(II) and structural Fe(III), bulk electron conduction, and Fe(II)-Fe(III) atom exchange.

In contrast, reactions of aqueous Fe(II) with clay minerals have received much less attention and descriptions of these reactions have been limited to surface or interlayer reactions such as ion exchange, surface complexation, and/or surface precipitation. Recently, however, we showed that interfacial electron transfer between aqueous Fe(II) and structural Fe(III) occurs in a clay mineral. Whether this observation can be generalized to all iron-bearing clay minerals and whether similar Fe atom exchange processes occur in clays as observed for the heterogeneous redox reaction in Fe(III) oxides is unclear.

Studies on chemical reduction of structural Fe in smectites have showed that the type of reductant as well as smectite structural properties determine the extent of Fe reduction, as well as the speciation of the resulting structural Fe(II). Here, we investigated the reaction of well-characterized smectites differing in structural Fe content, location of structural Fe (octahedral vs. tetrahedral), and location of excess layer charge (octahedral vs. tetrahedral) with aqueous Fe(II), a reductant abundant in natural anaerobic environments.

More specifically, we used stable isotope specific Mössbauer spectroscopy to determine the extent of reduction of structural Fe in smectites after exposure to aqueous  $^{56}\text{Fe(II)}$ , which is transparent in Mössbauer spectra. Experiments with aqueous Fe(II) enriched in  $^{57}\text{Fe}$  were also carried out to determine the exchange of stable Fe isotopes between aqueous Fe(II) and structural Fe(III) in smectites. Different pools of Fe(II) and Fe(III) were investigated by sequential extraction procedures and solid reaction products were characterized with X-ray diffraction and electron-based microscopic methods.



## **Coupled Biological and Micro-XAS/XRF Analysis of *In Situ* Uranium Biogeochemical Processes**

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Biogeochemical processes play a critical role in reductive uranium immobilization in groundwater found at former uranium mining and processing locations throughout the United States and other parts of the world. While microbiological and molecular studies have brought insights regarding enzymes and candidate microbes involved in bioimmobilization in contaminated aquifers, we have not yet correlated the identity and distribution of organisms associated with metal redox transformations, functionality of extracellular proteins in these systems, and complex mineral-microbe processes influencing uranium immobilization at the micrometer scale. The objective of our newly funded exploratory two-year project is to merge existing toolsets to query microbial location and distribution in conjunction with geochemical alterations in sediments. Our work is currently exploring methodologies for labeling subsurface microorganisms in the presence of sediments using fluorescent dyes and nanomaterials coupled to visualization using microprobe x-ray absorption and fluorescence spectroscopy (micro-XAS/XRF) analysis. We are also bridging efforts with other currently funded DOE projects and the Rifle IFRC (PI's Mailloux, Ranville, and Williams) with the goal of extending our research scope to include arsenic biogeochemistry. This merged approach will enable the co-visualization of microbe-metal processes in sediments and provide a mechanism to increase our understanding of heterogeneities, redox state, mineral form, and targeted biostimulation. The work has implications both for fundamental field-scale biogeochemical science as well as engineering applications and monitoring tools targeting low-cost, minimally invasive remediation of contaminated aquifers.

## Molecular Mechanisms of the *mer* Operon and Hg(II)-Ligand Interactions: Combined Experimental and Computational Studies

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Mercury resistant bacteria exert a strong influence on lowering toxic methylmercury levels at contaminated sites, with the proteins and enzymes of the *mer* operon functioning as an efficient detoxification system. The *mer* operon confers resistance to mercury by encoding specific genes that facilitate uptake of mercuric species, Hg-C cleavage of organomercurials, and reduction of Hg(II) to Hg(0). Combined experimental and computational approaches are used to investigate molecular mechanisms of the *mer* operon, including intramolecular transfer and reduction of Hg(II) by the mercuric reductase MerA, the roles of specific amino acids in the conformational transitions in MerR triggered by Hg(II) binding, and the energetics of Hg(II)-ligand binding interactions.

MerR responds specifically to nanomolar levels of Hg(II) by activating the transcription of *mer* genes. Binding of Hg(II) to MerR was found previously to induce a significant reorientation of the two DNA-binding domains relative to each other, which leads to underwinding of operator DNA followed by transcription. Four MerR mutants, Y27F, Y46F, K99T, and M106I, have been expressed and purified, and SAXS experiments are being performed to characterize the effects of these point mutations on the conformation of MerR. The results will reveal the roles of these key amino acids in propagating allosteric changes at the Hg(II) binding site to the DNA binding domains. MerA converts Hg(II) to less toxic Hg(0), and a pair of cysteines, C464 and C465, is involved in delivering Hg(II) to another pair of cysteines, C42 and C47, at the active site prior to reduction. The mechanisms of intramolecular Hg(II) transfer in MerA are not fully understood, as they are likely to be coupled to specific protonation/deprotonation events. A multi-step quantum mechanical/molecular mechanical (QM/MM) approach is applied to explore and compare the energetics of several possible Hg(II) transfer pathways. Multiple protonation states are considered, and the roles of various active site residues and solvent molecules are hypothesized. Solvation is expected to contribute significantly to the interaction energies involving Hg(II) and affect the relative stabilities of different states during the allosteric transition in MerR and the intramolecular Hg(II) transfer in MerA. However, such information has been lacking. To further determine the effects of solvation on the interactions involving Hg(II), density functional theory and polarizable continuum models are used to compute aqueous binding free energies for a number of complexes containing Hg(II). Excellent agreement with relative experimental data is achieved for both hydration and ligand binding free energies. The standard deviation of the average error (STDEV) in the binding free energies is around 1 kcal mol<sup>-1</sup> using Solvent Model D (SMD) with two explicit water molecules for hydration of anions and Hg(II)-ligand complexes. The binding free energies are analyzed in terms of local and long-range contributions. It is demonstrated that solvation provides a key driving force for effectively enhancing the interactions between Hg(II) and functional groups in low-dielectric media.

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

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Subsurface radionuclide and 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 ions, such as the short-lived radionuclide  $^{90}\text{Sr}$ , is co-precipitation in calcite. We have previously found that that nutrient addition can stimulate microbial ureolytic activity, that this activity accelerates calcite precipitation and co-precipitation of Sr, and that higher calcite precipitation rates can result in increased Sr partitioning. 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 September 2010 experimental campaign at the Integrated Field Research Challenge (IFRC) site located at Rifle, CO was based on a continuous recirculation design; water extracted from well M-07 was amended with urea and molasses and re-injected into up-gradient well M-02. The recirculation experiment was followed by 14 months of geophysical and groundwater monitoring. Cross borehole electrical conductivity tomography indicated a very conductive, high porosity layer overlying a low conductivity unit within the recirculation cell. An increase in electrical conductivity, primarily in the top layer, was observed 40 days after the start of urea recirculation and was likely the result of urea hydrolysis occurring predominantly in the upper portion of the recirculation cell. This interpretation is supported by analysis of sediments from a core (BS-11-2) collected between the injection and extraction well 10 months following urea injection which showed high *ureC* gene copy numbers as well as much higher laboratory measured  $^{14}\text{C}$  urea hydrolysis rates in the upper portion of the recirculation cell compared to the deeper portion of the cell.

Long term groundwater sampling of the injection and extraction wells showed an initial increase in urea concentration associated with injection activities followed by decreasing urea concentration and associated increases in ammonium and dissolved inorganic carbon (DIC) following the termination of injection. Preliminary estimates of urea loss yielded a first order rate constant for urea hydrolysis of  $0.2 \text{ day}^{-1}$ . This value is approximately 7 times higher than estimated for previous field experiments conducted in eastern Idaho. Additionally, DIC carbon isotope ratios were measured for the groundwater. Injected urea had a  $\delta^{13}\text{C}$  of  $-40.62 \pm 0.36 \text{ ‰}$  compared to background groundwater DIC  $\delta^{13}\text{C}$  of  $-16.62 \pm 0.27 \text{ ‰}$ . Observed decreases in groundwater DIC  $\delta^{13}\text{C}$  of up to  $-19.75 \text{ ‰}$  suggested that up to 41% of the post injection DIC resulted from urea hydrolysis. Aquifer solids incubated within the treatment zone retained high ureolytic activity 10 months after the cessation of active urea amendment. Sediments from background cores BS-01 collected in 2010, sediments recovered following 10 months incubation in experimental wells B-01, M-02, and M-04, and core BS-11-2 are being processed for trace metal speciation in different operationally defined sediment fractions as well as determination of the  $\delta^{13}\text{C}$  for carbonate minerals.

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

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The project goal is to examine the role of phosphohydrolases in naturally occurring subsurface bacteria for the purpose of promoting the immobilization of uranium through the formation of insoluble uranium phosphate minerals. Our prior work focused on pure culture and soil column studies that utilized contaminated soils from the DOE Oak Ridge Field Research Center (ORFRC), demonstrating that microbial phosphatase activity liberated sufficient concentrations of inorganic phosphate ( $\text{PO}_4^{3-}$ ) to promote uranium-phosphate mineral formation under oxic and anoxic conditions at different pH values (pH 5.5 and 7). Current objectives are to: (1) examine the diversity of the microbial communities present in Area 2 soil slurry incubations and examine treatments [glycerol-2-phosphate (G2P) or glycerol-3-phosphate (G3P) amendments at pH 5.5 or pH 6.8] conditions that promote intracellular polyphosphate formation, (2) examine the biochemistry of multiple acid phosphatases identified in the completed genomes of the ORFRC *Rahnella* strain Y9602 and reference strain *Rahnella aquatilis* ATCC 33071, and (3) examine the application of positron emission tomography (PET) to track subsurface bacteria in soil column studies. In collaboration with DOE LBNL investigators, we have examined microbial community dynamics of ORFRC Area 2 subsurface microbial populations responding to G2P and G3P amendments with the PhyloChip microarray. Within 36 days, treatments with G3P and G2P yielded 9.1 mM and 4.7 mM  $\text{PO}_4^{3-}$ , respectively. Treatments at pH 6.8 enriched members of the phyla *Crenarchaeota*, *Euryarchaeota*, *Bacteroidetes*, *Flavobacteria*, *Sphingobacteria*, and *Proteobacteria* while treatments at pH 5.5 enriched in the phyla *Crenarchaeota*, *Euryarchaeota*, and *Proteobacteria*. Electron microscopy of soil slurry treatments indicates that G2P (pH 5.5) enhanced intracellular polyphosphate formation. Our two *Rahnella* genome sequencing projects (*Rahnella* sp. Y9602 and *Rahnella aquatilis* ATCC 33071) led by JGI have been completed. Comparative genomic studies between the two strains are underway to examine possible influences the contaminated ORFRC had on the Y9602 strain. Five candidate low-molecular weight acid phosphatases have been cloned and biochemical analysis of substrate range(s) are underway and will be used to examine alternative phosphate substrates as well as allow for real-time gene expression analysis of cultured and mixed community phosphate-solubilizing studies.

Our current collaboration with DOE BNL researchers has examined the use of PET imaging as a method to visualize subsurface microbial processes. Our recently published studies demonstrated that the phosphate solubilizing *Rahnella* sp. Y9602 labeled with 2-deoxy-2- $^{18}\text{F}$ fluoro-D-glucose could be visualized in real-time after being introduced to soil columns. These initial studies demonstrate that metabolically active subsurface microorganisms could be visualized under varying soil column conditions relevant to the ORFRC. The combined multiphase approach of pure culture and soil slurry studies coupled to genome-enabled studies will provide a greater understanding of microbial community dynamics involved in phosphate-mediated U(VI) sequestration and biomineralization.

## The Molecular-Cellular-Field Continuum of Mercury Detoxification

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Stewardship requires controlling bioavailability  $\text{MeHg}^+$  and  $\text{Hg}^{2+}$ , the substrate for methylation. Effective Hg detoxifying proteins have evolved in many bacteria and archaea. Enhancing removal or sequestration of  $\text{Hg}^{2+}/\text{MeHg}^+$  by naturally Hg resistant (HgR) bacteria means knowing how these proteins work. We study HgR genes of  $\gamma$ -proteobacteria and actinobacteria abundant in high Hg areas of the ORR and report here on the mechanisms of the *mer* reductase and demethylase and regulatory proteins that optimize their expression to rapidly and completely convert  $\text{Hg}^{2+}$  or  $\text{RHg}^+$  to volatile, poorly biomagnified  $\text{Hg}^0$ .

**ACTINOBACTERIAL MerA/MerB:** Relatives of *Streptomyces lividans* are found in high Hg regions of the East Fork Poplar Creek of the ORR. We study their co-evolved MerA/MerB proteins. *S. lividans* MerA (SLMerA) lacks the tethered NmerA domain found in  $\gamma$ -proteobacterial MerA, and its MerB (SLMerB) lacks a cysteine of proteobacterial MerB essential for  $\text{Hg}^{2+}$  transfer to NmerA, but has a distinct C-terminal cysteine pair. To examine the role of this cysteine pair in  $\text{RHg}^+$  binding and  $\text{Hg}^{2+}$  release, we generated an active site mutant with only the C-terminal cysteine pair (SSCCSLMerB) and a mutant with only the active site cysteines (CCAASLMerB). Results with wild type SLMerB and the mutants will be presented.

**Hg ISOTOPE FRACTIONATION BY PURIFIED MerA:** Distinct signatures in Hg isotope fractionation for different chemical processes are important for distinguishing biotic and abiotic contributions to natural Hg cycling. Kritee et al (2007) measured Hg isotope fractionation by intact cells expressing *mer* genes and proposed that MerA is largely responsible for the observed effects. To test this in vitro, we're measuring Hg isotope fractionation with a purified  $\gamma$ -proteobacterial MerA. Experimental design and progress will be presented.

**PROTEOBACTERIAL REGULATION OF *mer* EXPRESSION:** In  $\gamma$ -proteobacteria, repressor-activator MerR holds RNA polymerase (RNAP) at the *mer* operator-promoter (MerOP) until  $\text{Hg}^{2+}$  provokes it to let RNAP transcribe. MerR binds MerOP and  $\text{Hg}^{2+}$  tightly, so anti-activator, MerD, must stop expression when all  $\text{Hg}^{2+}$  is reduced. We've made MerD monoclonal antibodies for in vitro interaction studies with MerR, MerOP and RNAP. In vivo RT-qPCR shows *mer* mRNA declines once  $\text{Hg}^{2+}$  is reduced if *merD* is wildtype and are currently testing *merD* mutants. We also find  $\text{Hg}^{2+}$  binds a 38 bp MerOP DNA in the absence of thiols (as in acute  $\text{Hg}^{2+}$  exposure) at 3 distinct, high affinity sites ( $K_{\text{form}} \sim 10^{20} \text{ M}^{-1}$ ). EXAFS shows expected nitrogen ligands but TA:AT slippage, the believed basis of  $\text{Hg}^{2+}$  binding in artificial systems, cannot occur in MerOP. Free bases don't bind  $\text{Hg}^{2+}$ , so DNA's high affinity must involve base-pairing or -stacking, an idea we are testing. We also find *E. coli* cells bind >3-fold more  $\text{Hg}^{2+}$  via N or O ligands than their available thiols. So, cellular DNA may be a large sink of  $\text{Hg}^{2+}$  with unknown turnover rate.

**ACTINOBACTERIAL REGULATION OF *mer* EXPRESSION:** Actinobacteria control the *mer* operon with a simple ArsR-type repressor. *S. lividans* MerR (SLMerR) is a distinct clade of the ArsR family. Homology models based on ArsR regulators with 3D structures show a candidate  $\text{Hg}^{2+}$  binding site in SLMerR's DNA binding helix, a radical departure from metal sites in other ArsR regulators. SLMerR over-expression proved toxic in *E. coli* which lacks the tRNA profile to translate actinobacterial mRNA, resulting in ribosome malfunction. With help from the UT-ORNL group we synthesized an *S. lividans merR* gene optimized for expression in *E. coli* and anticipate good production of this unusual ArsR-type regulator.

## Identifying Mechanisms of Toxic Metal Stress with Global Proteomics

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MeHg<sup>+</sup> and Hg<sup>2+</sup> directly inactivate proteins by binding to their cysteine or selenocysteine residues. As previously reported (Polacco, et al. MCP, 2011), we have devised a high-throughput global proteomics method using the 7 stable isotopes of Hg to identify proteins of *E. coli* most vulnerable to forming stable adducts of phenylmercury (PhHg<sup>+</sup>; a proxy for methylmercury). Analysis of the dataset of ~1.62 million total observed MS2 spectra continues and here we report establishment of a MySQL database for these data, an overview of the biological findings, independent confirmation of the stability of Hg-peptide adducts under the LC-MS/MS conditions used, and initial work extending this method to the Hg<sup>2+</sup>-methylating bacterium, *Desulfovibrio* ND132.

**Hg EXPOSOME DATABASE:** Unlike transcriptomic's MIAME standards, there is no agreed protocol for storage, sharing, and reporting of basic proteomics data, much less those with adducts. So, using the relational database freeware, MySQL, we built our own repository and analytical tools. Housed on a dedicated server at the University of Georgia, the database records every experimental aspect from cell culture thru spectra collection to identification and quality scoring with three widely used algorithms. It is available to all members of our collaboration by password.

**OVERVIEW OF BIOLOGICAL FINDINGS:** From three complete biological replicates (developed via five full scale pilot experiments) we identified by two or more peptides 1301 of *E. coli* MG1655's 4249 encoded proteins (WISC\_ASAP); an additional 261 proteins were identified by multiple observations of a single high quality peptide for a total of 1562 rigorously identified proteins (37% of total). There are 3654 cysteine-containing proteins encoded by MG1655 (86% of total); they vary widely in abundance and conditions for expression. We observed 303 cysteine-containing proteins modified by Hg (19.4 % of identified proteins) and 85% of these have at least one cysteine that is highly modified (Def: >50% of observations have Hg-modification). These Hg-vulnerable proteins represent all metabolic functional groups, and especially energy generation, translation and amino acid biosynthesis (Zink et al, in preparation). Bulk cell properties altered by PhHg or Hg exposure include thiol homeostasis, electrolyte balance, and free iron (LaVoie et al, in preparation).

**STABILITY OF Hg-PEPTIDES TO PROTEOMICS:** The 4-fold lower recovery of cysteine containing proteins than expected has several possible physiological causes, including their differential occurrence under the aerobic growth conditions used and natural differential abundance. These can be resolved via ontological analysis underway; although absolute abundance is unknown for many proteins, it can be inferred from their functional class. Possible technical factors include differential stability to pre-column procedures, column conditions, and MS conditions. We're using our database to examine cysteine peptide/protein yield variation related to pre-MS variables. Experiments with pure Hg-adduct peptides show they are stable to the MS conditions.

**Hg EXPOSOME OF A METHYLATING BACTERIUM:** To identify proteins involved in Hg<sup>2+</sup> uptake and methylation we've first grown *Desulfovibrio* ND132 facultatively without Hg<sup>2+</sup> to test the efficacy of our modified proteomics method on it. The results (~1350 identified proteins of 3455 encoded) were slightly less than work on pyruvate or fumarate grown *Desulfovibrio* G20 (1900 identified proteins of 3258 encoded). Optimization of growth conditions for maximum methylation is underway and, since this project requires exposure to bivalent Hg<sup>2+</sup>, we are adapting our Hg-proteomics method to identify the distinct peptides of pairs of Hg-crosslinked peptides. This work will also uncover cysteine peptides vulnerable to being missed because they are crosslinked.

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

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Successful bioremediation of U in the subsurface depends on the long-term stability of U(IV). As a strategy to immobilize U, injection of electron donors to the subsurface to stimulate U(VI) reduction is being tested. During the process, Mn and Fe oxides sequentially undergo reductive dissolution. Once the injection of electron donor is ceased, however, zones where U(IV) precipitated may return to oxidizing conditions and the stability of U(IV) affected by the formation of strong oxidants such as  $\text{MnO}_2$ . The focus of our project is 1) to determine how certain groundwater constituents affect the coupled Mn(II)/U(IV) oxidation process, 2) to examine the fate of U once it is oxidized by  $\text{MnO}_2$  in both lab and field settings, and 3) to quantify the effects of physical and chemical parameters on  $\text{MnO}_2/\text{UO}_2$  interaction.

The oxidation of U(IV) by Mn(II)-oxidizing bacteria and Mn oxides has been shown in laboratory experiments to occur very rapidly. To assess the effect of groundwater constituents typical of the Rifle field site and the form of U(IV) on coupled Mn(II)/U(IV) oxidation, we investigated the effect of  $\text{O}_2$  and common groundwater chemical species ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{HCO}_3^-$ ) on Mn(II) and U(IV) oxidation by a model Mn(II)-oxidizing organism, *Bacillus* sp. SG-1 spores. Increasing  $\text{O}_2$  resulted in increased levels of U(IV) oxidation which were attributable to both direct oxidation of U(IV) by  $\text{O}_2$  and to the formation of biogenic  $\text{MnO}_2$  which subsequently oxidized U(IV). Interestingly,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{HCO}_3^-$  showed varying effects on coupled Mn(II)/U(IV) oxidation by exerting its impact on either Mn(II) or U(IV) oxidation.

To understand the reaction between U(IV) and  $\text{MnO}_2$ , biogenic  $\text{UO}_2$  or monomeric U(IV) were embedded in agarose gels with SG-1 spores. 65% and 74% of the total U was oxidized in gels containing  $\text{UO}_2$  and monomeric U(IV) respectively. Additionally, more loss of U was observed from gels incubated with  $\text{O}_2$  in the absence of Mn(II), suggesting that the impact of Mn oxidation is U(IV) immobilization. Bulk XAS results showed that the majority of U after Mn(II) oxidation was in the U(VI) form. MicroXAS analyses showed, two main particle types associated with U(VI), single particles or aggregates of U(IV) and Mn oxide particles.

In order to gain a field-relevant understanding of U(IV) oxidation by Mn oxides, several columns were deployed in the Rifle field site, under Mn-oxidizing conditions. These columns contained sediment from the Rifle site that had been pre-loaded (*in situ*) with U(IV). Total digestion of the sediments carried out prior to deployment and after 2 months showed a large decrease (from 40–65%) in the amount of U present in sediments that received higher levels of Mn(II), indicating that Mn(II) oxidation may play a role in the oxidation of U(IV) species.

Finally, laboratory experiments have been performed to understand the physical and chemical factors controlling the interaction of  $\text{MnO}_2$ - $\text{UO}_2$ . Experiments with a multi-chamber reactor with a permeable membrane that prevented direct contact between  $\text{MnO}_2$ - $\text{UO}_2$  indicated that physical contact or close proximity is required for effective oxidation of  $\text{UO}_2$  by  $\text{MnO}_2$ . When contact was allowed, an increase in the  $\text{MnO}_2:\text{UO}_2$  ratio significantly increased the  $\text{UO}_2$  dissolution rate. The product of  $\text{MnO}_2$ - $\text{UO}_2$  interaction, U(VI), was substantially adsorbed to  $\text{MnO}_2$  and the release of Mn was less than expected based on the stoichiometry of the reaction.

## Firmicutes and Their Roles in Uranium Immobilization

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Bioremediation of uranium in subsurface is a great challenge. To evaluate the natural occurring uranium-removing bacterial populations, we have anaerobically enriched uranium contaminated soil sediments (FW107, FW102-2, and FW102-3) collected from ORNL iFRC site (S3 area). Electron acceptors,  $\text{NO}_3^-$ ,  $\text{Fe}^{3+}$ , and  $\text{U}^{6+}$ , were supplemented in the enrichments as selection factors. We previously reported the shifts of bacterial community profiles in enrichments. After two serial transferring and enriching, bacterial 16S rRNA gene survey revealed that  $\text{Fe}^{3+}$  and  $\text{U}^{6+}$  supplemented enrichments shared similar community structures. Genus *Pelosinus* was found dominant in  $\text{Fe}^{3+}$  and  $\text{U}^{6+}$  enriched FW107 microcosms. This group of bacteria was also present in FW102-2 and FW102-3 enrichments but substantially less abundant. Unclassified *Clostridiales* and *Geobacter* groups were most abundant in  $\text{Fe}^{3+}$  and  $\text{U}^{6+}$  supplemented FW102-2 microcosms, respectively. Genus *Geobacter* was dominating the FW102-3 community after it was enriched with  $\text{Fe}^{3+}$  and  $\text{U}^{6+}$ . Hence, we suspect that Firmicutes might play a significant role in subsurface uranium removal.

With our goal of identifying unknown uranium immobilizers in mind, we have isolated 54 bacterial isolates from  $\text{Fe}^{3+}$  and  $\text{U}^{6+}$  supplemented enrichments. The growth rates of these isolates at 25°C vary from 6 hours to 22 hours per generation. All isolates belong to phylum Firmicutes and can be categorized into four groups, *Clostridium XI*, *Clostridium XIVa*, *Clostridium sensu stricto*, and *Pelosinus*, based on their 16S rRNA gene sequences. Majority of the sequences in *Clostridium XIVa* group were identical to the 16S rRNA gene sequence of *Desulfotomaculum guttoideum*, which is a rarely studied sulfate-reducing bacterium. At 95% to 97% similarity, sequences in *Clostridium sensu stricto* group were closely related to *Clostridium acetobutylicum*, *Clostridium butyricum*, and *Clostridium puniceum*. While *Clostridium acetobutylicum* was recently reported as a uranium reducing bacterium, the other two were mostly studied for their chemical producing ability, such as butanol and 1,3-propanediol. The *Pelosinus* isolates we have identified were closely related to *Pelosinus sp.* strain UFO1, which was recently reported for its ability in multi-mode uranium immobilization. Out of 54 isolates, only one was identified as *Clostridium venationis* in group *Clostridium XI*. However, no studies have been reported on this species. In light of these taxonomy information and literature reports, we are in the process of evaluating the uranium immobilization rate by these isolates. Preliminary studies have shown that these isolates are resistant to 250  $\mu\text{M}$  of  $\text{U}^{6+}$ . We also performed rep-PCR to select the representative isolates to determine their  $\text{U}^{6+}$  removal rates. Currently, we are studying the  $\text{U}^{6+}$  immobilization by these representatives as well as the co-cultures of these isolates to evaluate the importance of mixed Firmicute populations in  $\text{U}^{6+}$  bioremediation.



## Differential Proteomics Analysis of GASP (Growth Advantage in Stationary-Phase) Phenotype of *Geobacter sulfurreducens* under “Famine” Conditions

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*Geobacter sulfurreducens* is used for bioremediation at heavy metal and radioactive waste contaminated sites. The organism typically encounter limited access to electron donors and/or electron acceptors in such environments. Therefore, gaining an understanding of how *Geobacter sulfurreducens* involved in bioremediation survive under such conditions is relevant for planning efficient bioremediation strategies. Previously we have demonstrated that *Geobacter sulfurreducens* displays five typical stages of growth: lag, log, stationary, death, and survival phases. The organism acquire a growth advantage adaptation that begin to emerge during the stationary phase; this adaptation is termed “growth advantage in stationary phase,” or GASP. The GASP strains can out-compete mid-log phase population when co-cultured under similar conditions. This work aims to study differential protein expression between different growth phases of *Geobacter sulfurreducens* grown under electron acceptor (fumarate) limiting conditions. We employed LC/MS- based iTRAQ methods to obtain information about relative changes in protein expression between mid-log, stationary and survival phases.

There were 80 proteins that were significantly up-regulated in survival phase organisms as compared to mid-log. Most of the up-regulated proteins (29%) were found to be involved in energy metabolism function. These included components of the ATP synthase and NADH dehydrogenase enzymes, as well as cytochrome c family proteins and all three components of the heterotrimeric fumarate reductase FrdCAB. In addition, several outer membrane proteins and putative lipoproteins involved in transport and binding were also up-regulated. About 40% of the total up-regulated proteins were found to be hypothetical or with unknown function.

We identified 66 proteins that were significantly down-regulated in survival phase organisms. Around 40% were ribosomal, transcription and translation proteins involved in protein synthesis. In addition 10 proteins (~15%) associated with energy metabolism, 5 (~8%) involved in nucleotide biosynthesis and 11 (~17%) hypothetical or unknown function proteins were also down-regulated. Two proteins that play a role in heme biosynthesis, the gene products of GSU3285 and GSU3453, 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. Of all the 146 proteins differentially expressed between mid-log and survival phases, the predicted cellular localization of 117 could be determined. A majority of the up-regulated proteins were predicted to localize in the membrane and periplasmic space, and most of the down-regulated proteins were predicted to be localized in the cytoplasm.

These results indicate that the composition of the inner and outer membranes of *G. sulfurreducens* changes drastically from mid-log to survival phase and may have important implications for organism’s survival under famine conditions. Fewer changes in protein expression between mid-log and stationary phases as compared to mid-log and survival phases, indicate that the significant changes in survival expression cannot be detected at early stationary phase. Thus, study of long-term cultures is necessary to truly appreciate the nature of *G. sulfurreducens* dwelling in famine environments. The genetic basis of the GASP phenomenon and survival capacity will be further investigated by genomic sequencing. We will also determine the energy charge and reducing equivalents involved to gain insight into the energy metabolism of the organism.

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

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One way to minimize the mobility of the  $\text{Tc}^{\text{VII}}$  oxyanion pertechnetate ( $\text{TcO}_4^-$ ) is to effect reduction under sulfidogenic conditions (generated abiotically by  $\text{Fe}^0$  or biotically) to form  $\text{TcS}_x$ , which is significantly slower to oxidize than  $\text{Tc}^{\text{IV}}\text{O}_2$ . In sediment systems,  $\text{TcS}_x$  and other precipitates may oxidize more slowly due to oxygen diffusion limitations to these low permeability precipitate zones. In addition, the  $\text{TcO}_4^-$  reduction rate may be more rapid in the presence of sediment because of additional reductive surface phases. This project aims to provide a fundamental understanding of the feasibility of immobilization of  $\text{TcO}_4^-$  as  $\text{TcS}_x$  in the vadose zone or groundwater by application nano zero-valent iron (nZVI), and sulfide or sulfate.

Biotic batch experiments have used the sulfate-reducing bacterium (SRB) *Desulfotomaculum reducens*. The iron sulfide mineral mackinawite was generated under these conditions, while vivianite was formed in nZVI only controls. The sulfide/bacteria-containing system consistently reduced aqueous pertechnetate rapidly (> 95% in the first hour), a rate similar to that for the sulfide-free, nZVI only system. Reduced Tc (aged for 3 months) generated in both SRB/nZVI systems was highly resistant to reoxidation. In reduced samples, Tc was found associated with solid phases containing Fe and S (*D. reducens*/nZVI) or Fe (nZVI only). Experiments using *D. reducens* without nZVI provided some additional insights. Firstly, stationary phase cultures were able to slowly reduce pertechnetate. Secondly, addition of pertechnetate at the beginning of cell growth (lag phase) resulted in a faster rate of Tc reduction, possibly indicating a direct (e.g. enzymatic) role for *D. reducens* in Tc reduction.

Abiotic batch experiments were conducted with  $\text{Na}_2\text{S}$  as the sulfide source. Pertechnetate reduction was rapid in the presence of sulfide and nZVI, although the rate was suppressed at the higher S/Fe ratios tested. This suppression appeared to be due to the formation of Tc-containing colloids. As with the biotic experiments, pertechnetate reduced under sulfidic conditions was highly resistant to reoxidation. The microscopic morphology of abiotically-transformed nZVI particles varied significantly with those in the biotic experiment, although mackinawite was formed in both systems (as indicated by  $\mu\text{XRD}$  and Mössbauer spectroscopy). Preliminary XAS analysis pointed to a mixture of Tc-O and Tc-S binding in the abiotic sulfide/nZVI system, while the major reduced solids under non-sulfidic conditions were  $\text{TcO}_2 \cdot n\text{H}_2\text{O}$ . Further investigation of these phases in abiotic and biotic samples are currently in progress using XAS.

The presence of sediment and advective flow to the  $\text{TcO}_4^-$ /nZVI/sulfide system results in additional processes occurring. Although the natural Hanford sediment used has sufficient available ferrous iron to slowly reduce  $\text{TcO}_4^-$ , under anaerobic conditions, that rate is orders of magnitude slower than reduction by nZVI/sulfide. Batch and 1-D column experiments showed that the  $\text{TcO}_4^-$  reduction rate increased with the sediment surface area (with the same nZVI mass). As in batch systems, column studies showed that the presence of sulfide with  $\text{TcO}_4^-$  at low (2-5 mM) concentrations increased the  $\text{TcO}_4^-$  reduction rate and high (10-30 mM) sulfide decreased the rate. This change is attributed to the formation of sulfide precipitates on the nZVI and sediment surfaces. Injection of low and high sulfide (i.e. pretreatment) prior to  $\text{TcO}_4^-$ /sulfide injection also greatly decreased the  $\text{TcO}_4^-$  reduction rate, likely decreasing the generation of ferrous iron from the nZVI. Although the high sulfide systems have slower Tc reduction rates, 190 times more Tc mass precipitated than in the low sulfide systems and the highest fraction of Tc mass remained immobilized.

## Microbiological-Enhanced Mixing Across Scales During *In Situ* Bioreduction of Metals and Radionuclides at Department of Energy Sites

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Bioreduction is being actively investigated as an effective strategy for subsurface remediation and long-term management of DOE sites contaminated by metals and radionuclides (i.e. U(VI)). These strategies require manipulation of the subsurface, usually through injection of chemicals (e.g., electron donor) which mix at varying scales with the contaminant to stimulate metal reducing bacteria. There is evidence from DOE field experiments suggesting that mixing limitations of substrates at all scales may affect biological growth and activity for U(VI) reduction.

Although current conceptual models hold that biomass growth and reduction activity is limited by physical mixing processes, a growing body of literature suggests that reaction could be enhanced by cell-to-cell interaction occurring over length scales extending tens to thousands of microns. Our project is investigating two potential mechanisms of enhanced electron transfer. The first is the formation of single- or multiple-species biofilms that transport electrons via direct electrical connection such as nanowires through biofilms to where the electron acceptor is available. The second is through diffusion of electron carriers from syntrophic bacteria to dissimilatory metal reducing bacteria (DMRB). The specific objectives of this work are i) to quantify the extent and rate that electrons are transported between microorganisms in physical mixing zones between an electron donor and electron acceptor (e.g. U(IV)), ii) to quantify the extent that biomass growth and reaction are enhanced by inter-species electron transport, and iii) to integrate mixing across scales (e.g., microscopic scale of electron transfer and macroscopic scale of diffusion) in an integrated numerical model to quantify these mechanisms on overall U(VI) reduction rates.

We are testing these hypotheses with five tasks that integrate microbiological experiments, unique micro-fluidics experiments, intermediate-scale flow cell experiments, and multi-scale numerical models. Continuous fed-batch reactors will be used to derive kinetic parameters for DMRB, and syntrophic/DMRB systems. They will also be used to develop an enrichment culture for elucidation of syntrophic relationships in a complex microbial community. Pore and continuum scale experiments using microfluidic and bench top flow cells will be used to evaluate the impact of cell-to-cell and microbial interactions on reaction enhancement in mixing-limited bioactive zones, and the mechanisms of this interaction. The microfluidic experiments will be used to develop and test a pore scale model that considers direct cell-to-cell interactions during U(VI) reduction. The pore scale model will be incorporated into a multi-scale hybrid model that combines pore scale modeling at the reaction interface with continuum scale modeling. We will validate the multi-scale model by comparison with continuum scale bench top flow cell experimental results, and then explore opportunities to extend this model to larger systems and eventually to field sites.

Work to date for this new project has investigated developing an appropriate system of syntrophs and metal reducers for use in follow-up experiments. Preliminary results indicate that *Anaeromyxobacter dehalogenans* has the capability to grow syntrophically with *Syntrophobacter fumaroxidans* using propionate as an electron donor and ferric citrate or ferrihydrite as an electron acceptor. Similarly, *Geobacter lovleyi* has grown with *S. fumaroxidans* using propionate and ferrihydrite only. In contrast, *G. sulfurreducens* has not been capable of syntrophic growth. Preparatory work needed for design and fabrication of microfluidic flow cells has been completed. Finally, we are starting to formulate the mathematical model for diffusion and reaction with a biofilm consisting of both syntrophs and metal reducers.

## Development of New and Integrated Stable Isotope Tools for Understanding Nitrogen–Uranium Interactions in Subsurface Environments

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As the most ubiquitous contaminant anion in sediments and groundwater at US DOE sites, high levels of nitrate ( $\text{NO}_3^-$ ) also co-occur with one or more priority contaminant metals or radionuclides. This project seeks to improve the understanding of the multifaceted interactions between the nitrogen cycle and cycling of the widespread radionuclide uranium (U) in order to better predict its fate and transport in complex subsurface environments. For example, while bioremediation efforts have primarily focused on immobilizing uranium through stimulation of microbial reduction of U(VI) to U(IV), this process is inhibited by the presence of  $\text{NO}_3^-$ . Furthermore, the potential for introduction of nitrate-contaminated groundwater poses a direct and serious threat to the longevity and stability of uranium immobilization in subsurface sediments directly through the oxidation of U(IV) by  $\text{NO}_3^-$  and/or the reductive intermediate  $\text{NO}_2^-$ , or indirectly by coupling of N and Fe cycling. As such, the interpretation of the results of field-scale bioremediation studies are often complicated by the fact that several competing processes may occur simultaneously.

Through the use of new and integrated stable isotopic tools, which represent naturally occurring spatial and temporal integrators of cycling processes, this project aims to improve the detection and monitoring of biogeochemical changes in subsurface environments, particularly those involving the coupling of nitrogen cycling to uranium oxidation and mobilization. Specifically, the use of coupled isotope systems (e.g.  $^{15}\text{N}/^{14}\text{N}$  and  $^{18}\text{O}/^{16}\text{O}$  and the ratio of their respective fractionation or  $^{18}\text{E}:^{15}\text{E}$ ) provides an even more powerful integrator for constraining the relative roles of simultaneously occurring cycling processes in the context of U(IV) oxidation.

Initial batch experiments were designed to characterize the kinetic isotope effects of abiotic  $\text{NO}_3^-$  reduction by Fe(II) as a starting point for understanding the potential for linkages among subsurface N, Fe and U cycling. Results have shown substantial reduction of  $\text{NO}_3^-$  by Fe(II) in the presence of iron oxyhydroxides mineral phases (goethite) and/or catalytic amounts of Cu(II). Kinetic isotope effects for  $\text{NO}_3^-$  reduction during these reactions have varied widely across experimental conditions ( $^{15}\text{E}_{\text{NO}_3} = 6.8$  to  $32.7\%$ ,  $^{18}\text{E}_{\text{NO}_3} = 3.4$  to  $19.7\%$ ). However, in all cases, abiotic  $\text{NO}_3^-$  reduction exhibited larger nitrogen isotope effects than oxygen isotope effects ( $^{18}\text{E}:^{15}\text{E} = 0.36$  to  $0.83$ ), which could suggest the involvement of abiotic Fe (II) based reduction of  $\text{NO}_3^-$  when observed in the environment. During the next phase of characterization, a full experimental series of 17 flow-through sediment columns, inoculated with subsurface sediment from a local study site, was run for approximately 60 days under anaerobic, reducing conditions. In a subset of columns, iron reduction was allowed to occur for the initial 30 days (allowing a buildup of Fe(II) sorbed onto mineral surfaces), followed by the addition of  $\text{NO}_3^-$ . Rapid  $\text{NO}_3^-$  and Fe(III) reduction was observed in all treatments. Results of  $\text{NO}_3^-$  stable isotope measurements from these flow-through experiments will be discussed in the context of potential linkages among subsurface N, Fe and U cycling. Based on these results, we posit that tracking changes in  $\text{NO}_3^-$  and  $\text{NO}_2^-$  concentration and  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  along with aqueous and solid phase U and Fe chemistry in future experiments will allow partitioning of the specific N reducing processes directly and indirectly related to U(IV) reoxidation and mobilization under advective flow.

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

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Microbially mediated metabolisms have been identified as a significant factor either directly or indirectly impacting the fate and transport of heavy metal/radionuclide contaminants. To date the role that viruses play influencing microbial mortality and the resulting community structure under varying redox conditions in subsurface sedimentary environments remains poorly understood. The objective of this project is to investigate viral infection of subsurface bacteria and the formation of contaminant-bearing viral particles. This objective will be approached by examining the following working hypotheses: (i) subsurface microorganisms are susceptible to viral infections by the indigenous subsurface viral community, and (ii) viral surfaces will adsorb heavy metals and radionuclides. In an effort to initially assess the significance of viral infection in subsurface microbial communities, the production of viral like particles in response to biostimulation of the microbial community was investigated by inoculating low nutrient culture medium containing acetate and nitrate with uranium and nitrate containing shallow subsurface sediment (25% mass/vol). No significant production of viral like particles was observed when acetate or nitrate was omitted from culture medium. Acetate was oxidized coupled to the reduction of nitrate to nitrite resulting in the increase in bacterial abundance and an initial increase in Viral Like Particles (VLP's) (Virus to Bacteria ration ca. 480 to 2,400 over the course of the study). During nitrate reduction to nitrite, VLP abundance increased from  $1.1 \times 10^5$  to  $4.6 \times 10^7$  VLP mL<sup>-1</sup> (ca. increase of 430x). The production of 9mM nitrite resulted in a lag in nitrite reduction and a subsequent decrease in bacterial abundance. This also corresponded to a decrease in VLP abundance ( $4.9 \times 10^6$  VLP mL<sup>-1</sup>). Interestingly when nitrite reduction resumed, VLP abundance again increased to  $2.6 \times 10^7$  VLP mL<sup>-1</sup> while bacterial abundance continued to decrease. In an effort to identify the viruses infecting indigenous bacteria, bacterial isolates were obtained. One nitrate reducing isolate, Alda10, was selected for further study. Analysis of the 16S rRNA gene sequence revealed the closest related organism in pure culture was *Pseudomonas fredricksbergensis* with 99% similarity. Mitomycin C induction of Alda10 resulted in the production of VLP's ( $1.9 \times 10^6$  VLPmL<sup>-1</sup>). Transmission electron microscopy of the VLPs revealed particles similar to filamentous phage. Together these results suggest a lysogenic infection of *Pseudomonas* sp. Alda10. The production of VLP's in groundwater has implications for metal and radionuclide transport. Initial experiments indicate that metals will adsorb to the surface of *Escherichia coli* phage T4. It is therefore necessary to establish the potential relationship(s) between viruses, subsurface microbial communities, and contaminant metals/radionuclides to provide sufficient scientific understanding such that DOE sites would be able to incorporate coupled physical, chemical and biological processes into decision making for environmental remediation and long-term stewardship by establishing viral-microbial relationships and the subsequent fate and transport of heavy metals and radionuclides.

## Investigation on U(VI) Sorption / Desorption on Nanopores Goethite, and Structures of Ferrihydrite Nano-Mineral

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Most reactive surfaces in clay-dominated sediments are present within nanopores (pores of nm dimension). The behavior of geological fluids and minerals in nanopores is significantly different from those in normal non-nanoporous environments. The effect of nanopore surfaces on U(VI) sorption/desorption and reduction is likely to be significant in clay-rich subsurface environments. Our research objective is to test the hypothesis that U(VI) sorption on nanopore surfaces can be greatly enhanced by nanopore confinement environments.

We investigated sorption and desorption of uranium on both natural nanoporous and non-porous goethite. U(VI) Desorption experiments of nanoporous goethite and non-porous goethite model systems show that all the sorbed U(VI) on non-porous goethite can be easily desorbed with low bicarbonate concentration. However, the most sorbed U(VI) on nanoporous goethite can not be desorbed even with high bicarbonate concentration. 50 mM bicarbonate solutions can only desorb ~ 10% of the sorbed uranium. The results also support that sorbed U(VI) in natural FRC sediments are bonded to both nanopore surfaces (strong sorption) and non-nanopore surfaces (weak sorption). Majority of sorbed U(VI) are associated with goethite nano-crystals. If we use Dithionite-Citrate-Bicarbonate (DCB) to remove goethite nano-crystals, it also removes more than 80% of U(VI). The amounts of labile Fe by BCD method is approximately 300  $\mu\text{mol/kg}$ .

Ferrihydrite nano-minerals are precursors for nanoporous goethite. Mechanism for highly reactive surfaces of ferrihydrite is not well explained, because ferrihydrite structure is still not well understood. We have determined structure and defects in a natural ferrihydrite using Z-contrast imaging. With spherical aberration-corrected scanning transmission electron microscopy (STEM), we can obtain locations of Fe atoms and vacancies in ferrihydrite directly from Z-contrast images with better than 0.1 nm spatial resolution. Two polymorphs are confirmed: ferrihydrite-4H (~ 2/3) and ferrihydrite-2H (~1/3). Intergrowth of the two polymorphs can also occur with (001) as interface. The 2H polymorph has ABAB packing for oxygen atoms, and the 4H polymorph has ABAC packing with doubled unit cell parameters along *a*- and *b*-directions with respect to the 2H polymorph. All Fe atoms occupy octahedral sites. Unit cell doubling along the *a*-, *b*-, and *c*-directions in ferrihydrite-4H is resulted from the ordering between Fe and vacancy in octahedral sites. Due to structural disordering between Fe and vacancies, ferrihydrite can be considered as 2-D crystals with two strong (*hkl*) diffraction peaks. The calculated XRD pattern based on the new structural models fits observed and reported XRD patterns well. Stoichiometry of 6-line ferrihydrite is close to  $\text{Fe(III)}_{0.75}(\text{O}, \text{OH})_2$  based on occupancy fitting, i.e., between  $\text{Fe(OH)}_3$  and  $\text{FeOOH}$ , instead of between  $\text{FeOOH}$  and  $\text{Fe}_2\text{O}_3$ . Z-contrast images of ferrihydrite sorbed with U(VI) show that some of the adsorbed U atoms are right on Fe-sites. It is proposed that structural disordering could be a reason for very reactive surfaces of ferrihydrite.

## Emulsified Vegetable Oil Stimulates Sulfate-Reducing Communities for U(VI) Reduction at a Contaminated Aquifer Via Pyrosequencing of Dissimilatory Sulfite Reductase Genes

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Sulfate reducing bacteria (SRB) are predominant and play an important role in accelerated, sustained reduction of U(VI) during bioremediation at contaminated sites, but the SRB community diversity, composition and structure are poorly understood. In this study, 454 pyrosequencing of dissimilatory sulfite reductase genes (*dsrA*) was used to analyze 44 groundwater samples collected from a U(VI)-contaminated aquifer (Oak Ridge, TN) undergoing bioremediation with an amendment of emulsified vegetable oil (EVO). Acetate was detected as a representative of the intermediates (e.g. H<sub>2</sub>, CO<sub>2</sub>, glycerol, propionate, butyrate) from EVO biodegradation, which stimulated the reduction of significant electron acceptors (sulfate, nitrate, Fe, Mn) and U(VI) in the seven downgradient wells during the entire experimental period of 269 days. A total of 82,537 sequences were obtained, and a total of 15,084 OTUs were defined at a 90% similarity level among all 44 samples. While a majority of the sequences (84%) were found to be *Deltaproteobacteria*, 0.4% were related to *Desulfotomaculum* in *Firmicutes*, and a considerable portion (21% at the genus level) was novel *dsrA* sequences. Significantly different SRB communities ( $P < 0.01$ ) were observed after EVO amendment. While novel *dsrA* sequences were predominant (98%) in the samples before EVO addition, *Desulfovibrio* and to lesser degrees, *Desulfobacterium* and *Desulfococcus* species overwhelmingly dominated the SRB communities after EVO injection. A temporal dynamics of these organisms relative to EVO amendment was observed, which was coordinated with the succession of microbial communities as well as EVO biodegradation and U(VI) reduction. When both species relative abundance and relative frequency of occurrence were taken into account, *Desulfovibrio* (60–80% of the SRB community) and *Desulfococcus* (20%) were indicator species from 4 to 31 days, and *Desulfobacterium* (50%) characterized the community at 80 days when extensive EVO degradation and U(VI) reduction occurred. *Desulfovibrio* species remained important indicators by 269 days when acetate production and U(VI) reduction were still detected, suggesting a long-term modification of the community. The indicators of the non-EVO samples were aligned with cultured *Desulfotomaculum* strains and the *Firmicutes*-like sequences recovered in this study, suggesting that *dsrA* genes of non-EVO U(VI)-contaminated environments may be phylogenetically distant from those enriched by EVO amendment. A regression analysis showed a significant influence of *Desulfovibrio* dominance over the U(VI) concentration in groundwater ( $P < 0.001$ ). *Desulfovibrio* species could play an important role in the accelerated and sustained U(VI) reduction at this site. Further analysis of *in situ* activity of *Desulfovibrio* and their relationship with U(VI)-reduction should provide more information for bioremediation of this contaminated DOE site.