DOE-ERSP PI MEETING:
Abstracts

April 16–19, 2007
Lansdowne, Virginia

Environmental Remediation Sciences Program (ERSP)

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U. S. Department of Energy
Environmental Remediation Sciences Program
Principal Investigators Meeting

Welcome to the annual 2007 Environmental Remediation Sciences Program (ERSP) Principal Investigators (PIs) meeting. The purpose of this meeting is to bring together all of the lead PIs and key Co-PIs in the program to share and review the results of funded research from the past year. This meeting allows program managers from the Environmental Remediation Sciences Division (ERSD) within the Office of Biological and Environmental Research (OBER) to gauge the progress and significance of the funded research, and it is also an important venue to showcase ERSP research to interested parties within DOE and other invited federal agency representatives. Additionally, these meetings should serve as an opportunity for funded PIs to view their research in the context of the entire ERSP portfolio. Past ERSP meetings have been very important venues for detailed discussion of research results among PIs, development of new research ideas, fostering new collaborations and discussion with ERSD program managers on future research efforts and/or initiatives within the program. In short, these meetings are an important resource for both program managers and PIs.

There will be only one ERSP PI meeting for 2007. In years past, ERSD has sponsored two PI meetings, one in the spring and a separate meeting in the fall that focused primarily on field research. However, this format tends to insulate laboratory-based research from the field research sponsored in the program and is incompatible with the ERSD view that laboratory-based research should progress towards understanding the relevant processes in natural environments at the field scale. Therefore the agenda for this year's PI meeting is well integrated with both lab-based and field-based projects, to allow for detailed discussion between PIs involved in each area.

In the agenda, you will notice a more relaxed format than in years past. This year's meeting spans four days, but is less heavily regimented in terms of oral presentations and allows ample time for informal group discussions and poster presentations. The intent of this format is to foster informal discussion of research among PIs and ERSD program managers—discussion that is a hallmark of previous ERSD-sponsored meetings. Morning sessions will be dominated by oral presentations from PIs chosen by ERSD program managers to communicate key topics of research within the program. There is ample time during lunch and in the early afternoon for small group discussions/meetings prior to convening again in the later afternoon for oral presentations on field research conducted at the Integrated Field-Scale Subsurface Research Challenge sites (IFCs). Formal poster sessions begin after dinner. Abstracts for all poster presentations are listed within this meeting booklet.

On behalf of the ERSD program managers and staff, we thank you for attending this year's PI meeting. We look forward to discussing the results of your research with you and your ideas for the future, and we hope that this meeting will continue as an important tradition for PIs in the program and serve as a valuable resource for your investigations.

Best Regards,

Mike Kuperberg
ERSD Acting Director

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ERSP Annual PI Meeting Preliminary Agenda
Lansdowne, VA
April 16–19, 2007

Objective: The purpose of this meeting is to bring together all of the lead PIs and key Co-PIs in the program to share and review the results of funded research from the past year. This meeting not only allows ERSD program managers to gauge the progress and significance of the funded research, but also provides a venue for PIs to view the entire ERSP portfolio and interact with colleagues working in similar science areas.

Monday April 16th, 2007

All Morning	Arrival of ERSD PIs, Co-PIs, ERSD program staff and guest speakers at the Lansdowne facility.
1:00 PM	Welcome/Opening Comments (ERSD Program Staff)
1:10 PM	Programmatic Overview & Outlook for the Future (ERSD Program Staff)
2:00 PM	EM Roadmap for the Future (M. Gilbertson, Dep. Asst. Sec. for EM)
2:30 PM	SERDP-ESTCP: Addressing DoD Environmental Issues through R&D (Bradley Smith, Exe. Dir.)
3:00 PM	***Break***
3:30 PM	**TBA**
4:00 PM	D.C. White Memorial Lecture (T. Phelps, ORNL)
5:00 PM	***Dinner***
6:30 PM	***Poster Session (Group A)***
8:00 PM	***Adjourn***

Tuesday April 17th, 2007

8:00 AM	Coffee and Breakfast
	Microbial Physiology and Env. Genomics of Radionuclide Reduction
8:30 AM	Molecular Analysis of Rates of Metal Reduction and Metabolic State of *Geobacter* Species During *In Situ* Uranium Bioremediation (D. Lovley, Univ. Massachusetts)
9:00 AM	**TBA**
9:30 AM	Identification of Molecular and Cellular Responses of *Desulfovibrio vulgaris* Biofilms under Culture Conditions Relevant to Field Conditions for Bioreduction of Heavy Metals (Matt Fields, Montana State Univ.)
10:00 AM	***Break***
	Contaminant Metal Biotransformation (Hg and U)
10:30 AM	Integrating the Molecular Machines of Mercury Detoxification into Host Cell Biology (Anne Summers, Univ. Georgia)
11:00 AM	Microbial Pathways for the Mobilization of Mercury as Hg(0) in Anoxic Subsurface Environments (H. Wiatrowski, Rutgers Univ.)
11:30 AM	Geochemical, Genetic, and Community Controls on Mercury Methylation (A. Palumbo, ORNL)
12:00 PM	***Lunch***
1:30 PM	***Free time***
3:00 PM	***Integrated Field-Scale Research Challenge: Old Rifle UMTRA Site*** (Phil Long, PNNL and Co-PIs) Agenda TBA
5:00 PM	***Dinner***
6:30 PM	***Poster Session (Group B)***
8:00 PM	***Adjourn***

Wednesday April 18th, 2007

8:00 AM	Coffee and breakfast
	Radionuclide Fate and Transport in the Subsurface
8:30 AM	**TBA**
9:00 AM	Influence of Microscopic Mass Transfer on the Reactivity and Stability of Uranium (C. Liu, PNNL)
9:30 AM	Uranium Immobilization via Phosphate Injection into the Subsurface at the Hanford 300 Area (D. Wellman, PNNL)
10:00 AM	***Break***
	Contaminant Fate and Transport Modeling and Simulation
10:30 AM	Microscopic Controls on the Desorption/Dissolution of Sorbed U(VI) and Their Influence on Reactive Transport (C. Steefel, LBNL)
11:00 AM	**TBA**
11:30 AM	Coupling Between Flow and Precipitation in Heterogeneous Subsurface Environments and Effects on Contaminant Fate and Transport (G. Redden, INL)
12:00 PM	***Lunch***
1:30 PM	***Free time***
3:00 PM	***Integrated Field-Scale Research Challenge: Hanford 300 Area*** (J. Zachara, PNNL and Co-PIs) Agenda TBA
5:00 PM	***Dinner***
6:30 PM	***Poster Session (Group C)***
8:00 PM	***Adjourn***

Thursday April 19th, 2007

8:00 AM	Coffee and breakfast
	Radionuclide Biogeochemistry (U and Tc)
8:30 AM	Technetium and Iron Biogeochemistry in Suboxic Subsurface Environments with Emphasis on the Hanford Site (J. Zachara, PNNL)
9:00 AM	Biogeochemical-Physical Process Coupling Influencing Contaminant Fate and Transport in the Subsurface (S. Fendorf, Stanford Univ.)
9:30 AM	Microcantilever Sensors for In Situ Subsurface Characterization (T. Thundat, ORNL)
10:00 AM	***Break***
	Mechanisms of Uranium Immobilization/Remobilization
10:30 AM	Investigations of coupled biogeochemical processes affecting the transformation of U: Integration of synchrotron-based approaches (E. O'Loughlin/K. Kemner, ANL)
11:00 AM	Long-Term Stability of Biogeochemically Reduced U and Cr in Contaminated Sediments (T. Tokunaga, LBNL)
11:30 AM	The effects of metal bio-oxidation on the fate and transport of uranium (J. Coates, UC-Berkeley)
12:00 PM	***Lunch***
1:00 PM	***Integrated Field-Scale Research Challenge: Oak Ridge Site*** (P. Jardine, ORNL and Co-PIs) Agenda TBA
3:00 PM	Closing Comments (ERSD Staff**)**
3:30 PM	***Meeting Adjourn***

ABSTRACTS

GENERAL ERSP ABSTRACTS

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

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

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This project tests the hypothesis that sedimentary lithofacies determine the geochemical and physical hydrologic properties that control reactive solute transport. The representative geochemical and physical aquifer properties selected for quantification in the proposed project are the properties that control carbon tetrachloride (CT) transport at the Hanford Site (Ringold Formation): hydraulic conductivity and reactivity (sorption distribution coefficient and transformation-rate constant). We are combining observations at outcrop analog sites (to measure lithofacies dimensions and statistical relations) with measurements from archived and fresh core samples (for geochemical experiments and to provide additional constraint to the stratigraphic model) to place local-scale lithofacies successions, and their distinct hydrologic property distributions, into the basinal context. Through this combination of observations and measurements, we will be able to estimate the spatial distributions of properties that control reactive solute transport in the subsurface.

During the first field season (summer 2006), we conducted field studies of the Ringold Formation outcrops and descriptions of selected archived core from the unconfined aquifer in the vicinity of the CT plume. The focus of this work was the examination of grain size, sedimentary structures, and preliminary composition (through hand-sample identification) within the clast supported gravel units of the Ringold. We noted and described the widespread occurrence of two types of geologic features that may have major implications for the transport of CT through the aquifer: iron-rich coatings *on* the sediments and carbonaceous matter *in* the sediments, including both leaf fragments and woody detritus. The spatial distribution of iron staining and carbonaceous matter are likely to be influenced by the geologic processes that controlled the initial deposition of the sediments and their later diagenesis. The geologic controls on these features could lead to lithofacies control of the reactive transport of CT, thus guiding the sample selection for studies of reactivity.

Subsamples of archived samples from three boreholes have been taken at various depths throughout the geologic profile, from the lithologic units represented in the CT plume. These subsamples will be used to evaluate CT sorption by lithofacies. Experiments to evaluate the apparatus and design for CT sorption by lithofacies have been completed. “Fresh” core samples from the Ringold Formation will be collected for geochemical studies later this spring (2007).

We have also developed a protocol for evaluation and validation of geostatistical software and analyses that will be employed for generating the numerical grids of physical and chemical aquifer properties. Progress has also been made on initial definition and testing of the reactive transport modeling approach and codes that will be used to evaluate the hypothesis.

Also critical to the project success for our University collaborators, we have recruited to the project a talented postdoctoral scholar, G. Wang, with expertise in the material properties that control organic solute sorption/desorption, as well as two graduate and undergraduate students with top qualifications and interests in CT reactivity and hydrostratigraphy.

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

William Apel[1] (PI), Brent Payton[2], Robin Gerlach[3], and Brady Lee[1]

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[3]Montana State University,

Our objective is to determine the effect of carbon and energy flow through simulated low level waste (LLW) environments on Cr(VI) and U(VI) migration from waste pits and trenches across the DOE complex. Metals and radionuclides can be mobilized by infiltration of water into LLW storage sites. Cellulolytic and noncellulolytic fermentative microorganisms have been chosen as the focus of this research, because their activity is a critical first step that we hypothesize will control subsequent fate and transport in contaminated natural systems. Microbial communities of lignocellulose degrading and fermenting microorganisms present in the subsurface of contaminated DOE sites can significantly impact migration by directly reducing and immobilizing metals and radionuclides, while degrading complex organic matter to low-molecular-weight organic compounds. These low-molecular-weight organic acids and alcohols can increase metal and radionuclide mobility by chelation (i.e., certain organic acids) or decrease mobility by stimulating respiratory metal-reducing microorganisms.

Our team has shown that:(1) a diverse bacterial population (species richness of 150 families), as determined by Phylochip analysis (LBNL), is present within cellulose containing surrogate waste forms retrieved from the Cold Waste Test Pit at the INL; (2) aerobic cellulose utilizing bacterial and fungal communities are present in the cellulosic wood waste material—several bacterial species (*Pseudomonas, Streptomyces,* two *Flavobacteria,* and *Pedobacter*) and one fungal species (*Lecythophora mutabilis*) were isolated and found capable of growth on media amended with cellulose; and (3) cellulose containing material has a high capacity for sorption of U(VI), but not for Cr(VI).

Our future research will focus on:

- Characterizing the production of fermentable substrates and low-molecular-weight organics from cellulosic debris in LLW by the activity of cellulolytic and noncellulolytic fermentative fungal and bacterial populations, and study their effect on the mobility of U(VI) and Cr(VI)
- Understanding the response of simple and complex microbial communities to carbon and electron flow through these natural and simulated LLW environments, using molecular microbial-ecology techniques along with monitoring fluctuations in concentrations of electron donors and acceptors
- Using this information to develop updated conceptual models for carbon and electron flow in LLW systems and the associated effect on Cr(VI) and U(VI) transport in the subsurface.

The Reaction Specificity of Nanoparticles in Solution

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Pacific Northwest National Laboratory, Richland, WA

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

Recent progress has been made on three fronts: (1) the aging (or diagenesis) of zerovalent iron (ZVI) nanoparticles in a water solution and the impact of this aging on reduction of CT; (2) the impact of metal additions to ZVI nanoparticles on the overall reaction rates and pathways for the breakdown of CT; and (3) theoretical determination of thermodynamic and kinetic properties for reactions of chlorinated hydrocarbons. A significant issue in the use of ZVI to remediate CT involves controlling the branching ratio, so that benign products such as formic acid (HCOOH) are formed, rather than toxic byproducts such as CF. Measurement of particle structures and reactivities at different stages of reaction with pure or contaminant-bearing water show that the processes that control contaminant degradation evolve with time. The ZVI nanoparticles are initially covered with an adherent iron-oxide coating, and the initial reactivity is low. As the oxide is exposed to water, the oxide becomes less coherent and the reactivity increases. After five days, the metal content in the nanoparticles decreases as more material is oxidized and the reaction rate slows. Nonetheless, a reasonably high reaction rate and good branching ratio persists for at least thirty days. Our most recent work involves the addition of different metal dopants to the nanoparticles at various stages of growth or processing to understand the importance of both dopant type and location on reactivity and reaction pathways. Measurements show that dopant location has a significant impact on reactivity.

A theoretical approach is being used to identify the most important reaction pathways that might be relevant in groundwater. Recent advances in *ab initio* electronic structure methods have the potential to help identify relevant environmental degradation reactions, by estimating the thermodynamic properties of all relevant contaminant species and intermediates for which experimental data are usually not available, and providing activation energies for relevant pathways. Using these methods, we have developed strategies to estimate the thermochemical and kinetic properties of reactions with chlorinated hydrocarbons. As an example, we have applied this approach to identify the reaction pathways and activation barriers for the dechlorination of chlorinated ethylenes.

Novel *In Situ* Incubation Techniques for Assessing Microbial Community Stability During and Following Biostimulation to Promote U(VI) Reduction

Brett R. Baldwin[1] (PI), Aaron D. Peacock[1], Charles T. Resch[2], Evan Arntzen[2], Amanda N. Smithgall[1], Jennifer L. Druhan[2], Margaret Gan[1], Philip E. Long[2], James P. McKinley[2], and David C. White[1]

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Biostimulation at the Rifle, Colorado, Uranium Mill Tailings Remedial Action (UMTRA) site promotes growth of *Geobacter* spp. and biological U(VI) reduction. However, oxidative dissolution of precipitated U(IV) remains an unresolved issue. Typically, U(VI) concentrations would rebound following consumption of bioavailable iron and the onset of sulfate reduction. Yet, after cessation of donor injection at the site, U(VI) concentrations have decreased and remained at approximately 20% of background levels for over one year. Postamendment U(VI) removal may be related to direct U(VI) reduction by sulfate reducers, generation of $FeS_{0.9}$, or sorption of U(VI) to biopolymers. An in-well sediment incubator (ISI) for field deployment of laboratory-stimulated native sediments was developed to evaluate the role of sulfate-reducing bacteria (SRB) in maintenance of bioreduced U(IV) under field conditions. An ISI containing Rifle aquifer background sediment (RABS) and laboratory-reduced RABS was deployed in a background monitoring well for 3 months. Following field incubation, the ISI was retrieved for analysis of the microbial community composition by phospholipid fatty acid (PLFA), denaturing gradient gel electrophoresis (DGGE), and real-time polymerase chain reaction (PCR) methods. PLFA profiles of ISI deployed reduced RABS showed loss of monounsaturated PLFA (the main PLFA of δ-*protoeobacteria* including *Geobacter*) following incubation in B-02. *Geobacter* spp. 16S rRNA gene copy numbers remained comparable to those of the reduced RABS, but overall, the microbial community of the reduced RABS responded to deployment in the background well. In a subsequent experiment, an ISI containing RABS was deployed in a downgradient well subject to prior biostimulation. An increase in both monounsaturated PLFA and δ-*proteobacterial* 16S rRNA gene copy numbers typical of reducing conditions at the site were noted, following deployment of RABS in the stimulated well.

Overall, the initial results demonstrated that in-well sediment incubation will permit expedient interrogation of the microbial community response to field environmental perturbations and will become a useful tool in examining microbial community control of bio-reduced uranium. Therefore, six additional ISIs containing sediments under iron-reducing and sulfate-reducing conditions were deployed at the Rifle site. ISIs will be retrieved during the June 2007 drilling event to compare changes in microbial community composition with those of the native formation.

Coupled Biogeochemical Processes Governing the Stability of Bacteriogenic Uraninite and Release of U(VI) in Heterogeneous Media: Molecular to Meter Scales

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

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The chemical stability of bacteriogenic "UO_2" is one of the seminal issues governing its success as an *in situ* waste form in remediated subsurface locations. Little detail is known about the structure and reactivity of this material, but based on comparison to its closest abiotic analog, UO_{2+x} ($0 < x < 0.25$), we expect that it is complex and disordered, likely to exhibit nonstoichiometry, and capable of structurally incorporating common groundwater cations and U(VI). These factors are expected to substantially impact its stability in ground water. Our four-institution team is conducting a systematic and coordinated characterization of: (1) the atomic- and nanoscale structures of bacteriogenic UO_{2+x} in the absence and presence of potentially important environmental cation dopants, (2) the equilibrium solubilities and dissolution rates of these materials, (3) the biogeochemical coupling of biologically mediated Mn cycling and UO_{2+x} oxidation, and (4) the influence of these molecular-scale processes on meter-scale release of U(VI) in sediments. This abstract describes results from Year 1 of this project.

Preparation of bacteriogenic UO_{2+x}: A prerequisite to this study is the development of a gentle aqueous cleaning method to separate the biooxide and organic components without altering the biooxides. A mild separation technique has been developed that overcomes challenges from the propensity of nanobacteriogenic UO_{2+x} to dissolve or ripen during NaOH treatment (as deduced from EXAFS and WAXS measurements). By varying the relative concentrations of divalent cation and carbonate as well as the pH, U(VI) was efficiently reduced in the presence of Mg, Mn, and Ca.

Atomic- and nano-scale structure of bacteriogenic UO_{2+x}: The local and long-range atomic structures and nanoscale structures of the wet oxides have been measured using EXAFS, WAXS, and SAXS. The unperturbed material exhibits a range of particle sizes with a mean around 4 nm. The oxygen shell is split, indicating distortion of the material, suggesting nonstoichiometry ($UO_{2.0}$ to $UO_{2.25}$) similar to abiotic UO_{2+x}, and the material contains strongly bound U(VI) at up to 10% of total U.

Dissolution kinetics of UO_{2+x}: Batch reactors and continuous-flow stirred tank reactors were used to study the dissolution kinetics of a synthetic UO_{2+x} as a function of pH, carbonate, and O_2 concentrations. The results will be compared to those for bacteriogenic UO_{2+x}.

Mn cycling and UO_{2+x} oxidation: We have cultivated twelve Mn(II)-oxidizing bacterial isolates from field sites. Initial studies of UO_{2+x} oxidation by bacteriogenic Mn oxides are being conducted with spores of the *Bacillus* sp. strain SG-1.

Future efforts: Dissolution rates and atomic structure of pure and cation-doped synthetic and bacteriogenic UO_{2+x} will be measured to assess the roles of dopants on structure and stability and the identity of strongly-sorbed U(VI). Mn(II)-oxidizing cultures will be used for studies of UO_{2+x} toxicity and oxidation and for inoculating future column experiments.

Reduction of Mercury (II) to Mercury (0) in Anoxic Enrichment Cultures Derived from FRC Sediments

Heather A. Wiatrowski, Yanping Wang, Pat Lu-Irving, Lily Young, Gerben Zylstra, and Tamar Barkay (PI)

Rutgers University, New Brunswick, NJ

A novel pathway for the reduction of Hg(II) to Hg(0) by metal-reducing microbes under anaerobic conditions was recently described (Wiatrowski *et al*., Env. Sci. Technnol. 40: 6690, 2006), suggesting that microbial activities may convert Hg(II) to Hg(0) in subsurface sediments. As Hg(II) sorbs strongly to sediments, and Hg(0) is a volatile gas, such a process could release sediment-bound Hg into groundwater. To test this hypothesis we established anoxic enrichment cultures with 5 g of subsurface sediment from the background area of the ERSD Field Research Center (FRC) (Oak Ridge, TN) under nitrate (10 mM) and iron (50 mM ferric oxyhydroxide) reducing conditions supplemented with 10 mM ethanol as a carbon and energy source. Nitrate-reducing enrichments were incubated for 24 days and assayed for Hg reducing potentials. Mercury, as $HgCl_2$, was added to a final concentration of 500 nM, and Hg(0) was collected in a trap of acidified potassium permanganate. An enrichment culture converted 35.9% of the added Hg to Hg(0) while 79.1% remained in the enrichment culture, suggesting Hg(II) reducing activities. In three control incubations containing gamma-irradiated sediments, $1.1 \pm 0.3\%$ of the mercury was recovered in the trap, and $87.1 \pm 1.9\%$ remained in the enrichments. Further passages of nitrate reducing enrichments also showed Hg(II) reducing activities. Initial evidence suggests that iron-reducing enrichments, after 60 days of incubation, reduce Hg(II) at higher rates than nitrate-reducing enrichments. These results suggest that there is a potential for the microbial mobilization of Hg as Hg(0) in anoxic subsurface sediments.

To analyze the community structure in nitrate enrichments, 16S rRNA clone libraries were constructed and analyzed by *Hae*III-*Rsa*I RFLP analysis. Three patterns were identified, two of which represented 93% of all clones and were most closely related (>98% sequence identity) to the *Betaproteobacteria* (*Massilia* spp., *Aquaspirillum* spp., *Zoogloea* spp.). The third RFLP pattern consisted of 16S rRNA gene sequences most closely related to *Herbaspirillum* spp., *Ramlibacter* spp., or *Curvibacter* spp. Pure cultures were obtained from the Hg(II) reducing nitrate enrichment by plating diluted samples on a nitrate-enriched artificial groundwater medium supplemented with different concentrations of Hg(II). Single colonies that were observed after a month of incubation were purified. One culture belonged to the *Bradyrhizobiaceae* and another to the *Paenibacillaceae* families. The mercuric reductase gene, *merA,* was PCR amplified from the genome of the bradyrhizobia-like strain; sequence analysis showed this *merA* to be most closely related to that of *Nocardioides* sp, a Gram-positive bacterium belonging to the *Actinomycetales*. These results show a limited diversity of the Hg(II) reducing denitrifying culture and suggest horizontal transfer of Hg resistance.

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

Alexander S. Beliaev (PI), Frank E. Löffler, Robert A. Sanford, Matthew J. Marshall, and Jim K. Fredrickson

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The overall scientific goal of the project is to elucidate the molecular mechanisms of radionuclide biotransformation by *Anaeromyxobacter dehalogenans*, a predominant member of indigenous microbial communities associated with contaminated subsurface environments. Further, this project explores how relevant environmental factors (e.g., co-contaminants, temperature, pH) affect these transformation reactions. *Anaeromyxobacter* spp. are facultative anaerobic dechlorinating δ-proteobacteria bacteria capable of enzymatically reducing a variety of metal and radionuclide contaminants. In resting cell reduction assays, *A. dehalogenans* strains 2CP-C and 2CP-1 rapidly reduce U(VI) or Tc(VII) to $U(IV)O_{2(s)}$ or $Tc(IV)O_{2(S)}$, using either hydrogen or acetate as electron donors. The subcellular localization of reduced UO_2 is predominantly extracellular, while TcO_2 nanoparticles are both periplasmic and extracellular. The U(IV) and Tc(IV) distribution patterns are similar to what is seen in *Shewanella*, suggesting that the reduction pathways may be similar in these organisms. Interestingly, strains 2CP-C and 2CP-1 displayed significant differences in the reduction rates of U(VI) or Tc(VII). Strain 2CP-1 reduces U(VI) and Tc(VII) at much faster rates than either strain 2CP-C or *S. oneidensis* MR-1. However, the subcellular localization of the UO_2 or TcO_2 nanoparticles was indistinguishable among the various *Anaeromyxobacter* strains.

Similar to other metal-reducing species, the metal reduction in *Anaeromyxobacter* is thought to be linked to a diversified electron transport chain. Based on the whole-genome sequence analysis, *A. dehalogenans* strains 2CP-C encodes a large number of redox-active proteins, including over 50 putative *c*-type cytochromes. Of particular interest is a gene cluster that includes three high-molecular-weight multiheme *c*-type cytochromes containing 16, 26, and 33 potential heme binding sites; two putative lipoproteins; and two tetratricopeptide repeat proteins (potentially involved in transport and/or assembly of electron transport machinery). Global transcriptome profiling indicated that 2CP-C expresses a cadre of high-molecular multiheme *c*-type cytochromes in response to solid metal electron acceptors. Notably, genes encoding the putative *c*-type cytochromes with 16 and 26 heme binding sites were specifically induced in the presence of Fe(III) oxide. To determine the role of these multiheme *c*-type cytochromes in metal reduction, we are developing and utilizing several genome level approaches, including utilization of site-directed and random mutagenesis approaches, to isolate metal-reduction-deficient mutants of strain 2CP-C. The results from efforts to develop a transposon-based random mutagenesis protocol and site-directed insertional and deletion mutagenesis methods will also be presented.

This study represents an important step towards the goal of characterizing the metal respiratory system of *A. dehalogenans* 2CP-C on a genomic scale. Current efforts are focused on the functional characterization of the *c*-type cytochrome genes upregulated during metal and radionuclide respiration.

Field-Portable and Automated Immunosensors for Hexavalent Uranium, Other Heavy Metals, and Chelators

Diane A. Blake[1] (PI), Haini Yu[1], Scott J. Melton[1], Nurettin Sahiner[1], and Robert Blake II[2]

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The goal of this project is to continue the development of new techniques for the rapid, automated identification of radionuclides, metals, and chelators that may contaminate surface and groundwater at DOE sites. A set of high-affinity, highly selective binding reagents (monoclonal antibodies) is being developed and refined to facilitate their use in two different immunosensors that can rapidly and accurately measure environmental contaminants in the field. Sensor technologies already in place will be the foundation for the development of additional assays for metal, radionuclide, and chelator contaminants.

The research objectives for this project and progress to date are as follows:

(1) To successfully deploy present immunosensors at DOE sites, by adapting the immunoassay procedures to site-specific geochemistries. Design and construction of the new field-portable immunosensor has been completed, and sensor function has been characterized using commercially available anticaffeine antibodies and soluble caffeine. A novel hydrogel (prepared by co-polymerization of N-vinyl 2-pyrrolidone/acrylonitrile and subsequent amidoximation) has been employed to specifically bind UO_2^{2+} in aqueous samples. Preliminary studies have shown that a 1 cm^3 piece of polymer will lower the UO_2^{2+} concentration from 1 ppm to below drinking water standards (30 ppb) in 100 mL of sample. We are devising a simple binding and elution strategy, based on a variation of this polymer, as a pretreatment strategy for the assay of surface and groundwater samples contaminated with UO_2^{2+}.

(2) To devise immunosensor-based assays for Pb(II), Hg(II), and/or Cr(III) in surface and/or groundwater. A bifunctional derivative of NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) has been covalently attached to a carrier protein and loaded with Cr(III). This Cr(III)-chelate-protein conjugate will be used as an immunogen for development of antibodies for Cr(III). Activities continue on the adaptation of an existing immunosensor-based assay for Pb(II) to environmental samples.

(3) To develop new technologies in antibody engineering that will enhance the immunosensor project. One of the monoclonal antibody used in the uranium immunosensor, clone #12F6, has been expressed as a recombinant protein. Molecular modeling and subsequent site-directed mutagenesis of selected amino acid side chains in the binding site have led to a new protein that binds to uranium with ~50% higher affinity than the original antibody. A Fab fragment of the 12F6 monoclonal antibody has been crystallized; we hope to use these crystals to solve the 3-dimensional structure of the 12F6 antibody. Extensive binding studies of a second antibody that recognizes chelated UO_2^{2+} (clone #8A11) have revealed novel synergistic activity after covalent modification (R.C. Blake II et al. (2007) *Biochemisty*: *46*,1573).

The Interaction of H_2O and NO_2 with Thin MgO(100) Films Grown on Ag(100) As Studied with Ambient Pressure Photoemission Spectroscopy

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The work described here was performed in the framework of the Stanford Environmental Molecular Science Institute (EMSI). The goal of the Stanford EMSI is to create fundamental molecular-level understanding of environmental interfaces and the important chemical and biological processes that occur at them. Using synchrotron-based spectroscopies under ambient temperatures and relative humidities, we are probing the coverage and chemical speciation of molecules, in particular water, at surfaces under realistic thermodynamic conditions.

Oxide surfaces have a particular relevance in environmental science, since water-oxide interactions play an important role in chemical, environmental, and biological systems. Even though these systems have been extensively studied using vacuum-based surface science techniques, the nature of the water-oxide interface under ambient conditions remains poorly understood. Because of its simplicity and environmental relevance, the MgO(100) surface has become a prototype for studying such interactions. However, basic issues such as molecular-versus-dissociative adsorption at the H_2O/MgO(100) interface have not been resolved. We have utilized Ambient Pressure Photoemission Spectroscopy at beamline 11.0.2 of the Advanced Light Source (at Lawrence Berkeley National Laboratory) to address such questions. This unique experimental setup is capable of performing photoemission and x-ray absorption experiments in the Torr pressure range, allowing measurements of the H_2O/MgO(100) interface under equilibrium conditions. We will present results relating to the degree of hydroxylation and uptake of H_2O on MgO(100) thin films grown on Ag(100), at room temperature and up to 1 torr of H_2O pressure. Further, we have studied the influence of film thickness on the reactivity of the MgO(100) film using NO_2 as a probe molecule. Our measurements show that films with thicknesses greater than 5 monolayers behave similar in nature to bulk MgO.

Anaerobic Biotransformation and Mobility of Pu and of Pu-EDTA

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The complexation of radionuclides by co-disposed ethylenediaminetetraacetate (EDTA) has enhanced their transport in sediments at DOE sites. The objectives of this project are (1) to determine the mobile form of Pu-EDTA (2) to determine the anaerobic biotransformation of Pu(IV) and Pu(III) in the presence of EDTA, and (3) to enrich for an anaerobic EDTA degrader.

Pu Thermodynamics: We reviewed all of the relevant published data, including our own, to develop reliable equilibrium constant values for the formation of Pu(OH)EDTA, $Pu(OH)_2EDTA^{2-}$, $Pu(OH)_3EDTA^{3-}$, and Fe(III)-EDTA. We developed a Pu(IV)-EDTA model to predict Pu behavior in geologic environments. In the presence of $Fe(OH)_3(s)$ or at relatively low Ca concentrations, EDTA is primarily complexed with Fe(III) or Ca(II) and does not complex Pu(IV). This suggests that Pu(IV)-EDTA complexes are not the mobile form of Pu in the environment and that Pu(III)-EDTA complexes are the mobile species.

Biological Reduction of Pu: Metal-reducing microorganisms have been widely investigated for their potential immobilization of toxic metals. However, Pu is chemically and radiologically toxic at much lower levels than other metals, and the solubility of Pu(III) is greater than Pu(IV). *Shewanella oneidensis* MR-1 rapidly solubilized Pu(IV) by reducing it to Pu(III) in the presence of EDTA (100% reduction within 2 days). Even in the absence of EDTA, the slow reduction of Pu(IV) by *S. oneidensis* MR-1 (5 orders of magnitude lower than with EDTA) is significant, because the amount of Pu solubilized still exceeds drinking water standards (15 pCi L^{-1} and 1×10^{-12} M). Similar reactions for the metal-reducing microorganism *Geobacter sulfurreducens* PCA are being evaluated.

Isolation of EDTA-Degrading Microorganisms: Semicontinuous enrichment systems were established by overlaying a shallow layer of aquatic sediments with minimal medium containing K_2EDTA as the sole carbon and energy source and $NaNO_3$ as the terminal electron acceptor. After 14 weeks, a stable enrichment was established. EDTA is metabolized to CO_2 and NH_3, and nitrate is reduced to N_2. We will gradually reduce the amount of the sediments in the system to isolate bacterial pure cultures capable of degrading EDTA under denitrifying conditions.

We conclude that Pu(IV)-EDTA will not be the mobile species in the environment, suggesting that Pu(III)-EDTA is the mobile species. The reduction (solubilization) of Pu(IV) by *S. oneidensis* MR-1 is greatly enhanced in the presence of EDTA, though its absence does not exclude the biological reduction of toxic quantities of soluble Pu. Biological systems capable of anaerobic reduction of EDTA do occur in the environment and remain to be fully characterized.

Aqueous Complexation Reactions Governing the Rate and Extent of Biogeochemical U(VI) Reduction

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Despite the promise of bioreduction as a remediation strategy, the factors that enhance or inhibit the rate and extent of biogeochemical U(VI) reduction under representative environmental conditions are not well defined. Previously, we reported the inhibition of bacterial U(VI) reduction in the presence of environmentally realistic concentrations of soluble calcium (Ca) (BROOKS et al., 2003). The effect was attributed to the formation of aqueous $CaUO_2(CO_3)_3^{2-}$ and $Ca_2UO_2(CO_3)_3^{0}$ species, although the precise mechanism of inhibition remains undetermined. Subsequent work has now demonstrated that the inhibitory effect of Ca is alleviated with the addition of EDTA as competing ligand for Ca. The effect is proportional to the concentration of ethylenediaminetetraacetate (EDTA). Measured pseudo-first-order rate constants for U(VI) bioreduction are negatively correlated with the fraction of U(VI) present as Ca-U(VI)-CO_3 species. Although bacteria effectively reduce U(VI) to U(IV) in the presence of EDTA, the biogenic U(IV) does not precipitate, but remains in solution as an U(IV)-EDTA complex—as confirmed by wet chemical, XANES, and EXAFS analysis. Reoxidation kinetics of biogenic U(IV) (either precipitated or U(IV)-EDTA) under atmospheric conditions (21% O_2) decreased with pH and in the presence of Ca or EDTA.

We have identified previously undescribed M-UO_2-CO_3 complexes for the remaining alkaline earth elements (M = Mg, Sr, Ba) and quantified their formation constants, enabling more complete speciation predictions for prepared and environmental samples. Complexes of $MUO_2(CO_3)_3^{2-}$ and $M_2UO_2(CO_3)_3^{0}$ are simultaneously formed for Ca^{2+} and Ba^{2+}, while Mg^{2+} and Sr^{2+} form only the $MUO_2(CO_3)_3^{2-}$ complex. Cumulative stability constants for the $MUO_2(CO_3)_3^{2-}$ complex, extrapolated to I = 0, are estimated to be ($\log\beta_{113} \pm s_{\log}$ 25.06 ± 0.07, 27.18 ± 0.06, 26.86 ± 0.04, and 26.68 ± 0.04 for Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+}, respectively. For $M_2UO_2(CO_3)_3^{0}$, the stability constants were measured to be ($\log\beta_{213} \pm s_{\log}$ 30.70 ± 0.05 and 29.75 ± 0.07 for Ca^{2+} and Ba^{2+}, respectively.

In the past 12 months, results of our investigations into U(VI) bioreduction suggest the following:

(1) At constant ionic strength (0.045 M) Mg in the range 1–5 mM slightly enhances U(VI) reduction, but the rate of U(VI) reduction decreased at 10 mM Mg. The extent of U(VI) reduction decreased with increasing [Mg].;

(2) The rate of U(VI) reduction decreased at ionic strengths above 0.045 M (0.1 and 0.275 M tested) when the ionic strength was adjusted with either NaCl or $MgCl_2$. Differences in the pseudo-first-order decay constants (k_1*) can exceed an order of magnitude.;

(3) EDTA alone significantly enhanced the U(VI) reduction rate. As [EDTA] increased (0, 0.05, 0.1, 0.5, 2.5 mM) k_1* increased (0.059 to 0.4 h^{-1}).;

(4) The observation that EDTA enhances U(VI) reduction led us to repeat experiments in which variable EDTA concentrations had been used to adjust the concentration of free Ca ($[Ca]_{free}$) at constant total Ca. EDTA was eliminated from the system, and variable amounts of Ca were added to match the $[Ca]_{free}$ in the previous experiments (0.25, 0.5, 0.75 mM). Calcium significantly lowered the U(VI) reduction rate (k_1* = 0.08, 0.011, 0.009 h^{-1}). The effect was significantly greater than that observed when EDTA was added to achieve similar $[Ca]_{free}$.;

(5) Although small amounts of Mg enhanced U(VI) reduction, adding Mg (1–5 mM) did not relieve the inhibitory effect of 0.5 mM Ca at constant ionic strength. The combination of 10 mM Mg + 0.5 mM Ca yielded slower U(VI) reduction than 0.5 mM Ca alone.

Reaction-Based Reactive Transport Modeling of Iron Reduction and Uranium Immobilization at Area 2 of the ERSD Field Research Center in Oak Ridge

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Our research is focused on developing mechanistic, phenomenological descriptions of important reactions, and mathematical formulations by which to model those reactions, for the *in situ* immobilization of uranium promoted via microbial iron(III) reduction. Experimental conditions have been designed to match those in saturated zone sediments at Area 2 of the ERSD Field Research Center (FRC) in Oak Ridge, TN. Our research has pursued three major objectives: (1) elucidate the mechanisms of reduction of solid-associated U(VI) in Area 2 sediment at the FRC; (2) evaluate the potential for long-term sustained U(IV) reductive immobilization coupled to dissimilatory metal-reducing bacterial (DMRB) activity in Area 2 sediments; (3) numerically simulate the suite of hydrobiogeochemical processes occurring in experimental systems, so as to facilitate modeling of *in situ* U(IV) immobilization at the field-scale. Our research is based on the following hypotheses: (1) the biological and chemical reduction of sediment-associated U(VI) is fundamentally controlled by its mineralogic and coordination environment; (2) the addition of humic substances can stimulate the reduction of solid-associated U(VI); (3) coupled Fe(III)/U(VI) reduction can be sustained in long-term flow-through reactor experiments, with hydrologic residence times comparable to those expected in pore domains colonized by DMRB in Area 2 sediments; (4) modest levels of nitrate input will not significantly inhibit coupled Fe(III)/U(VI) reduction in Area 2; and, (5) the kinetics and thermodynamics of simultaneous biogeochemical reactions can be described by reaction-based kinetic and equilibrium formulations, where rate formulations/parameter estimates derived from batch experiments will be applicable to flow-through reactor experiments.

Research progress has been made in all of the areas; however, we will present our most recent research results on the bioavailability of U(VI) sorbed to a variety of specimen mineral phases relevant to Area 2. We have measured and modeled the sorption of U(VI) to alumina, gibbsite, and illite in the presence and absence of humic substances. We have characterized the sorbed U(VI) species by X-ray absorption spectroscopy (EXAFS, conducted at Argonne) and by fluorescence spectroscopy (TRLIFS, conducted at PNNL) and found four distinct, similar molecular-scale coordination environments from eight separate samples tested. Specimen minerals with sorbed U(VI) were again prepared, and bioreduction of solid-phase U(VI) was tested with *Geobacter sulfurreducens* in an artificial groundwater representative of Area 2 amended with acetate of the electron donor. In all cases, a fairly large fraction of the solids-associated U(VI) was not available for reduction, and in one case no solids-associated U(VI) was reduced. In all but one case, dissolved U(VI) was completely reduced. We are attempting to describe how the different molecular-scale coordination environments of solids-associated U(VI) control its bioavailability.

Integrated Nucleic Acid System for In-Field Monitoring of Microbial Community Dynamics and Metabolic Activity

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With the accelerated development and use of nucleic acid microarray technology, there is considerable interest in applying existing (off-the-shelf) microarray methods and devices in uncharacterized sample backgrounds. Uncharacterized sample backgrounds create both a sample-preparation and data-interpolation challenge for the practical use of microarrays. The sample-preparation challenge results from the co-extraction of soluble environmental constituents that interfere with molecular techniques (including polymerase chain reaction [PCR] amplification, hybridization, and fluorescent detection) and the preponderance of unknown and uncharacterized nontarget organisms in the biological background. The data interpolation challenge is that theoretical and experimental data show that mismatched targets preferentially bind to microarray probes under nonequilibrium hybridization conditions, exacerbating the problem of false positive detection. Depending upon the nucleic acid purification and labeling strategy, nontarget sequences can also contribute to increased local and global background, degrading overall system (sample-to-answer) performance and dynamic range.

One strategy to address cross-hybridization is to remove unpredictable probes from the array. Another is to increase the total number of probes on an array and statistically compare the signal intensity between perfectly matched (PM) and single-base-mismatched (MM) duplexes. A third approach is to generate posthybridization thermal dissociation curves for every probe on the array. By themselves, however, these techniques do little to address the (fluidic or automated) nucleic acid sample preparation challenge or simplify the attendant analytical process or instrumentation. Based on our prior work with oligonucleotide-coated particles as automated affinity purification matrices, we reasoned that multiplexed affinity purification and thermal dissociation *prior to* biochip hybridization would simplify uncharacterized sample admixtures, thereby minimizing or eliminating sample interferents, improving hybridization specificity on a microarray detector, and minimizing or eliminating the need for posthybridization thermal dissociation analysis. Effort in FY06 therefore focused on developing an integrated thermo-affinity sample preparation subcircuit for sample purification and enrichment that is consistent with a field-portable form factor and analytical processes, and evaluate the efficacy of thermo-affinity sample preparation on model admixtures of varying complexity.

Femtomole quantities of oligononucleotide and synthetic gene targets were effectively enriched from sample admixtures at a ratio of 1 target to 10,000 nontarget molecules. The extent and incidence of false positive hybridization and average signal:noise ratios for nontarget microarray probes was significantly improved after multiplexed thermo-affinity sample purification. The proof-of-principle results and thermo-affinity approach provide a new, relatively simple method for incorporating nucleic acid sample preparation into microfluidic structures and analysis systems. We will also present proof-of-principle results for incorporating Dr. Yi Lu's Pb and U DNAzymes into a field-portable microarray format.

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

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Our work aims for a predictive-mechanistic understanding of the coupling between mineral weathering and contaminant (Cs, Sr, I) fate in caustic waste-impacted sediments across space, time, and geochemical gradients, an understanding that encompasses the heterogeneity observed at the Hanford Site. Our specific objectives are: (1) to assess the molecular-scale mechanisms responsible for time-dependent sequestration of contaminants (Cs, Sr, and I) during penetration of waste-induced weathering fronts; (2) to determine the rate and extent of contaminant release from the sorbed state; (3) to develop a reactive transport model based on molecular mechanisms and macroscopic flow experiments [(1) and (2)] that simulates adsorption, aging, and desorption dynamics.

Based on prior studies of model clay mineral systems, we postulated that contaminant uptake to Hanford sediments would reflect concurrent adsorption and co-precipitation effects. Our experimental work has focused on (1) determining molecular modes of Sr and Cs sequestration by coupling macroscopic measures of weathering with microscopic and spectroscopic characterization, and (2) developing bench-scale saturated and unsaturated flow-through reaction systems to determine hydraulic properties and contaminant sorption kinetics. Sediments reacted from 1 day to 1 year in batch systems with synthetic tank waste leachate (STWL) were characterized for contaminant uptake and sorbent-sorbate transformation using XAS, XRD, SEM/EDS, HRTEM, DRIFT, Si, Al, Sr or Cs NMR, and TG/DTA. Sorption of Sr exceeded that of Cs by about 3 times. The fraction of nonexchangeable Cs increased slightly over time despite fluctuations. Strontium became progressively recalcitrant to desorption from 92 days to 1 year, with spectroscopic and microscopic characterizations indicating sequestration in neoformed feldspathoid-type phases. Cs- and Sr-containing solid phases after 1 year of reaction include recalcitrant cancrinite and sodalite, and a smaller fraction of extractable $SrCO_3(s)$. The fraction of $SrCO_3(s)$ increased with an increase in PCO_2 from *ca*. 0 to $10^{-3.44}$. Although Cs uptake is strongly influenced by adsorption to high-affinity sites of native micaceous minerals, batch desorption kinetics are slower than those from both unimpacted sediments and Cs-sorbed cancrinite. Numerical modeling of batch Cs desorption indicates that a slow desorption reaction, such as $Cs^+ \rightarrow Na^+$ exchange in sodalite, is required to fit the data.

Results indicate that while both Sr and Cs are incorporated into neoformed precipitates, Sr uptake and lability appears more sensitively dependent on the trajectory of mineral transformation in the vadose zone. Unsaturated column experiments showed greater retardation of Sr relative to Cs in two columns with different effective saturation (25 and 37%). Further, greater retardation of Sr occurred at higher water saturation because of enhanced rates of mineral transformation. The kinetics of contaminant desorption from STWL-reacted sediments are being studied in ongoing saturated and unsaturated column experiments, to probe the effects of contaminant concentration, PCO_2 and sorbate aging in the presence of STWL, and the influence of pore-water chemistry present during the desorption/dissolution process.

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

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

We have coupled a surface-complexation modeling framework with a transport code and are testing the code's performance. We are currently working with Professor Zheng's group at the University of Alabama to develop methods to integrate the RT3D model within the latest version of MT3DMS. Also, we are currently exploring methods to run the reactive transport code in a PC-based parallel computing system. We have derived a new analytical formulation for solving multispecies transport equations coupled with a radioactive decay chain. We have completed batch studies to evaluate the oxidation and sorption reactions of As (III) and As(V) on MnO_2 minerals. The batch results were then successfully scaled to predict column-scale transport observed in soil columns containing various concentrations of MnO_2 minerals. We have developed methods to synthesize goethite-coated sand (GCS) in a controlled setting. The sand was characterized and used to study uranium sorption under batch and sequential batch conditions. We have predicted uranium adsorption onto GCS at different equilibrium pH values, using the surface complexation modeling (SCM) approach. The eventual goal is to perform large-scale column experiments and predict the fate and transport of DOE-site-specific metal contaminants using the SCM parameters derived from batch experiments.

On the modeling front, we plan to complete the numerical modeling effort to integrate the surface complexation modules within various multispecies transport simulators, including RT3D. We will complete our analytical modeling work and extend the ideas to create analytical solutions for both Dirichlet and Cauchy boundary conditions with the source term decaying at the boundary itself. On the experimental front, we will run a set of sequential batch reactors (SBR) using multiple reactors in series to evaluate As(V) and U(VI) reactive transport onto GCS under different solid-to-solution ratios. The experimental results will be simulated using the surface complexation modeling approach, and the transport model will then be used to design column and soil box experiments. We will also study the effect of phosphates during the adsorption of As(V) and U(VI) onto GCS, and we will predict their behavior using reactive transport modeling for batch, SBR, column and soil-box systems.

Anaerobic Uraninite Bio-Oxidation

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A proposed strategy for the remediation of uranium (U) contaminated sites is based on immobilizing U by reducing the oxidized soluble U, U(VI), to form a reduced insoluble end product, U(IV). Owing to the use of nitric acid in the processing of nuclear fuels, nitrate is often a co-contaminant found in many of the environments contaminated with uranium. Recent studies indicate that direct biological oxidation of U(IV) coupled to nitrate reduction may exist *in situ*. Direct biological oxidation of reduced metals (Fe(II) and U(IV)) coupled to nitrate reduction at circumneutral pH has recently been recognized in several environments, as well as in pure culture. Several phylogentically diverse mesophilic bacteria have been described as capable of anaerobic, nitrate-dependent Fe(II) oxidation (NFOx). Our recent identification of a freshwater mesophilic, lithoautotroph, *Lutiella nitroferrum* strain 2002, capable of growth through NFOx, presents an opportunity to further study metal bio-oxidation. Continuing physiological studies revealed that in addition to Fe(II) oxidation, strain 2002 is capable of oxidizing U(IV) (4 μM) in washed cell suspensions, with nitrate serving as the electron acceptor. Under growth conditions, strain 2002 catalyzed the oxidation of 12 μM U(IV) within a two-week period. Cultures poisoned with sodium azide, an electron transport inhibitor, demonstrated limited oxidation (7 μM) similar to pasteurized cultures, supporting the direct role of electron transport in U(IV) bio-oxidation. It is currently unknown whether strain 2002 can couple this metabolism to growth. The growth of *L. nitroferrum* strain 2002 utilizing another metal, Fe(II), as the sole electron donor, was previously demonstrated. Assuming 100% efficiency for the conversion of energy from autotrophic nitrate-dependent Fe(II) oxidation into biomass, *L. nitroferrum* strain 2002 required 0.096 kJ for cell division. The amount of U(IV) (~12 μM) that strain 2002 oxidized under similar autotrophic growth conditions yields 0.0019 kJ, enough energy for the generation of ATP (5.31×10^{-23} kJ ATP^{-1}), but not enough energy for cell replication as calculated (0.096 kJ) assuming a similar metabolism. The conservation of energy via this metabolism not only has direct implications for U mobility but also for cell maintenance and survival of microorganisms stimulated in nitrate and U co-contaminated subsurface sediments.

In addition to *L. nitroferrum* strain 2002, a nitrate-dependent Fe(II) oxidizing bacterium isolated from U contaminated groundwater, *Diaphorobacter* sp. strain TPSY, was also capable of nitrate-dependent U(IV) oxidation (8 μM over 24 hours, pseudo-first-order rate constant of 0.12 ± 0.02 hr^{-1}) in washed cell suspensions. Further biochemical investigation of nitrate-dependent U(IV) oxidation in strain TPSY revealed the expression of several putative high molecular weight proteins specific to this metabolism. Most-probable-number enumeration of the nitrate-dependent U(IV) oxidizing microbial community in sedimentary environments revealed a microbial community similar in number to NFOx, ranging from 93 to 2,398 cells g^{-1} sediment in both contaminated and uncontaminated sediments, including subsurface sediments from the ERSD Field Research Center in Oak Ridge, TN, and Longhorn, Texas, lake sediments and agricultural field soil. Together with the previously described metabolic ability of *Geobacter metallireducens* and *Thiobacillus denitrificans*, these data indicate that anaerobic U(IV) oxidation is a ubiquitous microbial metabolism.

Anaerobic, Nitrate-Dependent Fe(II) Bio-Oxidation: A Column Study

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Most-probable-number enumeration revealed nitrate-dependent Fe(II) oxidizing microbial communities in groundwater and subsurface sediments on the order of 0–2.04×10^3 cells mL^{-1} and 2.39×10^2–1.17×10^3 cells (g wet sediment)$^{-1}$, respectively. The isolation of a nitrate-dependent Fe(II) oxidizing isolate, strain TPSY, from the MPN enumeration series initiated from groundwater collected from the ERSD Field Research Center (FRC), Area 2, in Oak Park, further supports the potential for an active nitrate-dependent Fe(II) oxidizing microbial community in FRC groundwater and sediments. Strain TPSY is a nitrate-dependent Fe(II)-oxidizing bacterium, capable of growth over a pH range of 4.5 to 9.0. Comparative analysis of the entire 16S rDNA sequence indicated that strain TPSY is a member of the beta subclass of the Proteobacteria. Strain TPSY is closely related to *Diaphorobacter nitroreducens*. As nitrate is often a co-contaminant found in these environments, these results indicate the potential for an active nitrate-dependent Fe(II) oxidizing microbial population *in situ*.

The efficacy of nitrate-dependent Fe(II) oxidation under advective flow was evaluated in a mesoscale column reactor packed with sterile low-iron sand amended with subsurface sediments collected from the FRC background field site (10% mass/mass). Continuous flow of minimal medium mimicked the natural groundwater. Periodic $FeCl_2$ and nitrate injections over a period of 49 days resulted in the retention of 95% of the iron (~20.3 mmol). Extraction of solid-phase Fe revealed a net increase in Fe(III) of 13.2 mmol above background Fe(III) content, indicating that 65% of the injected Fe(II) was oxidized. Differential solubility analysis of 0.5 M HCl-extractable Fe and 3 M HCl-extractable Fe indicated that the oxidation product was crystalline in nature, since only 20% was soluble in 0.5 M HCl. This formation of crystalline biogenic Fe(III) oxides is consistent with our previous studies. Periodic injections of nitrate and acetate did not result in significant changes in Fe(II) or Fe(III) throughout a control column.

Most probable number enumeration of the nitrate-dependent Fe(II) oxidizing microbial community indicated that the Fe(II) and nitrate injections stimulated a significant community (7.41×10^5 cells g^{-1} sediment) concurrent with the region of Fe(III) oxide precipitation. Small-subunit 16S rDNA clone libraries revealed that the poorly understood Acidobacteria phylum was stimulated in this region, representing a significant proportion (21%) of the microbial community. The biogeochemical role of Acidobacteria in soils and sediments is poorly understood. Physiological screening of a pure culture Acidobacteria representative, *Geothrix fermentens*, revealed that a member of the Acidobacteria phylum was capable of anaerobically oxidizing Fe(II) coupled to nitrate reduction under nongrowth conditions. Pasteurized control cultures exhibited neither Fe(II) oxidation nor nitrate reduction. This result supports the stimulation of Acidobacteria identified in the small-subunit 16S rDNA clone library. Together, these results indicate that the environmentally ubiquitous Acidobacteria play a role in anaerobic, nitrate-dependent Fe(II) oxidation in these subsurface sediments.

Nitrate-dependent Fe(II) oxidizing microorganisms not only prevail in surface environments, but are also present in subsurface environments, as demonstrated in the MPN enumeration series initiated from groundwater and sediment samples collected from the FRC. Thus, these microorganisms demonstrate the potential for an active nitrate-dependent Fe(II)-oxidizing microbial community *in situ* in subsurface environments, such as the FRC. Together, these results demonstrate that native subsurface sediments harbor microbial communities capable of nitrate-dependent Fe(II) oxidation under advective flow. The biogenic formation of reactive Fe(III) oxide minerals capable of immobilizing heavy metals and radionuclides presents a plausible bioremediative strategy for contaminated subsurface environments.

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

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Significant quantities of metals and radionuclides are contained in thick unsaturated zones at several DOE sites in the western United States. In many cases, this contamination has migrated to underlying groundwater, sometimes decades after being released into the subsurface. Because of the prohibitive costs associated with physically removing the contamination, the only remedy to this problem is to develop methods for long-term, *in situ* stabilization of the contamination in the vadose zone. Our research focuses on developing a method of introducing gaseous compounds to stimulate precipitation of stable phosphate mineral phases in the vadose zone to remove contaminants (specifically ^{90}Sr and uranium) from pore waters and thus minimize further transport to groundwater. Preliminary studies have demonstrated that biological precipitation of phosphate minerals can be stimulated under unsaturated conditions by injection of triethyl phosphate (TEP) gas. Microorganisms hydrolyze the TEP, producing inorganic phosphate and, under the right conditions, catalyzing the precipitation of phosphate minerals.

Our initial results indicate that a mixed culture of aerobic heterotrophic microorganisms enriched from sediments from the Vadose Zone Research Park at the Idaho National Laboratory are capable of degrading low concentrations of TEP. The degradation rate increases significantly when ethanol is added as a supplemental carbon and energy source. Additional experiments are planned to optimize rates of TEP consumption under varying chemical and physical conditions with different nutrient amendments (e.g., N_2O as a source of nitrogen). The results of these studies will be the basis for unsaturated column experiments designed to test different delivery methods for the TEP and other nutrients, and to estimate potential rates of phosphate mineralization in the vadose zone. Precipitates formed during these experiments will be analyzed using X-ray micro-beam techniques (e.g., X-ray absorption, micro-diffraction) to detect and identify neo-formed phosphate minerals, and to confirm uptake of strontium and uranium added to culture solutions. Microbial activity will be monitored using high-density 16S ribosomal RNA microarrays to determine the response of microbial communities to the injection of gas mixtures. The chemical and isotopic compositions of reactants and products will be measured during all experiments to develop a method for tracking *in situ* microbial utilization of TEP and other gas phase compounds used to stimulate precipitation of phosphate minerals. Computer simulations of all experiments will be performed, using the multicomponent reactive transport code TOUGHREACT, to model and quantify gas reactive transport and gas-pore water-sediment interactions, including mineral precipitation. This modeling effort will help us to gain a better understanding of the coupling between microbial processes, geochemical reactions, and water and gas transport.

Characterizing the Catalytic Potential of Bacteria Isolated from Contaminated Subsurface Environments of the Hanford Site

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Our objective is to characterize the relationship between metal-reduction, metal-assimilation, and bacterial radiation and desiccation resistance for environmentally relevant bacteria of the Hanford Site. In a previous study using inductively coupled plasma mass spectrometry, we found that resistant and sensitive bacteria had significantly different intracellular metal concentrations, lending support to the role of manganese and iron in recovery from radiation and desiccation. We extended those studies with x-ray fluorescence microprobe analysis, which is suited for trace metal profiling and quantification because of its inherent elemental sensitivity of approximately 0.1–10 parts per million. The most resistant cells contained about 300 times more Mn and three times less Fe than the most sensitive cells, where the highest regional Mn concentrations were associated with the cytosol. The current work investigated the functional consequences of this disparity, showing that high cytosolic Mn and low Fe concentrations facilitate resistance by protecting proteins, but not DNA, from ionizing radiation and other conditions which elicit redox-related toxicity. These findings offer a novel perspective on the long-cryptic nature of bacterial resistance mechanisms, shifting the focus of toxicity and resistance away from DNA damage and repair toward a potent form of protein protection. High intracellular concentrations of Mn ions are known to alleviate oxidative stress in several bacterial species and can interact with different reactive oxygen species, depending on the oxidation state of Mn and its binding with organic acids. *In vitro*, we found that although Mn ions failed to protect DNA from hydroxyl radicals generated during irradiation, Mn(II) ions prevented enzyme damage. To understand the nature of Mn(II) protection *in vivo*, we compared the levels of radiation- and desiccation-induced oxidative protein damage in the most resistant cells, including *Deinococcus* and *Arthrobacter* spp., with the most sensitive cells, including *Shewanella* and *Pseudomonas* spp. Whereas sensitive cells with the lowest Mn to Fe concentration ratios had high levels of protein oxidation, resistant cells with the highest ratios had no detectable protein oxidation. Our new model of radiation and desiccation toxicity opens up novel avenues for cellular protection in diverse settings. Given that many bacteria with favorable bioremediation functions, such as *Shewanella oneidensis*, are extremely sensitive to radiation and desiccation, the new insight provided by *Deinococcus radiodurans* on how to survive oxidative stress conditions might prove useful in efforts to harness sensitive bacteria for bioremediation. The work also points to how protein oxidation could be used as a specific stress indicator for understanding the physiology of subsurface microorganisms catalyzing contaminant transformation.

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

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The focus of this project is the development of scientifically defensible approaches for upscaling reactive transport models (RTM) through a detailed understanding of U(VI) desorption from natural sediments across several spatial scales: bench, intermediate, and field. Uranium-contaminated sediment was collected from the alluvial aquifer at the DOE UMTRA site near Naturita, Colorado, which was also the site of field experiments. Sediment for the laboratory experiments was excavated from an area measuring 2 m by 6.5 m by 1 m. This area was selected based on particle size distributions measured on 26 cores collected from the alluvial aquifer immediately downgradient of the highest U(VI) concentrations at the site. The excavated sediment had particle sizes that ranged from silt to gravels, and the U(VI) concentration in the groundwater from a nearby well was 7.1 uM. The sediment was initially sieved through a 12 mm screen and then further separated into <2 mm, 2–4 mm and 4–12 mm size fractions in the field. The <2 mm material is being used in an initial set of batch, column and intermediate-scale laboratory experiments. Initial experiments at all three scales are being conducted with synthetic groundwater having an initial alkalinity of 0.6 meq/L. Kinetic batch desorption experiments containing either 25 g/L or 1,000 g/L of sediments suggest that the rate of U(VI) desorption was smaller for the higher sediment concentration. A laboratory column experiment is being prepared by packing the column with a paste created from the <2mm sample and the same synthetic groundwater used in the batch desorption experiments. An intermediate-scale tank (2.44 m × 1.22 m × 7.6 cm) was also uniformly packed with the <2 mm sediment and capped with 8 cm of clay, to simulate 2-D confined groundwater flow. Hydraulic head was measured throughout the tank through sampling ports installed through bulkhead fittings in the tank wall. Modeling using the code CRUNCH is under way to simulate the U(VI) behavior in the intermediate-scale tank and to design future tank experiments. Hypothetical modeling results show that the time required to flush U(VI) from a physically and chemically heterogeneous simulated aquifer was significantly longer than the time required for a homogenous simulate aquifer, even though both simulations had the same average properties.

In addition, a small-scale (6 m) tracer test site was installed to examine U(VI) desorption under natural and forced gradient conditions. The experimental tracer test site consisted of 34 multilevel wells; cores were collected at 22 of the wells for analysis of physical and geochemical properties. A U(VI) desorption experiment was conducted at the test site in November 2006 by injecting 750 L of uncontaminated groundwater containing 6.3 mM NaBr into four upgradient multilevel wells and then monitoring downgradient wells for Br and U(VI).

The findings from these multiscale investigations will provide a basis for a methodology to upscale the understanding of processes gained at the pore scale to effective rates of the same processes occurring at the field scale. Development of this methodology will lead to a scientifically defensible approach in conceptual model development for multicomponent RTM at contaminated DOE sites, leading to predictive U(VI) transport simulations with reduced uncertainty.

Isotopic Tracers for Biogeochemical Processes and Contaminant Transport: Hanford, Washington

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Our goal is to use isotopic measurements to understand how contaminants are introduced to and stored in the vadose zone, and what processes control migration from the vadose zone to groundwater and then to surface water. We have been using the Hanford Site in south-central Washington as our field laboratory, and our investigations are often stimulated by observations made as part of the groundwater monitoring program and vadose zone characterization activities there. Understanding the transport of contaminants at Hanford is difficult because of the presence of multiple potential sources within small areas, the long history of activities, the range of disposal methods, and the continuing evolution of the hydrological system. Observations often do not conform to simple models and cannot be adequately understood with standard characterization approaches, even though the characterization activities are quite extensive. One of our objectives is to test the value of adding isotopic techniques to the characterization program, which has the immediate potential benefit of addressing specific remediation issues, but more importantly, it allows us to study fundamental processes at the scale and in the medium where they need to be understood. We will focus our presentation on two recent studies at the waste management area (WMA) T-TX-TY, which relate to the sources and transport histories of vadose zone and groundwater contamination and contaminant fluid-sediment interaction.

WMA T-TX-TY presents a complicated picture of mixed groundwater plumes of nitrate, ^{99}Tc, Cr^{6+}, carbon tetrachloride, etc. and multiple potential vadose zone sources such as tank leaks and disposal cribs. The scientific questions arise because different contaminants were introduced to the vadose zone at different times and in differing ways, they are arriving at the water table at different times and in different places, and in some cases are concentrated in groundwater but below the water table. The advantage of studying this site is that there has been extensive subsurface sampling, both in the aquifer and in the vadose zone, there are abundant complementary characterization data, and there are records of the contamination events. As a field experiment (although unintended), it is moderately well controlled and uniquely long term.

An immediate concern was the observation of increasing ^{99}Tc concentration in groundwater monitoring wells near WMA T, with no clear indication of the source. In this case, we determined that, although the Tc itself is not labeled, the associated nitrate is, through its $\delta^{15}N$ and $\delta^{18}O$. We find that nitrate from low-level waste, high-level waste, and natural sources can be distinguished, and that the nitrate associated with the Tc is from high-level waste. Uranium isotopic data for a contaminated vadose zone core from the nearby WMA TX links the U contamination to a nearby single shelled tank. Also in WMA T, the U contamination is from the major leak of the T-106 tank in 1973. Uranium and strontium isotopic data on vadose zone cores provide evidence for chemical interaction between high-pH waste fluid and sediment.

Our ongoing research focuses on Cr isotopes to evaluate remediation performance and options relating to Cr^{6+} contamination at the Hanford Site. We are also investigating isotopic signatures of sulfate ($\delta^{34}S$ and $\delta^{18}O$) to trace sources of co-contaminants, and to probe redox processes affecting the mobility of redox-sensitive metals like Cr.

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

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Uranium reduction. *Shewanella putrefaciens* strain 200 respires a wide range of compounds as a terminal electron acceptor. The respiratory versatility of *Shewanella* is attributed in part to a set of *c*-type cytochromes with widely varying mid-point redox potentials (E'_0). A point mutant of *S. putrefaciens*, originally designated Urr14 and here renamed CCMB1, was found to grow at wild-type rates on electron acceptors with high E'_0 [O_2, NO_3^-, Fe(III)-citrate, MnO_2, Mn(III)], yet was severely impaired for growth on electron acceptors with low E'_0 [NO_2^-, U(VI), DMSO, TMAO, fumarate, γ-FeOOH, SO_3^{2-}, $S_2O_3^{2-}$]. Genetic complementation and nucleotide sequence analyses indicated that the CCMB1 respiratory mutant phenotype was caused by mutation of a conserved histidine residue (H108Y) in a protein that displayed high homology to *Escherichia coli* CcmB, the permease subunit of an ABC transporter involved in cytochrome *c* maturation. Although CCMB1 retained the ability to grow on electron acceptors with high E'_0, the cytochrome content of CCMB1 was <10% of the wild-type strain. Periplasmic extracts of CCMB1 contained slightly greater concentrations of the thiol functional group (-SH) compared with the wild-type strain, an indication that the E_h of the CCMB1 periplasm was abnormally low. A *ccmB* deletion mutant was unable to respire anaerobically on any electron acceptor, yet retained aerobic respiratory capability. These results suggest that the mutation of a conserved histidine residue (H108) in CCMB1 alters the redox homeostasis of the periplasm during anaerobic growth on electron acceptors with low (but not high) E'_0. This is the first report of the effects of Ccm deficiencies on bacterial respiration of electron acceptors whose E'_0 nearly span the entire redox continuum.

Technetium reduction. A rapid mutant screening technique was also developed to identify *S. oneidensis* respiratory mutants unable to reduce Tc(VII) as anaerobic electron acceptor. The Tcr mutant screening technique was based on the observation that wild-type *S. oneidensis* produced a black Tc(IV) precipitate on its colony surface during growth on Tc(VII)-amended agar, while colonies arising from mutagenized cells did not. Tcr mutants unable to produce the black precipitate were subsequently tested for anaerobic growth on an array of three electron donors and 13 alternate electron acceptors. The Tcr mutants displayed a broad spectrum of anaerobic growth deficiencies, including several that were unable to reduce Tc(VII) with hydrogen or lactate as electron donor, yet retained the ability to reduce Tc(VII) with formate. The putative Tcr mutants were subsequently grown either aerobically or anaerobically in minimal medium with fumarate as electron acceptor. After aerobic growth, Tcr mutants Tcr-9, Tcr-17, and Tcr-18 were unable to reduce Tc(VII) with either lactate or H_2. Tcr-9, however, displayed the ability to reduce Tc(VII) with formate as electron donor, while Tcr-17 and Tcr-18 did not. After anaerobic growth with fumarate as electron acceptor, Tcr-17 was unable to reduce Tc(VII) with any electron donor. Tcr-18 was unable to reduce Tc(VII) with lactate or formate (yet displayed activity with H_2), while Tcr-9 reduced Tc(VII) with all three electron donors. These results indicate that Tc(VII) reduction in *S. oneidensis* proceeds via electron transport pathways that are electron donor-specific and whose expression depends on the electron acceptor used for growth. Genetic complementation analysis is currently under way to identify the genes required for Tc(VII) reduction activity.

Heterogeneity in Bioreduction and Resulting Impacts on Contaminant and Microbial Dynamics

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Soils and sediments are complex mineral assemblages hosting a diverse microbial community within a convoluted physically heterogeneous setting. As a consequence, biogeochemical processes will exhibit high spatial variability, owing to chemical conditions dictated by the local mineralogical and physical environment. Bioreductive stabilization of contaminants is dependent on the convoluted coupling of biological, chemical, and hydrologic processes that will vary spatially from the micro- to macroscale. In response to the probability of coupled processes governing reactive transport of contaminants, the objective of our project is to determine processes controlling metal fate and transport within chemically and physically heterogeneous systems.

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

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

Structural Role for Flagella in Biofilm Formation and Stability in *Desulfovibrio vulgaris* Hildenborough

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One of our main objectives in this work is the identification of key genes necessary for biofilm formation and maintenance. This identification will provide insight into cellular responses to heavy metals when cells are grown as surface-adhered populations. *Desulfovibrio* spp. are model SO_4-reducing bacteria (SRB), and recent work has observed *Desulfovibrio* spp. at field sites contaminated with chromium and uranium. SRB biofilms have been shown to reduce heavy metals, but little is known about the cellular events that lead up to biofilm formation and development in SRB, including the cellular material and gene products used to promote and maintain cell adherence under sulfate-reducing conditions. Biofilm formation was observed on glass slides submerged in a CDC reactor that contained a defined medium and a dilution rate of approximately 0.09 h^{-1}. Significant levels of carbohydrates were not detected in biofilms grown in continuous mode, and less than 2 ug/cm^2 of carbohydrate was detected at any stage of biofilm growth tested. Biofilms cultivated in batch and continuous culture appeared similar, and both biofilms contained long filaments interconnected between the cells and the surface. The images revealed a monolayer of cells, and the biofilm maintained a constant cell number throughout cultivation. The filaments remained throughout the cultivation of the biofilms, and our recent results suggested that the extracellular filaments were flagella. Transcriptomic analyses of the biofilm cells revealed that most up-expressed genes could be classified in the COG categories of energy production and conversion, followed by signal transduction mechanisms, cell motility, secretion, and hypothetical proteins. With respect to flagella, *D. vulgaris* has six putative flagellin proteins, but only one novel putative flagellin was up-expressed in biofilm cells compared to planktonic cells. *D. vulgaris* ATCC 29579 (wild-type) and three mutants, Δ*fla*G, Δ*fli*A, and ΔMP were grown in batch mode in a defined medium with lactate and sulfate and biofilms were allowed to form on glass slides. Initial results indicated that Δ*fla*G mutants were motile, while the ΔMP and Δ*fli*A mutants were deficient in motility. The filaments, possibly a form of modified flagella, were present within wild-type biofilms, but fewer were seen in Δ*fla*G, and were almost completely lacking in the Δ*fli*A and ΔMP mutants. Crystal violet staining revealed that Δ*fla*G, Δ*fli*A, and ΔMP mutants produced (respectively) 5-fold, 2-fold, and 3-fold less biofilm compared to the wild-type. In addition, transcriptomic analysis indicated that *fla*G was the only novel flagellin that was up-expressed in biofilm cells compared to planktonic cells. The data indicated that *D. vulgaris* Hildenborough biofilms had unique gene expression patterns compared to both exponential and stationary-phase cells, that biofilms maintained simple monolayers, and that a significant carbohydrate matrix was not required for biofilm formation or maintenance. In addition to initial attachment, unique flagella appear to be involved in biofilm maturation and stability.

Natural Gene Transfer to Develop Resistance to Metal Toxicity in Microbial Communities at the ERSD Field Research Center in Oak Ridge

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The ERSD Field Research Center (FRC) at Oak Ridge, Tennessee, contains extreme geochemical environments that place a number of stresses on the extant microbial community, including low pH (e.g., pH < 4), nitrate concentrations that can exceed 100 mM, and the occurrence of heavy metals and radionuclides (U and other actinides). Removal of U(VI) from groundwater by biostimulation is the most promising remediation strategy. U(VI) can be microbiologically immobilized via reduction from U(VI) to insoluble U(IV) by (for example) *Desulfovibrio* sp., *Geobacter* sp. and *Shewanella* sp. Nitrate, however, serves as a competing and energetically more favorable electron acceptor for these bacteria, that along with toxic heavy metals will impede *in situ* uranium bioremediation. This project seeks to determine if adding nitrate-reducing bacterial strains, genetically enhanced with heavy-metal-resistant traits, results in more efficient uranium reduction under selected *in situ* conditions.

Soil contaminated with uranium and nitrate from FRC Area 3 was homogenized and incubated anaerobically under continuous flow-through conditions, with ethanol as carbon source. By incubating these soils under a variety of *in situ* conditions including influent groundwater containing elevated nickel and nitrate concentrations, we looked for improved uranium reduction efficiency, resulting from (1) adaptation of the indigenous population to the presence of toxic co-contamination and/or (2) bioaugmentation of nitrate-reducing organisms genetically engineered with heavy-metal-resistance genes.

The results show that the indigenous community adapts to the implemented selection pressure of heavy metals, added carbon sources, and high nitrate concentrations. Phylogenetic analyses of 16S ribosomal DNA obtained from the soils reveal that the microbial population evolved from initially αProteobacteria and Actinobacteria, which were not heavy metal resistant, to a community dominated by heavy-metal-resistant, nitrate-reducing β-, γ- Proteobacteria, Sphingobacteriaceae and sulfate reducing *Clostridiaceae*. Bioaugmentation with a nitrate-reducing *Pseudomonas* species and its Ni-resistant derivatives did not have a measurable effect at the functional level, although it did induce changes in the population composition. The indigenous FRC community did, however, evolve under biostimulation to one that efficiently uses nitrate and sulfate as electron acceptors. This evolution also selected strains that are known to reduce uranium. During the final year of this project, the U(VI) immobilization efficiency of the biostimulated community will be characterized, and Ni-resistant isolates will be tested for resistance to other metals.

Molecular Mechanism of Uranium Reduction by *Clostridia* and Its Manipulation

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The overall objective of this research is to elucidate, systematically, the molecular mechanisms involved in the reduction of uranium by *Clostridia*. We propose to (1) determine the role of hydrogenases in uranium reduction, (2) purify the enzymes involved in uranium reduction, (3) determine the mechanisms of reduction, e.g., one or two electron transfer reactions, and (4) elucidate the genetic control of the enzymes and cellular factors involved in uranium reduction.

We investigated the physiological conditions and biochemical mechanisms of uranium (VI) reduction by *Clostridium* sp. (ATCC 53464), *C. sphenoides* (ATCC 19403), *C. acetobutylicum* (ATCC 824), and *C. pasteurianum* (ATCC 7040). Among the strains tested, *Clostridium* sp. showed the highest U(VI) reduction at the rate of 0.1 μmol U(VI) h^{-1} μg^{-1} protein. This organism fermented glucose with the production of organic acids, CO_2 and H_2, and reduced U(VI) under acidic conditions. The pH of the culture did, however, have a significant effect on uranium reduction, with pH 5–6 being the optimal pH in most cases. *Clostridium* sp. showed the strongest ability to reduce uranium and was less affected by culture pH compared with other strains. Our results showed that while the ability to reduce uranium is a common phenomenon among *Clostridia*, the differences in their ability to reduce it is evident among the strains tested. Our results also suggest that hydrogenase plays an important role in U(VI) reduction. Initial studies show extracelluar reduction of Fe (III) to Fe (II) and U(VI) to U(IV) by *Clostridium* sp.

Molecular engineering to improve U(VI) remediation by *Clostridia* and *Pseudomonas* has dealt with two approaches. One is to look for reductases similar to our previously characterized ChrR proteins that are useful in increasing the bioremediation potential of *Clostridia*. A *C. acetobutylicum* quinone oxidoreductase was cloned in *Escherichia coli*, and the enzyme purified to homogeneity. The activity was lower than the other reductases we have examined. But since it can conceivably function better in *Clostridia*, which are valuable bacteria for bioremediation, further studies are in progress with this enzyme with respect to its overproduction in these bacteria and its potential for improvement by directed evolution and tests in *C. acetobutylicum*. Since we have already greatly improved ChrR enzymes (latest activity 258,336 nmole substrate converted/mg protein/min), we have focused major attention at removing permeability barriers in *P. putida*, so as to exploit the full potential of the improved activity of this enzyme in intact bacteria. Attempts have been made to over-express OprF, which is a major porin in this bacterium, as well as the sulfate transporter in the cytoplasmic membrane. There appeared to be problems with proper folding of the protein when over-expressed. An alternate approach is concerned with knocking out the gene *imp4213*, which plays an important role in the outer membrane permeability.

Mechanisms of Actinide Microbial Transformations

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The overall goal of this research is to determine the mechanisms involved in the microbial dissolution and stabilization of actinides. This includes investigating the fundamental aspects of microbially catalyzed radionuclide transformations (oxidation/reduction reactions, dissolution, precipitation, chelation).

Reductive dissolution of Pu(IV) to Pu(III). We determined the ability of *Clostridium* sp. to reduce Pu(IV). Analysis of Pu oxidation state by solvent extraction technique and X-ray absorption near edge structure (XANES) analysis confirmed that Pu(IV) was reduced to Pu(III); microfiltration (<0.03 □m) of the supernatant showed that the Pu remained in solution. The effect of direct and indirect mechanisms on biotransformation of Pu was determined in medium with and without cells. Although Pu(IV) can be solubilized to a limited extent by organic acid metabolites and low pH, dissolution of Pu in the presence of bacteria is primarily due to reduction of Pu(IV) to Pu(III).

Characterization and biodegradation of Pu-citrate. The molecular association of Pu(IV) with citric acid was determined by electrospray ionization-mass spectrometry (ESI-MS) and extended X-ray absorption fine structure (EXAFS) analyses. The molecular structures at pH 6 consist of a mixture of monoligand 1:1 Pu:citric acid complex; biligand 1:2 Pu:citric-acid complex $[PuO(cit)_2]^{4-}$ and a dimeric 2:2 Pu:citric acid complex. The mononuclear biligand complex was the predominant form present in solution. The rate and extent of citric acid metabolism by *Pseudomonas fluorescens* in the presence and absence of Pu(IV) was determined. Citric acid (10^{-4} M) in the absence of Pu was metabolized completely at a rate of 4.9 μM/h. With 10^{-6} and 10^{-8} M Pu, we observed a slight decline in the rate and extent of citrate degradation compared to the sample without Pu. In both samples, citrate was 96% degraded at the rate of 4.0 μM/h and 3.8 μM/h, respectively. Speciation of the Pu, following bacterial metabolism of citric acid using solvent extraction technique and microfiltration, indicated the Pu was present as colloidal Pu(IV) species.

Mobilization and immobilization of actinides from contaminated soil. Synchrotron scanning transmission X-ray microscopy analysis of a soil sample obtained from the Nevada Test Site showed that the Pu distribution is localized to a small area of the sample (~500 nm). The effect of indigenous bacterial activity on mobilization of Pu, Am, and U in the soil under both aerobic and anaerobic conditions was observed. In the presence of glucose, there was a gradual increase in concentration for all actinide species in solution. However, in the presence of citric acid, an initial increase in actinide in solution was observed, followed by its rapid precipitation. This decrease was concomitant with metabolism of the citric acid. These results show that under appropriate conditions, bacteria play a significant role in regulating the mobility of actinides.

Biogeochemical Mechanisms Controlling Reduced Radionuclide Particle Properties and Stability

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Uranium and technetium are the major risk-driving contaminants at Hanford and other DOE sites. These radionuclides have been shown to be reduced by dissimilatory metal-reducing bacteria (DMRB) under anoxic conditions. Laboratory studies have demonstrated that reduction results in the formation of poorly soluble hydrous oxides, $UO_{2(s)}$ and $TcO_2 \cdot nH_2O_{(s)}$, that are believed to limit mobility of these radionuclides in the environment. The mechanisms of microbial reduction of U and Tc have been the focus of considerable research in the Environmental Remediation Sciences Program (ERSP). However, relatively little is known regarding the precipitation mechanism(s), reactivity, persistence, and transport of biogenic $UO_{2(s)}$ and $TcO_{2(s)}$. The goal of this research project is to elucidate the principal biological and geochemical mechanisms that govern the biomineralization and reactivity of these redox reactive contaminants.

Shewanella oneidensis MR-1 *c*-type cytochromes were shown to be essential for the reduction of U(VI) and formation of extracellular UO_2 nanoparticles. In particular, an outer membrane (OM) decaheme cytochrome was confirmed to be capable of directly transferring electrons to U(VI), as determined by *in vitro* assay with purified protein. Additionally, deletions of OM cytochromes (OMCs) significantly affected the *in vivo* U(VI) reduction rate relative to wild type MR-1. Biogenic UO_2 nanoparticles accumulated extracellularly to high densities in association with a complex exopolymeric substance (EPS) that contained multiple elements of the OM, polysaccharide, and heme-containing proteins. Analysis of MR-1 strains containing mutations in putative polysaccharide biosynthesis genes suggested that some mutants containing altered polysaccharide (exo- and/or lipopolysaccharide) had significantly different rates of U(VI) reduction than MR-1. Subcellular localization studies using these mutants indicated that differences also exist in the formation of EPS-associated UO_2 nanoparticles (UO_2-EPS) relative to the wild type. These studies indicate that while EPS production is not required for U(VI) reduction, it could influence the formation of UO_2-EPS structures and subsequently affect the environmental behavior of the biogenic $UO_{2(s)}$ nanoparticles.

In contrast to U(VI), the microbial reduction of $Tc(VII)O_4^-$ is generally considered to be catalyzed by hydrogenase. Owing to our recent discovery of metal-reducing *Shewanella* in Hanford Reach Columbia River sediments, where ^{99}Tc is predicted to migrate into in the future, we investigated the role of putative MR-1 hydrogenases in $Tc(VII)O_4^-$ reduction. As suggested by our results, the NiFe hydrogenase was involved in the H_2-driven reduction of $Tc(VII)O_4^-$ presumably through a direct coupling of H_2 oxidation and Tc(VII) reduction. Interestingly, the deletion of both hydrogenases did not completely abolish the cells ability to transfer electrons to Tc(VII), suggesting an alternative mechanism of electron transfer to Tc(VII) was present in MR-1. To investigate this possibility, we used a combination of genetics, analytical TEM, and *in vitro* assays with purified cytochromes to deduce that the OMCs, MtrC and OmcA, catalyzed the reduction of $Tc(VII)O_4^-$ to nanoparticulate $TcO_2 \cdot nH_2O_{(s)}$ with lactate as the electron donor. These studies represent important steps towards determining the principal biological and geochemical mechanisms that govern the biomineralization and reactivity of these redox reactive contaminants.

Investigating Ultrasonic Diffraction Grating Spectroscopy and Reflection Techniques for Characterizing Slurry Properties

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Our objectives in this work are to: (1) investigate the new technique of ultrasonic diffraction grating spectroscopy (UDGS) for the measurement of particle size and to develop a method for measurement on-line and in real time; and (2) investigate the reflection of an ultrasonic shear wave for the measurement of viscosity for on-line real-time measurement.

In recent results, the ultrasonic beam produced by the "send" transducer travels through the solid and strikes the back of the grating, with the front of the grating in contact with the slurry. The angle of the transmitted beam increases as the frequency of the beam decreases. The frequency at which it reaches 90° is called the critical frequency. Slightly below the critical frequency, the existence of a transmitted beam is not possible, and the energy is transferred to other beams, such as that reflected to the "receive" transducer, where the signal shows a peak at the critical frequency. During this transition at the critical frequency, the ultrasound interacts with particles in the slurry. The peak in the receive transducer monitors this interaction, and the peak height varies according to the particle size. Data have been compared with two theoretical models: an inertial model and a scattering model. Results have been published (*Ultrasonics 44* [2006] 1385–1393), and a manuscript descibing nine diameters of polystyrene spheres for transmitted spectral orders m = 1 and m = 2 is ready to be submitted for publication.

Some important results that will be presented:

(1) Measurement of two peaks yields the particle size and the concentration of the slurry.
(2) The velocity of sound in the slurry is determined from the critical frequency.
(3) A peak height calibration can be used for determiningthe weight percent solids in a slurry.
(4) The data for some particle sizes compare well with the inertial model, and other sizes with the scattering model. Thus, the inertial model can be investigated *without* the observation of scattering. Such studies can probe the basic interaction of ultrasound with particles and lead to a greater understanding of attenuation measurements.
(5) Results using solutions of differing viscosity and the possibility of a sensor will be presented.
(6) The research for viscosity measurements has been published in *Ultrasonics* (*44*, 2006).

Future work includes research with gratings having critical frequencies of 13 MHz and 20 MHz, investigation of a new method for increasing particle size sensitivity, and using glass spheres for particles in addition to polystyrene spheres.

Field-Integrated Studies of Long-Term Sustainability of Chromium Bioreduction at Hanford 100H Site

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The overall objectives of this project are: (1) to investigate coupled hydraulic, geochemical, and microbial processes affecting Cr(VI) immobilization and its transformation into nontoxic Cr(III) complexes in groundwater, using an injection of a slow-release polylactate compound (HRC™), and (2) to determine the biogeochemical conditions needed to minimize Cr(III) reoxidation. To demonstrate the feasibility of a cost-effective remediation technology for bioimmobilization of Cr(VI) in groundwater, we have conducted a series of bench-scale and field-scale treatability studies. A three-well system, comprised of an injection well, an upgradient monitoring well, and a downgradient monitoring well, was used for conducting *in situ* biostimulation, one regional flow (no-pumping) Br-tracer test, and five pumping tests, along with a Br-tracer injection. Field measurements were conducted using a multiparameter flow cell to collect hourly data on Br concentration, temperature, pH, redox potential, electrical conductivity, and dissolved oxygen (DO). Groundwater sampling was conducted by pumping through specially designed borehole water samplers. Cross-borehole radar and seismic measurements were carried out to assess the site background lithological heterogeneity and the migration pathways of HRC byproducts through groundwater after the HRC injection.

In August 2004, HRC was injected into Hanford sediments (over a depth interval from 44 ft to 50 ft) to stimulate immobilization of Cr(VI). Within the next 2–3 weeks, the HRC injection induced a 2-order-of-magnitude increase in biomass—up to more than 10^7 cells ml^{-1}, which remained high for almost 2 months before dropping to background conditions. Using sediment samples, we determined the presence of several types of bacteria, including *Bacillus/Arthrobacter and Geobacter* species. These bacteria are known to withstand high concentrations of heavy metals, metabolize recalcitrant chlorinated compounds, and reduce or sorb hexavalent chromium. The HRC injection induced the onset of reducing biogeochemical conditions—redox potential decreased from +240 to -130 mV, and DO was completely removed. We have found that the HRC breakdown products caused the microbial population to sequentially deplete oxygen, nitrate, iron(III) sulfate (SO_4 was reduced, but never completely depleted), and other competing electron acceptors needed for the transformation of Cr(VI) species to Cr(III) species precipitated on soil particle surfaces. Sulfate and iron microbial reducers apparently maintain Cr(VI) reduction below the drinking water standards in the injection well for more than 2.5 years. Cr(VI) concentration in the monitoring well decreased below drinking water MCL and remained at this level for about 2 years, and remained below upgradient (background) concentration. It is very likely that some HRC or its byproducts still remain in the area surrounding the injection well.

Thus, we have shown that under field conditions at the Hanford 100H site, a single application of HRC led to enhancing Cr(VI) immobilization using naturally occurring microorganisms. Adding HRC to a contaminated aquifer may be a low cost and effective approach to treating Cr(VI)-contaminated aquifers at Hanford and other DOE sites. Ongoing research is focused on a comprehensive data analysis, continuation of field observations in existing and new boreholes, and the development of a 3D reactive transport code, TOUGHREACT-BIO, to simulate coupled biological and geochemical processes.

Spectroelectrochemical Sensor for Pertechnetate Applicable to Hanford and Other DOE Sites

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The general aim of our work currently funded by DOE is the design and implementation of a new sensor technology that offers unprecedented levels of specificity, needed for analysis of the complex chemical mixtures found at DOE sites nationwide. This project involves a very successful collaboration between scientists at the University of Cincinnati (UC) and several at the Pacific Northwest National Laboratory (PNNL) and the Environmental Molecular Sciences Laboratory (EMSL). The goal of the work is the continued development of a sensor for ^{99}Tc that is applicable for characterizing and monitoring the vadose zone and associated groundwater. The single focus is pertechnetate, TcO_4^-, which is considered to be the dominant species in the vadose zone and ground water. The sensor will have the capability for on-site monitoring, either by immersion in subsurface water for continuous monitoring, or for the immediate analysis of collected samples. The project will build on the substantial progress of a well-established UC-PNNL collaboration that provides the wide range of expertise needed for success: spectroscopy, electrochemistry, device fabrication, thin film technology, synthetic inorganic chemistry, experience with Tc, and facilities for handling radioactive isotopes.

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

- Fluorescence offers a means of dramatically increasing the sensitivity of the spectroelectrochemical sensor, and we have demonstrated a limit of detection ($< 10^{-12}$ M) that is 100 times lower than that needed for pertechnetate.
- TcO_4^- preconcentrates in sensor films containing anion exchange polymers and can be electrochemically reduced. This is the first step in operation of a spectroelectrochemical sensor for TcO_4^-.
- Prepared lower oxidation-state Tc-complexes fluoresce at ambient temperatures in sensor films, and these complexes exhibit reversible redox processes leading to fluorescence modulation, which is the second step in operation of a spectroelectrochemical sensor.
- The spectroelectrochemical sensor and associated instrumentation for either absorbance or fluorescence modes are portable and easily transported to and used at DOE sites.

Our continued work incorporates three specific tasks:

Task 1, Refinement of the chemically selective reagent-containing sensor film for TcO_4^- sensing
Task 2, Development of the TcO_4^- reductive trapping mechanism
Task 3, Development and evaluation of prototype sensors.

Tasks 1 and 2 will pave the way for development of prototype sensors and associated instrumentation. These will be used to evaluate and improve critical performance characteristics, such as limit of detection, range, response time, and reversibility. Optimized prototypes will be used to demonstrate sensor performance on TcO_4^- standards and samples from the vadose zone and subsurface water at the Hanford Site.

Biogeochemical Cycling and Environmental Stability of Pu Relevant to Long-Term Stewardship of DOE Sites

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Central to understanding the environmental behavior of Pu in vadose- and saturated-zones, as well as waste streams, is the contribution of microbial communities to Pu speciation. This research addresses the principal mechanisms by which naturally occurring microbial communities regulate transformations in Pu chemical speciation; such changes may lead to either enhanced Pu immobilization or its release from immobile phases and subsequent transport.

This year, the research group examined the ability of natural organic matter or extracellular polymeric substances (EPS) to: (1) reduce Pu(V) to Pu(IV); (2) enhance the sorption of Pu to particles or colloids; (3) affect the rate of sorption of Pu (Pu(V) v. Pu(IV)) in batch systems.

The relative hydrophobicity of EPS was investigated for its hydrophobic contact area (HCA) using HIC-HPLC. Results show a significant decrease of HCA for the case in which the protein content of the EPS was significantly reduced through proteinase and pronase treatment. Results suggest that the effect of EPS on Pu immobilization by particle surfaces depends not only on the sequence of addition for the system component, but also the biochemistry of the EPS.

Another primary effort this year was the continued investigation of the role of indigenous soil bacteria to alter the physico-chemical state of ^{239,240}Pu that has been resident in Rocky Flats, Colorado, soils for ~30 years. Batch incubations of Rocky Flats soils with glucose show that Pu availability and mobility can be associated with transformations occurring to soil iron minerals (e.g., ferrihydrite to siderite). In contrast, static columns filled with Rocky Flats soils showed little enhancement of ^{239,240}Pu mobility; this result, relative to the batch incubations, is likely caused by the buffering ability of the soils and the resistance to iron phase transformations. In contrast, ^{241}Pu, added as a tracer for isotope dilution, showed increased Pu solubility upon incubation.

The evaluation of Pu(IV) complexation by selected bacterial exudates shows that Pu complexation decreases in the order *P. fluorescens* > *S. putrefaciens* > *Clostridium sp.* Static column experiments (e.g., 2E-11 M Pu (IV), 2640 g/L sand, pH = 8, I = 0.01 M NaCl, 40 mg / L EPS) have shown that the complexation of Pu with EPS prior to injection into the column substantially decreases Pu sorption and retardation relative to systems not precomplexed. In addition, when EPS solutions (40 mg/L EPS) were brought into contact with Pu (IV) sorbed to the mineral surface for an extended period, between 4% and 18 % of the sorbed Pu (IV) could be remobilized. The extent of remobilization is dependent on both system history and kinetics. Ultrafiltration of effluent samples (3 kDa MWCO) indicates that remobilized Pu (IV) was primarily found in the form of Pu-EPS complexes.

Multiscale Characterization and Prediction of Coupled Subsurface Biogeochemical-Hydrological Processes

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Our research objectives are to advance solutions for remediation of DOE contaminated sites. Approaches are needed that can elucidate and predict reactions associated with coupled biological, geochemical, and hydrological processes over a variety of spatial scales and in heterogeneous environments. Our goals are to explore and develop the use of geophysical methods (complex electrical, SP, radar, and seismic) to obtain quantitative estimates of the end products associated with iron reduction and sulfate reduction, and to combine such advanced monitoring information with reactive transport models to improve the understanding and predictability of associated subsurface transformations. Our research is geared toward the Rifle, Colorado, IFC site, where the interplay between iron and sulfate reduction is believed to be of critical importance to the sustainable reduction of U(VI).

Our research includes numerical, experimental, and theoretical approaches, and has focused in this second year of our project on four different components. The first key component includes exploring the spatiotemporal change in geophysical responses to the onset and evolution of transformations associated with iron and sulfate reduction, as well as with reoxidation processes. To date, comparison of synchronous geophysical and biogeochemical measurements, collected at both the laboratory and the field scales during biostimulation experiments, have illustrated the potential of complex resistivity and spontaneous potential measurements for tracking microbially mediated sulfide production.

The second component of the project that we have advanced this year is the development of the petrophysical relationships and estimation framework needed to use these geophysical responses to quantitatively estimate biogeochemical properties or processes. We have explored the use of a modified Cole-Cole and double-porosity model for relating the IP and seismic attributes, respectively, to properties of sulfide production, and we have developed a stochastic framework for estimation and evolution of geochemical properties using the time-lapse geophysical datasets and the petrophysical models. We have used the developed framework to estimate the mean grain size and volume of evolved precipitates over space and time associated with a sulfate reduction biostimulation column experiment.

Simulations of transport associated with the same column experiment have been performed as the third key component of our project, and suggest that inclusion of certain processes, such as chemotaxis, are important for predicting the system behavior. Comparison of the results obtained using the biogeophysical framework and the transport simulations provide important insights that are being used to improve both approaches.

A final component of our work this year has focused on the field-scale subsurface characterization of the galleries associated with the Rifle 2004 and 2005 flow cells using integrated hydrogeological and geophysical datasets. This characterization will provide the baseline information needed to parameterize the field-scale reactive transport model, and as such provides the first step in our field-scale effort to both predict and remotely monitor complex subsurface transformations associated with U(VI) bioreduction at the Rifle Site.

Stability of U(VI) and Tc(VII) Reducing Microbial Communities to Environmental Perturbation: Development and Testing of a Thermodynamic Network Model

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The overall goal of this project is to develop and test a thermodynamic network model for predicting how substrate additions and environmental perturbations affect the composition and functional stability of subsurface microbial communities. The overall scientific hypothesis is that a thermodynamic analysis of the energy-yielding reactions performed by broadly defined groups of microorganisms can be used to make quantitative and testable predictions of the change in microbial community composition and system geochemistry (including contaminant chemistry) that occur when a substrate is added to the subsurface or when environmental conditions change. A list of the major microbial groups responsible for observed microbial activity in published experiments has been compiled. These include major microbial processes (e.g., aerobic respiration and denitrification) as well as those involved in contaminant transformation (e.g., U(VI) and Tc(VII) reduction). The output from this activity is a thermodynamic database containing the chemical stoichiometry and standard state free energy change that defines the growth of each group (e.g., denitrifiers, iron reducers). The thermodynamic database is combined with existing geochemical data and used to predict equilibrium reaction paths that show the coupled changes in microbial community composition and system geochemistry that occur when amendments are added to the subsurface.

Simulations are being performed to investigate the effect of ethanol additions on uranium and technetium bioimmobilization for the major subsurface environments at the ERSD Field Research Center (FRC) in Oak Ridge, Tennessee (Areas 1 and 3 with neutral pH and low nitrate groundwater; and Area 2 with low pH and high nitrate groundwater); at Old Rifle, Colorado; and at Hanford 100 H in Washington. Inputs include measured chemical quantities on sediment and groundwater; outputs include predicted changes in groundwater and sediment chemistry and microbial community composition. Model predictions have been consistent with laboratory and field observations and provide important insights into the role of specific microbial groups (esp. denitrifiers) on overall system response. Model predictions for long-term experiments in small-scale microcosms and intermediate-scale physical models, and for short-term small-scale field experiments, are being compared with biomarker data collected on groundwater and sediment samples by our group and others. Initial comparisons are made on total biomass and groups with known functional genes. Particular emphasis is placed on denitrifiers, sulfate reducers, and iron reducers, because these make up the largest portion (> 90% in some cases) of the entire community following substrate addition. We are actively collaborating with several ERSP investigators to apply model simulations to other experimental systems.

This project is clearly showing that the ability to predict the effects of donor addition on change in microbial community composition is essential for creating conditions that favor the long-term stability of bioreduced contaminants. The longer-term significance of this project will be to provide a comprehensive theoretical framework for designing and interpreting complex field experiments and to aid in "bridging-the-gap" between basic research and field applications**.**

Reduction and Reoxidation of Soils During and After Uranium Bioremediation

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This research focuses on the remobilization of uranium by different oxidants—dissolved oxygen, nitrate, and Fe(III)—after U has been precipitated biologically, and the role of reduced iron phases, FeS, and biomass in its long-term stability within groundwater after the injection of an electron donor has been discontinued.

The research focused on long-term sediment column experiments involving the biological removal of uranium from groundwater under low (9 μM) and high (6 mM) sulfate concentrations and subsequent reoxidation. Aquifer sediment was collected from the background area of the Old Rifle UMTRA site in Colorado, and then dried and sieved (<2 mm) before being packed into 15 cm long × 5 cm diameter columns. Each column was supplied a feed media containing 30 mM bicarbonate, 20 μM U(VI) (as uranyl acetate), 3 mM acetate, (as well as other trace nutrients), either 9 μM or 6 mM sulfate, and purged with CO_2/N_2 gas (20:80). Biostimulation was facilitated by the addition of the Fe(III)- and U(VI)-reducing microorganism, *Geobacter metallireducens*.

Significant U(VI) reduction occurred upon acetate addition in all column experiments. *In situ* X-ray Absorption Near Edge Structure (XANES) spectroscopy performed on a flowing column confirmed that the majority (>90%) of the uranium was present as U(IV). Iron and sulfate reduction occurred concurrently with U(VI) reduction. Though sulfate reduction was detected in all experiments, increasing the sulfate concentration from 9 μM to 6 mM changed the system from Fe(III)/methanogenic-dominated to sulfate-dominated reducing conditions. No difference in the U(VI) removal rate was detected for the low and high sulfate concentration experiments during the 70-day bioreduction experiments. In the low sulfate columns, the majority of the uranium precipitated within the first third of the column, whereas the Fe(II) accumulation occurred toward the end of the column. Mössbauer spectroscopy is currently being performed to determine which Fe(III) phases were reduced during biostimulation.

The columns were reoxidized by discontinuing the supply of acetate and either replacing the influent anaerobic gas with a gas mixture containing 20% oxygen or adding 1.6 mM nitrate to the influent media. In the low sulfate columns, both oxygen and nitrate resolubilized the majority (88% and 97%, respectively) of the uranium precipitated during bioreduction within 54 days. *In situ* XANES analysis performed on flowing-column-supplied oxygen showed that within the reach of the oxygen front, U(IV) in the sediment was transformed to U(VI) on the order of minutes. Nitrate-dependant uranium oxidation occurred significantly faster than oxygen-dependant uranium oxidation, which was attributed to the higher reactivity of oxygen with other reduced compounds. Uranium remobilization was significantly slower in the high sulfate columns, suggesting that, under these conditions, a combination of $Fe(III)/SO_4^{2-}$ reducing conditions was more successful in preventing U(IV) resolubilization during short-term oxic conditions, without compromising the rate of U(VI) bioreduction during biostimulation.

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

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In this project, lysimeter studies had previously been conducted to identify the controlling chemical processes influencing Pu(IV) mobility through the vadose zone. Five 52-L lysimeters filled with sediment collected from the Savannah River Site (SRS) were amended with well-characterized, solid Pu sources and left exposed to natural precipitation for 2 to 11 years. Pu-XANES analysis conducted on $Pu^{IV}(NO_3)_4$ and $Pu^{III}Cl_3$ amended lysimeters sediments (a red clayey sediment, pH = 6.3) recovered at the end of the study contained essentially identical Pu distributions: approximately 37% Pu(III), 67% Pu(IV), 0% Pu(V), and 0% Pu(VI). All three Pu(III) and Pu (IV) lysimeters also had near-identical sediment Pu concentration profiles, in which >95% of the Pu remained within 1.25 cm of the source after 11 years (the other 5% of Pu moved at an overall rate of 0.9 cm yr^{-1}). As expected, Pu moved more rapidly through the Pu(VI) lysimeter, at an overall rate of 12.5 cm yr^{-1}. Solute transport modeling of the sediment Pu concentration profile data in the Pu(VI) lysimeter indicated that some transformation of Pu into a much less mobile form, presumably Pu(IV), had occurred during the course of the two year study. This modeling also supported previous laboratory measurements showing that Pu(V) or Pu(VI) reduction was five orders of magnitude faster than corresponding Pu(III) or Pu(IV) oxidation. The slow oxidation rate (1×10^{-8} hr^{-1}) was not discernable from the Pu(VI) lysimeter data that reflected only two years of transport, but was readily discernable from the Pu(III) and Pu(IV) lysimeter data that reflected 11 years of transport.

Although plutonium mobility was very limited, its mobility was greatly influenced by resolubilization under oxidizing conditions. The mechanism for this resolubilization is not known. The hypothesis governing future work is that Pu transport through the vadose zone is controlled by its oxidation state, which in turn is controlled by biogeochemical processes closely related to fluctuations in pore-water level and composition. This hypothesis suggests that Pu remains largely immobile most of the time—except for short but very important periods when essentially all of the transport occurs in the oxidized form. Thus, Pu mobility consists of a series of very short bursts of rapid mobility, interspersed by much longer periods during which the sediment naturally immobilizes the Pu through reductive precipitation.

Going forward, the overall objectives of this proposal are to (1) identify and quantify the abiotic processes responsible for Pu mobilization under vadose zone conditions and evaluate the likelihood that biological processes contribute to Pu mobilization in the vadose zone; and (2) develop a fully transient vadose zone model to describe Pu transport and calibrate it to existing 2- or 11-year vadose zone data. The approach will involve the use of well-characterized sediments that have been in contact with Pu for 24-years and expose these sediments to various unsaturated moisture and chemical regimes, including steady-state and wet-dry cycling. Pu oxidation states will be determined by XAS and a solvent extraction method; Fe-mineralogy by Mössbauer and TEM; and microbial community structure by 16S-rRNA based qPCR. The benefit of this work to DOE is that it will: (1) reduce the uncertainty and therefore the cost associated with Pu remediation and long-term stewardship, by improving the accuracy of the presently used models; and (2) identify the range of vadose zone conditions under which Pu desorption may be expected to occur and represent them in a transport model designed to represent long-term processes.

Monitoring Uranium Transformations Determined by the Evolution of Biogeochemical Processes

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Researchers have commonly assumed that reduction of U(VI) to U(IV) under anaerobic conditions was sufficient to sequester uranium in the subsurface as uraninite, due to the low solubility of uraninite and ease of its formation in simple laboratory experiments. Using X-ray absorption spectroscopy (XAS), however, our group and others have shown that products of biostimulation are more complicated than previously thought: We have observed that as the complexity of the system increases, the nature of the products becomes increasingly difficult to predict. Another method for reducing the complexity in natural sediments is to make measurements on length scales that are smaller than that of the natural heterogeneity. The objective of this proposal is to develop novel X-ray based techniques to monitor contaminant transformations during the biogeochemical evolution of the highly heterogeneous subsurface material at the ERSD Field Research Center (FRC), Oak Ridge. These measurements will give us "eyes" into the sediments, so that we can observe the local atomic distribution around U change. We will use static microcosms (SMs) as our system by which to monitor U transformations in a consistent sample volume during the evolution of biogeochemical processes. This system resembles field conditions in that mass transfer is dominated by diffusion.

The specific hypotheses of the proposed research project are as follows: (1) X-ray monitoring of U transformations can be accomplished in consistent, unique regions of SMs, throughout the depth of the sediment, while the anoxic integrity is maintained and system integrity is preserved for up to three years. (2) Field-relevant biogeochemical processes result in U transformations (changes in U valence state and U speciation) within SMs; our X-ray measurements can monitor these transformations. (3) The dominant biogeochemical processes in SMs composed of field-relevant materials depend on incubation time and the bicarbonate levels in the system. Low levels of bioreducing activity over longer periods of time will lead to more stable forms of immobilized U. The presence of Fe(II) is a marker for the development of bioreducing conditions and an indicator of the valence state and speciation of U. We will apply microprobe X-ray fluorescence (μXRF) to map the spatial distribution of key elements, and microprobe XAS (μXAS) to monitor changes in U valence state and U speciation as they occur in an intact, undisturbed microcosm. This work will enable identification and a basic scientific understanding of the dominant biogeochemical mechanisms affecting the chemical transformations of U. These mechanisms greatly influence U immobilization and/or remobilization in the subsurface.

Ecological Interactions Between Metals and Microbes That Impact Bioremediation

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Our objectives in this project are to (1) determine the distribution of phylotypes and metal-resistance genes at the scale of spatial heterogeneity observed in microbial community activity; (2) determine the environmental effects on community responses to Cr(VI) contamination; (3) determine the role of mobile elements that confer Cr resistance; and (4) identify the novel physiological and genetic bases for bacterial resistance to Cr(VI).

Arthrobacter sp. FB24 was isolated from soils contaminated with metals (Cr and Pb) and aromatic hydrocarbons. This bacterium is most notable for its resistance to extreme concentrations of Cr(VI) (200 mM). The genome of strain FB24 has recently been sequenced and analyzed. A cluster of putative Cr resistance genes is located on a 96kb plasmid. More detailed research has been conducted employing a proteomics approach. Whole cell protein extracts were prepared from log-phase cells grown in 0.2X nutrient broth amended with 0 mM, 5 mM, or 20 mM Cr(VI). Using two-dimensional gel electrophoresis, approximately 700–800 proteins were detected. Gel comparisons revealed up-regulation of 5 proteins and down-regulation of 6 proteins with the 5 mM Cr samples; whereas 9 proteins were up-regulated and 10 were down-regulated in 20 mM Cr. Of these, 4 proteins were differentially expressed in both conditions. Several spots were excised from replicate gels and identified by tandem mass spectrometry. Two of the proteins down-regulated in both Cr conditions were phosphoenolpyruvate (PEP) carboxykinase and transketolase. Glycerol kinase, which serves in both lipid biosynthesis and as a means for glycerol to enter glycolysis, was up-regulated in both Cr conditions. Cystathionine beta-synthase was up-regulated in 20 mM Cr. This protein functions in cysteine biosynthesis, sulfur metabolism, and the formation of acetate, which can enter the tricarboxylic acid cycle. Two other proteins had similarities to genes of unknown function. The down-regulation of proteins involved in carbon metabolism, and the up-regulation of proteins that may offer alternative energy sources, are consistent with the growth aberrations when *Arthrobacter* sp. FB24 is grown in increasing concentrations of Cr.

A chromate sensitive mutant (D11) was obtained by successive culturing in nonselective media, and this strain was shown to have lost plasmid-borne putative chromate resistance genes by both PCR and Southern hybridization analyses. These genes include ChrB (40% similarity), two chromate ion transporters (51% and 53% similarity to published ChrA), and a probable Cr-resistance signal peptide (34% similarity). When exposed to Cr(VI), the Cr-sensitive mutant accumulated three times as much Cr(VI) as the wild type, consistent with the operation of an efflux pump in conferring Cr-resistance. Wild-type levels of Cr-resistance were recovered in the mutant by mating with wild-type FB24. At 10.6 kb region the FB24 megaplasmid that contains the putative Cr-resistance genes was cloned into an *Arthrobacter* plasmid and introduced into Cr-sensitive strain D11. Resistance to Cr(VI) concentrations up to 5 mM was restored. Real-time reverse transcriptase PCR was used to assess gene expression as a function of chromium concentration. The putative Cr-resistance genes showed a dose-dependent response, with 8- to 100-fold increases in expression relative to cells not exposed to Cr(VI).

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

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The overall goal of the proposed project is to closely couple cutting-edge microbiological and biogeochemical approaches to provide a mechanistic understanding of the functioning of subsurface microbial communities with a high bioremediation potential. The focus will be on microbial populations (metal- and nitrate-reducing prokaryotes) that mediate electron flow in subsurface sediments, thereby controlling the transport and transformation of radionuclides. For acidic subsurface sediments exposed to mixed contaminants, such as those at the ERSD Field Research Center at Oak Ridge, Tennessee, there is as yet no consensus on the predominant, "metabolically active" community members that catalyze radionuclide immobilization *in situ* or the distribution of active community members across changes in the relevant environmental parameters likely to control bioremediation potential.

The proposed research will: (1) isolate and characterize novel anaerobic prokaryotes from subsurface environments exposed to high levels of mixed contaminants (U(VI), nitrate, sulfate), (2) elucidate the diversity and distribution of metabolically active metal- and nitrate-reducing prokaryotes in subsurface sediments, and (3) determine the biotic and abiotic mechanisms linking electron transport processes (nitrate, Fe(III), and sulfate reduction) to radionuclide reduction and immobilization. Mechanisms of electron transport and U(VI) transformation will be examined under near *in situ* conditions in sediment microcosms, flow-through columns, and in field investigations. We will employ new cultivation insights along with state-of-the-art high-throughput techniques (e.g., microcapsules and flow cytometry) to isolate novel microorganisms. The activity or function of subsurface microbial communities will be directly linked to their phylogenetic structure, using stable isotope probing and message RNA analysis of structural and functional genetic markers. Population dynamics of the metabolically active microbial groups will be monitored in laboratory and field experiments where physicochemical conditions (pH, redox) and terminal electron-accepting processes (TEAPs) are manipulated. Pathways of nitrogen transformation will be quantified using a state-of-the-art combination of dissolved gas analysis by membrane inlet mass spectrometry (MIMS) and stable nitrogen isotope analysis. The transformation of Fe(III) minerals will be characterized using a suite of physical and wet chemical methods, including variable temperature Mössbauer spectroscopy. Through determination of the microbially mediated reaction mechanisms under near *in situ* conditions, the proposed project will provide important inputs, in the form of rate measurements and abundance of metabolically active microbial groups, for reactive transport models that predict the fate of radionuclides in the contaminated subsurface of DOE sites. Therefore, the project will deliver on the basic science to drive long-term stewardship and site closure.

Soil-Bacterial-Community Dynamics in the Presence of Plutonium and Uranium

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Our objectives were to compare the effects of Pu and U at low concentrations on soil bacterial communities, and to identify active bacterial species in the presence of actinide. Natural soil species found to be resistant to Pu toxicity, that are active in the presence of the actinide, are candidates for further study of actinide/bacterial interactions. We conducted short-term, replicated laboratory time course experiments where soil was exposed to Pu or U, at actinide concentrations spanning 10^{-3}M to 10^{-7}M, and incubated under aerobic or anaerobic conditions. A suite of DNA- and RNA-based methods were used to monitor changes in the bacterial community. These biological measures were coupled with analysis of actinide speciation and partitioning in soil/aqueous phases.

In results this past year, microbial biomass was reduced 10–20 fold in soils exposed to 10^{-3} and 10^{-4} M Pu(VI)HCl, but no reduction in biomass was observed at lower Pu concentrations. Biomass reduction was not observed in soils treated with U(VI)HCl. The addition of Pu(VI)HCl to soil at 10^{-7} M had little observable effect on the composition of the bacterial community. Concentrations between 10^{-6} and 10^{-4} M caused shifts in the bacterial community after a 2-week incubation. At concentrations in which overall biomass was not affected, the most pronounced effect on community composition appeared to be caused by the chloride anion rather than the actinide. Over and above the effects of chloride, the bacterial community also shifted in response to the Pu, with a final composition dominated by several alpha-Proteobacterial species. DNA and RNA-based analysis of the 16S rRNA gene from the same soil samples provided very different images of the bacterial community during Pu exposure. Clone/sequence libraries of the total bacterial community only rarely detected members of the *Geobacteriaceae*, *Desulfotomaculum* lineage 1, or *Shewanella* species groups, in any of the actinide-treated or control samples. In a direct survey for these groups with specific primers using quantitative PCR, we found that 79% of the samples were positive for *Desulfotomaculum* lineage 1. At concentrations of 10^{-4} or 10^{-5} M, only 1.5 to 3.3% of the Pu added as Pu(VI)HCl at pH 4.0 or as soluble Pu(VI) hydroxides at pH 7.0, remained in the soil solution after 2 days and did not change over 2 weeks. In all treatments, the pH of the soil solution rapidly returned to 6.5–6.9 after Pu addition. The Pu(VI) form could not be detected in the soil solution or in Pu desorbed from the soil matrix, suggesting that the available Pu had been reduced to lower oxidation states.

Taken together, our soil studies suggest that Pu(VI)HCl caused a general toxicity response at concentrations above 10^{-4} M Pu. Most of the actinide rapidly adsorbed (or precipitated) to the soil matrix, although it may still be bio-available. At subtoxic concentrations, the major bacterial community shift appeared due to the chloride counterion. Bacterial composition changed in the presence of low concentrations of Pu, but remained highly diverse.

Upscaling Pore-Scale Lattice-Boltzmann Simulations for Multicomponent Reactive Transport

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The objective of this work is to investigate the role of pore-scale heterogeneity on macro-scale continuum representations of reactive flows in porous media. Volume averaged multicomponent reactive transport simulations, based on pore-scale lattice Boltzmann (LB) equations, are compared to single and multiscale continuum model simulations. Reactions included in the simulations involve both homogeneous aqueous complexing reactions and heterogeneous reactions with minerals. Heterogeneous reactions include mineral precipitation and dissolution, ion exchange, and surface complexation. These latter reactions are incorporated into the LB method through boundary conditions imposed at the mineral surface. Generally, it is found that sufficiently fast reaction rates lead to concentration gradients at the pore scale and thus are sensitive to the local velocity field. Simulations are carried out for a channel representing a fracture and more complex porous media. Results for the channel are compared with Generalized Taylor-Aris dispersion theory. Results for more complex structured media are compared with multiscale continuum model simulations. In general, we find that a multiscale continuum formulation is required to fit volume-averaged pore-scale results. However, in some cases, multiscale processes may be fit, using a single continuum model employing effective parameters that are not directly measurable. Provided sufficient resolution of the pore-scale geometry can be obtained, the pore-scale model can be used to determine the form of continuum formulation (single, dual, or multiple continua) that best fits the upscaled pore-scale simulation and, simultaneously, provide parameters needed for constitutive relations appearing in the multiscale continuum formulation. It is suggested that a multiscale continuum approach may help explain the observed discrepancy between laboratory and field-derived reaction rates, by explicitly representing distinct transport domains through separate interacting continua which could be responsible for the formation of preferential pathways. Finally, multiscale effects of some 3D realistic media are also analyzed.

Proteomic Characterization of the Changes in Community Structure and Dynamics Before and After Amendment at DOE-Relevant Sites

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Microbial function is dictated by the proteins expressed in an organism. While the limits of function are defined by the genetic makeup of an organism, the dynamic nature of the proteome directs the adaptation of the organism to its environment and confers actual function. Such is also the case on a broader scale in microbial communities, where microbes interact, compete, and act upon the changing environment. Thus, integral to the improvement of microbially mediated contaminant transformation at field sites is the in-depth characterization of the components of the communities, the activity and function of each of the components, and how they interact with each other. The application of proteomic techniques coupled with activity assays has the potential to deepen the understanding of microbial function as related to metal transformation. To this end, this proposal aims at leveraging current state-of-the-art proteomics capabilities at PNNL with ongoing work at the Hanford 100H site, located north of Richland, Washington, and the Uranium Millings Tailings Remedial Action (UMTRA) site in Rifle, Colorado—to characterize the changes in community structure and activity and related proteomic changes to specific genome changes and metal-reduction activities.

Stimulating microbial environments with amendments has the potential to be an effective strategy for the *in situ* remediation of contaminated sites. Such is the case at both the Old Rifle site and the Hanford 100H site. The main goal of work at these sites is to examine how groundwater amendments of organic acids affect the bioreduction of a particular metal. In both cases, the community structure in the groundwater changes after the amendment, though the specific functions of these communities and the components in them are not clearly understood. Leveraging existing projects at both sites aimed at defining the components of the community and microarrays on the metagenomic scale, this proposal will add depth of characterization by contributing the proteomic and biochemical metal assays.

The specific hypotheses center on the induced changes in the community structure and activity upon the addition of an amendment into the groundwater, and the characterization of these changes by high-throughput proteomic techniques and biochemical assays. Once the changes in community protein complement, before and after amendment, are determined, these changes can be linked back to specific biochemical activity of the community as a whole and will lend insight into the specific interactions between members of the community.

Influence of Microscopic Mass Transfer on Reactivity and Stability of Uranium(VI)

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Uranium at Hanford and other DOE sites has been in-ground for relatively long periods (i.e., 30 years or more at Hanford), allowing U(VI) migration toward and concentration in sediment regions that are influenced by slow reactions and diffusion processes. Recent characterization has revealed that sorbed U(VI) typically exists in complex, microscopic, intragrain domains in Hanford sediment. Sorbed U(VI) in intragrain domains desorbs/dissolves to undersaturated pore waters through a microscopic mass-transfer process, one that couples desorption/dissolution reactions that occur in intragrain regions where static water (and U) resides, with biogeochemical reactions that occur in water filled, intergrain spaces. The objective of this research is to investigate the microscopic mass transfer process under three biogeochemical scenarios: (1) aqueous complexation of U(VI) under conditions representative of monitored natural attenuation (MNA); (2) microbially induced reduction of aqueous U(VI) to U(IV) by direct enzymatic activity or biogenic reductants, such as Fe(II); and (3) U(VI) dissolution and reprecipitation by chemical ligands. The results will be used to: (1) evaluate the reactivity and stability of intragrain U(VI), (2) predict the future fate and reactive transport of U(VI); and (3) provide a scientific base for evaluation of MNA or selection of engineered remedial approaches.

This new ERSP project will investigate the uranium mass-transfer process by integrating microscopic and spectroscopic measurements, laboratory experiments (batch and flow-through reactors), and numerical modeling. Four sediments representative of different U(VI)-sediment associations at Hanford site will be used. One sediment contains uranyl-silicate precipitates within intragrain microfractures; a second contains U(VI) coprecipitated with calcite; a third contains micron-sized particles of metatorbernite [$Cu(UO_2)_2(PO_4)_2 \cdot 8(H_2O)$], or autunite-group minerals in secondary grain coatings; and the fourth contains adsorbed U(VI) in aggregates of clay-sized phyllosilicates. The research will use <2.0 mm sediment samples because U(VI) is concentrated in this size fraction (Zachara et al., 2005). Our study will begin with U(VI) desorption experiments in batch and flow-through reactors, using the contaminated sediments and aqueous solutions that are representative of different conditions at Hanford. The goal of these experiments is to determine the rate and extent of U(VI) release from the intragrain spaces under MNA and establish numerical models to describe coupled intragrain U(VI) desorption/ dissolution, mass transfer, and aqueous U(VI) speciation without complications from microbiogic activity and/or precipitation. Experiments will then be performed with bacterial cultures enriched from Hanford or PO_4^{3-}, to determine the rate and extent of U(VI) release from intragrain regions under the influence of microbiologic activity and ligand-promoted chemical precipitation, and to evaluate the subsequent reactivity and stability of U. Microscopic and spectroscopic characterizations of mineral phases, U speciation and distribution, associations with sediments, and numerical model development and simulations will be integrated in each of the investigations. Important sediment grains or minerals that control reactions and/or mass transfer will be isolated for better characterization, to determine(for example) solubility, diffusivity, and other mass-transfer properties.

Anaeromyxobacter spp. Catalyze Multiple Pathways That Affect Uranium Mobility

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The project goals are to characterize U(VI) reduction in *Anaeromyxobacter* spp. and evaluate their contributions to U(VI) immobilization. *Anaeromyxobacter* spp. are versatile delta-proteo-bacteria that couple the oxidation of lactate, acetate, formate, or hydrogen to a variety of electron acceptors, including metals and radionuclides, oxidized nitrogen species, oxygen, or ortho-substituted halophenols. The application of real-time quantitative PCR (qPCR) demonstrated that *Anaeromyxobacter* spp. grow at the expense of these electron acceptors, including U(VI). The growth yields with U(VI) as electron acceptor were lower than expected, based on the free-energy change associated with the U(VI)/U(IV) redox couple, suggesting that this reduction is inefficient or imposes an additional cost to growing cells.

Culture-independent, 16S rRNA gene-targeted methods demonstrated that *Anaeromyxobacter* spp. are distributed in the Oak Ridge Environmental Remediation Sciences Division Field Research Center (FRC) sediments, and that multiple *Anaeromyxobacter* strains coexist. Enrichment cultures established with FRC sediments yielded several U(VI)-reducing *Anaeromyxobacter* isolates (collaboration with J. Kostka, Florida State Univ.). Physiological tests with the *Anaeromyxobacter* isolates showed that ferric oxides and nitrate were reduced to ferrous iron and ammonium, respectively. Under electron-donor-limiting conditions, these cultures oxidized ferrous iron back to ferric iron. With some strains, the transient appearance of nitrous oxide (N_2O) was observed, which *Anaeromyxobacter* spp. use as an electron acceptor. These findings suggest that at least some *Anaeromyxobacter* spp. are capable of coupling ferrous iron oxidation to the reduction of oxidized nitrogen species. Microbes with such activity may influence the fate of U(VI), because nitrate and nitrite, which are co-contaminants at the FRC, contribute to U(IV) reoxidation, and ferric-oxides sequester uranium, owing to sorption processes. The quantitative analysis of the *Anaeromyxobacter* population at an FRC site test plot that received ethanol additions demonstrated that *Anaeromyxobacter* spp. respond to biostimulation, indicating that these organisms are active at the FRC. These findings suggest that native *Anaeromyxobacter* spp. are key players in catalyzing redox processes that directly and indirectly affect the fate of uranium at the FRC.

Sustained Removal of Uranium from Contaminated Groundwater Following Stimulation of Dissimilatory Metal Reduction: Potential Role for Biosorption

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A surprising development in the ongoing field studies at the Environmental Remediation Sciences Program study site in Rifle, Colorado, is that although the addition of acetate to the groundwater stimulated microbial reduction of U(VI) to U(IV), there is an additional, previously unexpected, mechanism for long-term removal of uranium from the groundwater. As reported previously, studies over four summers have repeatedly demonstrated that addition of acetate to the groundwater stimulates the growth of *Geobacter* species and the reduction of U(VI) to U(IV), effectively removing uranium from the groundwater. It was previously expected that when acetate amendments were stopped and oxygenated groundwater reentered the zone in which U(IV) had been precipitated, that the U(IV) would be reoxidized with the release of U(VI) back into the groundwater. However, when high concentrations of acetate were added to the groundwater, stimulating sulfate reduction as well as dissimilatory metal reduction and increased growth of microorganisms, then U(VI) continued to be removed from the groundwater, for up to 1.5 years following the time when the additions of acetate were stopped. This unexpected, continued long-term removal of U was replicated in sediments from the Rifle site incubated in flow-through columns. Speciation of uranium in the sediments revealed that, after the additions of acetate was stopped, U(VI) was not being removed via reduction of U(VI) to U(IV), but rather via adsorption of U(VI) to the sediments. The capacity for U(VI) adsorption was lost if the sediments were heat-sterilized either by autoclaving or with pasteurization. This suggested that intact microorganisms might be required for U(VI) adsorption. Analysis of 16S rRNA gene sequences in the sediments in which U(VI) was being adsorbed indicated that members of the *Mollicutes* were the predominant organisms, whereas no *Mollicutes* sequences were detected in background sediments that did not have the capacity to adsorb U(VI). This suggested that the U(VI) adsorption might be caused by the presence of these organisms.

To further evaluate this phenomenon, a comparative U(VI) adsorption study was performed. This study included *Geobacter uraniumreducens*, a species isolated from the Rifle site; *Desulfosporosinus meridiei*, which is representative of the sulfate reducers that predominate when sediment Fe(III) becomes depleted; and *Acholeplasma palmae*, a representative member of the *Mollicutes*. Cells were suspended in a salt buffer, at circumneutral pH, with uranium concentrations in the environmentally relevant range. Initial results demonstrated that *A. palmae* adsorbed U(VI) more rapidly than *G. uraniumreducens*. However, both organisms effectively adsorbed U(VI) within 4 hours. In contrast, *D. meridiei* had limited sorption capacity. Further pure culture studies are under way. Moreover, we will investigate whether, once adsorbed, the U(VI) can be reduced by other organisms if acetate is reintroduced into the Rifle sediments. This unexpected enhanced adsorption of U(VI) onto sediments following the stimulation of microbial growth in the subsurface may potentially enhance the cost effectiveness of *in situ* uranium bioremediation.

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

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A promising strategy for the *in situ* bioremediation of radioactive groundwater contaminants is to stimulate the activity of dissimilatory metal-reducing microorganisms to reductively precipitate the contaminants. Previous molecular analyses have clearly indicated that *Geobacteraceae* are the primary agents for metal reduction when dissimilatory metal reduction is stimulated in the subsurface, and that even when contaminant metal levels are high, electron transfer to insoluble Fe(III) oxides accounts for ~ 99% of the growth of the *Geobacteraceae*. When Fe(III) oxides are depleted, the growth and activity of *Geobacteraceae* stop, and U(VI) is no longer reduced. These results demonstrate that to design strategies to optimize *in situ* bioremediation of metals, it is imperative to understand how *Geobacteraceae* transfer electrons to insoluble Fe(III) oxides. Previous studies demonstrated that *Geobacter* species express pili on one side of the cell, specifically during growth on Fe(III) oxides. Deleting the gene for PilA, the structural pilin protein, inhibits Fe(III) oxide reduction, and atomic force microscopy analysis suggested that the pilin were electrically conductive. These results suggest that the *Geobacter* pilin function as microbial nanowires, serving as the final conduit for electron transfer between the cell and Fe(III) oxides.

To further evaluate the conductive properties of the pili, *Geobacter sulfurreducens* was grown on graphite electrodes that permit direct measurement of the extent of extracellular electron transfer. Deleting the *pilA* gene had no impact on current production when cells were maintained at low biomass levels, so that cells were in direct contact with the electrode surface. At high current levels, wild-type cells formed thick (>50 μm) biofilms. The *pilA* mutant could not. However, the pilin-deficient mutant formed thick biofilms on graphite surfaces when fumarate was provided as an electron acceptor. These results demonstrated that pilin are not required as a structural feature for biofilm formation and suggested that the requirements for pilin for high levels of current production were related to their electrical conductivity.

Further evidence for electrical conductivity through the biofilms was obtained in a split electrode system in which two gold electrodes were separated by a 50 μm nonconducting gap. Conductivity across the gap was characterized with AC spectral measurements, from 1 Hz to 100 kHz. Conductivity across the gap increased as the biofilms from the two electrodes merged and bridged the gap, further suggesting that the biofilms are conductive. Similar studies on the *pilA* mutant are under way. The conductivity of the pili are also being electronically evaluated by masking electrodes with a nonconductive surface with pores that prevent direct contact of the cells with the electrode, but allow passage of the pili.

In a genetic approach, amino acid residues in PilA are being systematically changed and the impact on extracellular electron transfer quantified. The ability of *pilA* genes from other microorganisms to complement the *pilA* mutant of *G. sulfurreducens* is being studied to determine whether other PilA proteins can provide conductivity.

Other proteins on the outer surface that are involved in extracellular electron transfer are also being further investigated. For example, a purification protocol for OmcS, an outer-membrane *c*-type cytochrome essential for Fe(III) oxide reduction, was developed, and the biochemical properties of this cytochrome are under study.

Molecular Analysis of the Metabolic State of *Geobacter* Species During *In Situ* Uranium Bioremediation

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To understand the factors controlling subsurface microbial processes, such as *in situ* bioremediation of metal contaminants, it is necessary to not only identify which microorganisms are responsible for these processes, but also to evaluate their *in situ* rates of metabolism and how different environmental factors affect the rate and extent of processes of interest. We are investigating the hypothesis that quantifying transcript levels for key metabolic and respiratory genes can provide insight into the metabolic state of the *Geobacter* species catalyzing *in situ* bioremediation of uranium-contaminated groundwater.

To identify genes whose expression levels could be linked to rates of respiration and/or environmental stresses that might limit the growth and activity of *Geobacter* species during growth in the subsurface, gene expression patterns were evaluated with whole-genome microarrays and quantitative RT-PCR in two species of *Geobacter*. This included *G. sulfurreducens*, which has been the subject of intense physiological investigation, and *G. uraniumreducens*, which was isolated from the Environmental Remediation Sciences Program study site in Rifle, Colorado, and is representative of the *Geobacter* species that predominate during *in situ* uranium bioremediation. For example, to identify genes potentially diagnostic of growth rate in the subsurface, both *Geobacter* species were grown in chemostats at different dilution rates. Genes that were more highly expressed at high growth rates than at low growth rates (*rpsC, accA, ftsZ, rapA, rpsO, rplL, secF,* and *purF*) were identified, as were a number of housekeeping genes whose expression was not affected by growth rate changes (*proC, rho, pyk, alaS, gluM, coaBC, pheA, glnA, hisH,* and *hisS).* Initial results from the analysis of transcript levels in the subsurface during *in situ* uranium bioremediation demonstrated that transcript levels of *rpsC* normalized against transcript levels of *proC* varied with rates of Fe(III) reduction in the subsurface. When *Geobacter* species grown either in chemostats or sterilized sediments from the Rifle site were stressed with 5%, or 10% oxygen for a day, there was a linear increase in transcript levels for oxidative stress genes such as *sodA, rbr, rbo, cydA, cydB, hybA,* and *hybB*. *Geobacter* species expressed the oxidative stress genes, *sodA* and *cydA* during *in situ* uranium bioremediation, suggesting that *Geobacter* species were exposed to oxidative stresses during the uranium bioremediation process. Other potential metabolic marker genes identified by genetic and gene expression studies included *gur1, gur2,* and *gur3*, genes for three putative sodium solute symporters that are highly conserved in the available genomes of *Geobacter* species and appear to be involved in acetate metabolism. Microarray and quantitative RT-PCR analysis of the expression of these genes indicated that *gur1* and *gur2* are more highly expressed when growth is limited by acetate availability than when there is an excess of acetate, whereas expression of *gur3* appears to be constitutive. Studies are under way to determine whether monitoring expression of these genes in the subsurface can provide an indication of when *Geobacter* species are limited by acetate availability. These results, coupled with results reported in the previous two years, suggest that it is possible to monitor the metabolic state of *Geobacter* species during *in situ* uranium bioremediation. This provides information on how to optimize the bioremediation strategy.

Catalytic DNA Biosensors for Radionuclides and Metal Ions with Parts-Per-Trillion Sensitivity and Million-Fold Selectivity

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We aim to develop new catalytic DNA biosensors for simultaneous detection and quantification of bioavailable radionuclides such as uranium and technetium, and metal contaminants such as lead and mercury. The sensors will be highly sensitive and selective, not only for different metal ions, but also for different speciation states of the same metal ion. They will be applied to on-site, real-time assessment of concentration, speciation, and stability of the individual contaminants before and during bioremediation, and for long-term monitoring of DOE contaminated sites.

To achieve this goal, we are employing a combinatorial method called "*in vitro* selection" to search from a large DNA library (~10^{15} different molecules) for catalytic DNA molecules that are highly specific for radionuclides or other metal ions, through intricate 3-dimensional interactions (such as in metalloproteins). Comprehensive biochemical and biophysical studies are being performed on the selected DNA molecules. The findings from these studies have helped to elucidate fundamental principles for designing effective sensors for radionuclides and metal ions. Based on the study, the DNA molecules have been converted to fluorescent or colorimetric sensors by attaching fluorescent donor/acceptor pairs or gold nanoparticles to them.

Using the approach described above, we have obtained catalytic DNA sensors for Pb(II) and U(VI). The uranyl sensor has a detection limit of 45 pM or 11 parts-per-trillion, a dynamic range up to 400 nM, and selectivity of over one million-fold over other metal ions (J. Liu et al., *Proc. Natl. Acad. Sci. USA* 104, 2056–2061, 2007).These sensitivity and selectivity rival those of instrumental analyses. Application of the sensor in detecting uranium in contaminated soil samples from ERSD's Field Research Center (FRC) at Oak Ridge has also been demonstrated. This is the first time that uranium has been recruited as an enzyme cofactor, and the sensor rivals the most sensitive analytical instrument for uranium detection. This work shows that the *in vitro* selected catalytic DNA can be used as a general way of obtaining sensors for radionuclides and metal ions with ultrahigh sensitivity and selectivity.

Comparative Biochemistry and Physiology of Iron-Respiring Bacteria from Acidic and Neutral pH Environments

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During the last year of research, we have made significant progress in several areas, including further characterization of cytochromes c, genome annotation, and biofilm physiology and imaging.

Electron Transport Proteins—Purification, Overexpression, and Characterization. Studies of native cytochromes c from *A. cryptum* have shown pH-dependent redox behavior, significant in that acidophilic redox proteins would be expected to behave differently from their neutrophilic homologs. We have also adopted an expression system used for *Geobacter sulfurreducens* polyheme cytochromes c. Results show successful expression of the *A. cryptum* 42 kDa membrane-associated cytochrome c, which can be purified by inclusion body solubilization and subsequent refolding. We have also produced antibodies against the 10.1 kDa periplasmic cytochrome c to perform immunopurification and immunolocalization analyses.

Chromium Enzyme Detection and Characterization A putative chromate reductase has been identified in soluble protein extracts of *A. cryptum* JF-5. Initial characterization of the reductase suggests that it is a multisubunit complex containing at least one c-type cytochrome and is expressed under aerobic conditions in the presence or absence of Cr(VI). A protein zymography method for detection of Cr(VI) reducing enzymes has been developed, and this method will be used in conjunction with genomics and directed proteomics methods to identify additional Cr(VI) reducing enzymes in this organism.

Genome Annotation of A. cryptum. A genome annotation jamboree was held in August 2006 to annotate the recently completed sequence (courtesy of JGI) of *A. cryptum*. Significant findings include the presence of an "extragenome" consisting of 9 plasmids containing mobility- and replication-related sequences, as well as sequences corresponding to mercury resistance and cation transport. Biofilm-related genes, encoding capsular exopolysaccharide synthesis and pili/flagella assembly, were present on the main chromosome. Several genes encoding NiFe-hydrogenase were found, as well as a modified TCA cycle (glyoxylate bypass) for conservation of carbon.

Biofilm Formation and Imaging Under anaerobic and aerobic growth, *A. cryptum* will form large rafts of cells, as visualized with DAPI, Live/Dead, and SYPRO orange stains. Both ConA and wheat germ lectins bound to the rafts of cells. A new fluorescent Fe(II) probe detected Fe(II) only in the anaerobically grown cells, confirming Fe-reduction. Flow cell studies on iron coupons resulted in microcolonies. Lectin stains show that under aerobic and anaerobic conditions, N-acetylglucosamine, N-acetylneuraminic acid, α-mannopyranosyl, and α-glucopyranosyl sugar residues are present. *A. cryptum* produces acid mucopolysaccharides only when grown anaerobically. Deconvolution microscopy (with Dr. Andy Neal, SREL) revealed that the most significant biofilm formation occurred either with a glass surface under aerobic conditions or an iron surface under anaerobic conditions.

Quantification of Hydrological, Geochemical, and Mineralogical Processes Governing the Fate and Transport of Uranium over Multiple Scales in Hanford Sediments

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Our research is motivated by the remediation and stewardship needs of DOE sites in which contaminant transport is influenced by anisotropic lateral flow and mineralogical and geochemical heterogeneities. The objective of our research is to provide validated scaling strategies that can be applied to existing contaminant distributions and migration scenarios at Hanford and related DOE sites. We will accomplish this through an integrated multiscale approach involving: (1) quantification of hydraulic, mineralogical, and geochemical transport parameters within small intact cores of unsaturated sedimentary layers, (2) upscaling of these parameters using a combination of numerical, composite medium, and fractal approaches to compute effective coupled hydraulic and uranium transport parameters, and (3) validation of the methodology by application of effective parameters to progressively larger scales consisting of intact multiple sedimentary beds that encompass both lateral and vertical transport of uranium. The unique aspects of our experiments include use of a state-of-the-art Ultra Rock Centrifuge (URC) to measure moisture retention and unsaturated hydraulic conductivity, novel surface analysis techniques (e.g., x-ray absorption and micro-Raman spectroscopy), and uranium transport experiments in a natural, layered, instrumented system.

Intact sediment samples will be collected at scales ranging from 20 to 300,000 cm^3 at Hanford in the spring of 2007. The flow and transport of nonreactive tracers and U(VI) will be investigated under partially saturated, transient conditions in 4 intact 2D sediment blocks (150,000–300,000 cm^3). Progress will be monitored within individual sedimentary layers as a function of lateral distance and vertical depth. Intact and disturbed samples (20 cm^3) collected from individual sedimentary beds that make up the large samples will be analyzed using the URC to determine their hydraulic properties.

For the first application of this technology to environmental problems, we will validate the URC method by comparing results with standard moisture retention methods and the widely used unsaturated flow apparatus (UFA) on Hanford vadose zone samples. Using adsorption isotherms and repacked columns, we will investigate the relationship between U(VI) solution geochemistry and the magnitude and speciation of U(VI) immobilized onto the solid phase. These results will be related to U(VI) transport on larger intact samples (8,000 cm^3) and ultimately to the large, intact 2D sediment blocks. Modeling efforts will consider several conceptual formulations of varying complexity and identify the formulation that yields minimum prediction uncertainty, considering intrinsic model prediction uncertainty and error propagation resulting from model parameter uncertainty. A combination of numerical, composite medium, and fractal approaches has been applied to Hanford subsurface materials to compute effective, upscaled hydraulic properties for layered heterogeneous media. These results demonstrate that the modeling approach is robust for steady-state flow, but more research is needed to determine the accuracy for transient flow. The approach will be used to compute effective, upscaled hydraulic and reactive transport parameters from the sedimentary layer scale to the largest sampled scale and ultimately to the field scale. Our results will consist of an integrated dataset and validated coupled hydrological, mineralogical, and geochemical process models at molecular to meter scales in field-relevant, intact sediments. New strategies for coupling reactive transport with complex hydrology will be developed that are directly applicable to the prediction and remediation of contaminant migration in layered systems throughout the U.S.

The Phytoremediation of Ionic and Methylmercury

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

The focus of our current research is to increase the rates of mercury uptake, transport aboveground, and hyperaccumulation in leaves. We have cloned and tested more than 20 genes from bacterial and plant sources in transgenic plants, and several produced exciting phenotypes. Two of the several plant *high affinity zinc transporter (ZIP)* genes tested increased mercury uptake in yeast, but only one of these, *ZIP7,* appears to enhance root mercury uptake in transgenic plants. Plants co-expressing *gamma-glutamylcysteine synthetase (ECS)* and *glutathione synthetase (GS)* have increased levels of mercury accumulation aboveground as a result of higher concentrations of glutathione and other thiol-peptides. Arabidopsis has a family of 14 glutathione conjugate pumps (GCPs) with the potential to enhance vacuolar storage of $(GS)_2$-Hg complexes. We have found that four of these are elevated in expression upon exposure to mercury and are good candidate genes in our phytoremediation strategy. In parallel, we have shown that the yeast GCP, *YCF1,* enhanced plant tolerance to mercury, but like the endogenous Arabidopsis genes, *YCF1* was poorly expressed in transgenic plants. Current efforts focus on rebuilding and mutagenizing *YCF1* to enhance the levels of full-length RNA, to increase protein synthesis rates on the rough endoplasmic reticulum, to increase rates of YCF1-loaded vesicle transport to vacuole, and to increase specific activity of mercury vacuolar transport. We hope in the next few years to be able to deliver a magazine of six well-characterized transgenes on a minichromosome to field adapted plants for the phytoremediation of mercury.

Nucleation and Precipitation Processes in the Vadose Zone During Contaminant Transport: Formation Kinetics of Uranium Silicates

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At many U.S. Department of Energy sites, radionuclides and toxic metals released to the subsurface have formed contaminant plumes that must be assessed for their potential risk to water supplies. Contaminant mobility is the sum of various processes, foremost of which are sorption of aqueous species at solid surfaces, nucleation and precipitation of secondary solids on surfaces or in confined spaces, and formation and transport of colloids. An example from the Hanford Site is the immobilization of uranium released from a subsurface waste tank (pH 10) by formation of Na-boltwoodite in microcracks in feldspar minerals (Liu et al., 2004, *Geochimica et Cosmochimica Acta 22*, 4519–4537, 2004; J.G. Catalano et al., *Environmental Science & Technology 38*, 2822–2828, 2004). The phase may have formed relatively quickly, in part because dissolution rates of primary minerals that provided components of the Na-boltwoodite would have been accelerated at the basic pHs of the leaked waste and vadose zone, as well as by the elevated temperature field emanating from the tank.

We are determining the range of chemical and physical conditions under which secondary silicate phases that can trap uranium form and evolve with time. Two sets of experiments to simulate homogeneous kinetics were conducted. In the first, solutions were prepared at pHs of 2 to 4 to maximize concentrations of aqueous uranium silicate species and minimize or remove the influence of hydrolyzed uranium and uranium carbonate species. In the second set, solutions were open to the atmosphere and precipitation was investigated at variable U/Si concentration ratios from pH 2 to 9. A third set of experiments simulating heterogeneous precipitation kinetics was conducted using plagioclase feldspar as the substrate and solution compositions similar to those of Hanford groundwater. Homogeneously precipitated solids were characterized using small angle X-ray scattering (SAXS) and high energy X-ray scattering (HEXS). Solids in experiment set 2 were also characterized by Attenuated Reflectance-Fourier Transform Infrared Spectroscopy (AT-FTIR). Precipitated U on feldspar was characterized using X-ray absorption spectroscopy (EXAFS). Solution compositional changes in experiment sets 1 and 3 were monitored by alpha-counting (U) and UV-visible spectroscopy (Si).

Solids from experiment set 1 show systematic increased uptake of U with increasing Si concentration, with a likely strong influence of precipitated amorphous silica. Solids from experiment set 2 show a sharp phase transition at around pH 6 from phases similar to those formed at low pH in the absence of CO_2 (experiment set 1) to phases similar to those observed at Hanford. Results from experiment set 3 currently are being analyzed.

We are constructing a 2D flow simulator with imaging capabilities that take advantage of uranium's fluorescence properties, for the purpose of investigating nucleation and growth in quartz and feldspar substrates under conditions of saturated and unsaturated flow. Final project results will provide fundamental kinetic data that can be used in reactive-transport models, by relating the variables of solution saturation state and substrate surface area to critical parameters that activate the formation of precipitates as colloids or thin films.

Targeted Deletion Mutagenesis of *Shewanella Oneidensis* MR-1 Outer Membrane Proteins Reveals a Varying Influence upon Cell Adhesion and Membrane Architecture

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The outer membrane (OM) proteome of dissimilatory metal-reducing bacteria continues to attract intense research attention. This group of bacteria is unusual in expressing cytochromes in the OM, thought to be an adaptation to the physical difficulty of gaining access to insoluble electron acceptors. OM proteins may influence the iron-reduction process via a variety of mechanisms. Central to the concept of direct electron transfer from the cell to iron oxide surface is the assumption that close association between the mineral and cell is required. This assumption requires that two competing requirements are met: first, that sufficient adhesive force is derived between the cell and mineral surfaces to confer cell attachment; and second, that the cell surface remains largely free of materials that would prevent the necessary close association between OM-bound cytochromes and Fe(III)-sites at the mineral surface. Employing targeted deletion mutants of three OM proteins, we present experiments designed to test the hypothesis that *Shewanella oneidensis* MR-1 OM proteins exert a significant influence upon cell-mineral interactions by functioning as adhesins and/or controlling the architecture of the OM surface. Static adhesion assays, in which washed, fumarate-grown cells of wild type and targeted deletion mutants were allowed to adhere to α-Fe_2O_3 surfaces under an anaerobic atmosphere, indicated that the OM cytochrome *mtrC*-, the OM porin *gspD*- and a putative autotransporter, *SO3800*-deficient cells adhered at significantly (α=0.05) lower densities than either wild type or cytoplasmic membrane bound thiosulfate reductase, *psrA*-deficient cells to α-Fe_2O_3. *SO3800*-deficient cells were the least able to adhere (cell attachment reduced to 20% of wild type levels). Electrokinetic cell behavior indicated that differences in adhesion could in part be explained by reduced outer membrane potential (Ψ_0) associated with the OM-protein-deficient strains (Ψ_0^{mtrC} =-3.8 mV, Ψ_0^{gspD} =-2.4 mV, Ψ_0^{SO3800} =-2.4 mV) compared to the wild type strain (Ψ_0^{MR-1} =-10.5 mV). It was also noted that both Δ*gspD* and Δ*SO3800* exhibited a reduced electron-donating (γ^-, Lewis base) component of surface free energy (γ^- Δ*gspD* = 30.5 mJ m^{-2}, γ^- Δ*SO3800* = 32.2 mJ m^{-2}) compared to wild type Δ*psrA* or Δ*mtrC* cells (γ^- all of approximately 45 mJ m^{-2}). Electrokinetic cell behavior combined with low-temperature HR-SEM also found that the surface of Δ*gspD* and Δ*SO3800* cells accumulated significantly more capsular material than the other strains.

These results demonstrate that OM proteins exert varying influence upon the physicochemistry of the cell surface. While all three OM proteins appear to contribute to the OM potential (thereby influencing electrostatic interactions with mineral surfaces), secretion system proteins (*gspD* and *SO3800*) or their allocrits also appear to contribute to the base component of polar interactions between the cell and mineral surface. Additionally, in contrast to nonsecretion-system-deficient strains (wild type, Δ*mtrC* and Δ*psrA*), secretion-system-deficient strains of MR-1 also appear to secrete extensive capsular polymers.

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

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The behavior of contaminant plutonium (Pu) in subsurface environments is impacted by the complex hydrogeology, by prevailing redox conditions at the contaminated site, and by direct and indirect bacterial processes. The overarching goal of this research effort is to advance our understanding of the complex biological processes that affect plutonium speciation and stability. We investigated plutonium accumulation through biofilms of aerobic bacteria and bacterial mineralization and immobilization via direct enzymatic reduction of Pu(VI)/Pu(V) by metal-reducing bacteria. We have also examined the accessibility of $Pu(IV)(OH)_4(am)$ to bacterial reduction and its implications for the fate and transport of plutonium in the environment.

The ability of *Geobacter metallireducens* GS15 and *Shewanella oneidensis* MR1 to reduce freshly prepared Pn(VI) and Pu(V) was examined under cell suspension and growth conditions. We found that cell suspensions of *G. metallireducens* GS15 and *S. oneidensis* MR1 reduce oxidized Pu (Pu(VI) and Pu(V)) to Pu(IV). The rate of plutonium reduction was similar to that of U(VI) reduction obtained under the same conditions. The rate Pu(VI)/Pu(V) reduction by a cell suspension of *S. oneidensis* MR1 was slightly faster than the reduction rate observed with a cell suspension of *G. metallireducens* GS15. The reduced form of Pu was characterized as colloidal Pu(IV). TEM images recorded by analyzing the solids obtained from the reduction of Pu(VI) by *S. oneidensis* MR1 show that plutonium precipitates as nanoparticles of crystalline Pu(IV) present at the cell surface, and also as aggregates of larger particles between cells. Analysis of the reduction products did not reveal the presence of Pu(III). However, when cell suspension of *G. metallireducens* GS15 and *S. oneidensis* MR1 were incubated with freshly precipitated $Pu(IV)(OH)_{4(am)}$, minor amounts of Pu(III) were observed. In cell suspensions without complexing ligands, added minor Pu(III) production was observed in cultures containing *S. oneidensis*, but little or no Pu(III) production was observed for cultures containing *G. metallireducens*. In the presence of EDTA, most of the $Pu(IV)(OH)_{4(am)}$ present was reduced to Pu(III) and remained soluble in cell suspensions of both *S. oneidensis* and *G. metallireducens*. When soluble Pu(IV)(EDTA) was provided as the terminal electron acceptor, cell suspensions of both *S. oneidensis* and *G. metallireducens* rapidly reduced Pu(IV)(EDTA) to Pu(III)(EDTA), with near complete reduction within 20 to 40 minutes depending on the initial concentration. Neither bacterium was able to use Pu(IV) (in any of the forms used) as a terminal electron acceptor to support growth.

These results have significant implications for the potential remediation of plutonium and suggest that strongly reducing environments where complexing ligands are present may produce soluble forms of reduced Pu species. However, in the absence of complexing ligands, the main form of plutonium produced by direct enzymatic reduction was the desired insoluble Pu(IV).

Coupled Microbial, Geochemical, and Mineralogical Controls on Biogenic Fe^{II} Speciation and Reactivity

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The current DOE strategy for treatment of radionuclide and heavy metal contamination in the subsurface relies heavily on *in situ* immobilization and stabilization. However, the successful application of bioremediation-based *in situ* treatments requires a thorough understanding of the key microbiological and geochemical processes controlling contaminant transformation and mobility in the subsurface. ERSP-funded research has already significantly advanced our understanding of the basic microbial and geochemical processes affecting the speciation and distribution of contaminants in subsurface environments. However, much remains to be learned about the key biogeochemical processes (e.g.,the redox cycling of Fe) that determine the rate and extent of metal and radionuclide immobilization and stabilization, particularly with regard to the coupling of biotic (microbial) and abiotic (geochemical) processes.

Therefore, the major objectives of this research are to determine which aspects of microbial physiology, solution chemistry, and Fe^{III} mineralogy are key in determining the distribution of Fe^{II} species (i.e., soluble Fe^{II} complexes, Fe^{II} complexes with the surfaces of organic and inorganic solid phases, and a host of mineral phases containing structural Fe^{II}, including magnetite [Fe_3O_4], siderite [$FeCO_3$], vivianite [$Fe_3(PO_4)_2$], green rusts, ferrous hydroxy carbonate, and Fe^{II}-bearing clays) resulting from dissimilatory iron reduction (DIR)—as well as to evaluate the reactivity of specific biogenic Fe^{II} phases with respect to the reduction of U^{VI}. Emphasis is on understanding the kinetics and mechanisms of these reaction(s) under conditions relevant to natural and engineered subsurface environments. These objectives will be met by completion of experiments designed to test the following hypotheses: (1) metabolic pathways involved in the utilization of electron donors for DIR influence the distribution of biogenic Fe^{II} phases; (2) for a given species of dissimilatory iron-reducing bacteria, changes in solution chemistry and Fe^{III} mineralogy affect the distribution of Fe^{II} products; (3) the rate, extent, and mechanism(s) of U^{VI} reduction by biogenic Fe^{II} are dependent on the structure of the biogenic Fe^{II} phase; (4) the rate, extent, and mechanism(s) of U^{VI} reduction by a given biogenic Fe^{II} phase are dependent on U^{VI} speciation; and (5) the "abiotic" reduction of U^{VI} to U^{IV} by Fe^{II} can be coupled to dissimilatory Fe^{III} reduction under conditions that promote the formation of reactive Fe^{II} species. The research we propose will significantly increase our understanding of the coupling of biotic and abiotic processes—specifically with respect to the speciation of uranium, and more generally to the speciation of other redox-active contaminants—under Fe^{III}-reducing conditions in the subsurface.

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

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

In a series of experiments at the ERSD Field Research Center (FRC) site in Oak Ridge, we have found consistently high rates of nitrate and uranium reduction (removal of U from solution) with some electron donors (e.g., glucose and ethanol). However, while methanol stimulation consistently promoted nitrate and sulfate reduction, U reduction was stimulated by methanol addition in only some of the experiments with different sediment samples. For example, of four samples taken within three meters of each other, there was only one sample in which U reduction was observed with the methanol addition. These results were repeatable and consistent between replicates. Thus, there appear to be sample-scale heterogeneities in the community structure for a relatively uncommon community able to reduce U when stimulated with methanol. Also, although the rate of U removal from solution was slower with methanol than with other electron donors, the extent (e.g., the percent of U as U(VI)) of U reduction in the total sediment-water microcosms was greater. Characterization of the microbial community using a variety of methods (phospholipid fatty acid [PLFA], terminal restriction fragment length polymorphism analysis [TRFLP], clone libraries) indicated that there were substantial differences in the community structure related to the type of electron donor added. In our experiments, where we added humics as well as electron donors, there were no significant effects on U reduction rates or extent, and minimal effect on community structure. Experiments to be completed this summer include tests of the concept of resource ratio theory using phosphate-enriched microcosms, and additional heterogeneity studies, including examination of vertical heterogeneity in the response of the microbial community.

Geochemical, Genetic, and Community Controls on Mercury Methylation

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The objectives of our research are to (1) delineate the genetic basis for mercury methylation in *Desulfovibrio*, (2) examine the biogeochemical controls on mercury methylation, and (3) translate the knowledge of the genetic basis and the understanding of the environmental controls (biogeochemical and community) influencing the mobilization and immobilization processes to the field. Mercury contamination is a serious concern at several DOE sites, including areas in Oak Ridge surrounding the ERSD Field Research Center. Mercury methylation is the most important geochemical process involved with mercury contamination, since monomethylmercury is a potent human neurotoxin. Sulfate-reducing bacteria (SRB) are key organisms in several processes that have significant consequences for humans, including production of monomethylmercury. However, the molecular mechanisms for mercury methylation are largely unknown. In addition, the ecological and microbial community interactions and biogeochemical controls of this process are not well known. This lack of knowledge severely limits the scope of, and confidence in, potential remediation activities.

To address this problem, we will link field and laboratory studies and use a genomic and environmental approach with mercury-methylating *Desulfovibrio* as a model bacterium. We will compare gene expression in *Desulfovibrio* species that methylate mercury with gene expression in those that do not, to focus in on the genes responsible for mercury methylation. Two candidates for the study are *Desulfovibrio desulfuricans* G20, a representative SRB and an organism whose genome has been sequenced by DOE, and the closely related *D. vulgaris,* which does not methylate. Prior to our studies, *D. desulfuricans* G20 was never tested for mercury methylation, but other *D. desulfuricans* strains have been reported to methylate mercury. We recently began producing a microarray for gene expression studies of *D. desulfuricans* G20 and have been using a microarray for gene expression studies of *D. vulgaris.* Microarray technology is an important tool that allows researchers to obtain insights into cellular processes by examining gene expression under various physiological states. Our approach to identifying the critical genes (e.g., a "mercury methylase") will benefit significantly from doing joint gene studies on closely related bacteria, some of which do not methylate mercury and some of which do. We have obtained numerous *Desulfovibrio* strains with described ability to methylate mercury. We have sequenced the full 16s RNA sequence of these strains to better establish phylogenetic relationships. We are in the process of (1) testing the strains for mercury methylation, in order to make a final selection of strains to be used and (2) designing the accompanying field studies, (3) testing mercury sensitivity and mercury-induced changes in gene expression in nonmethylating *Desulfovibrio*. Our combined approach is designed to yield significant advancements in the understanding of *in situ* mercury methylation processes and controls.

Bioremediation Approaches for Nitrate-Independent Uranium Reduction

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Bioremediation strategies for uranium contaminated DOE sites, such as the ERSD Field Research Center (FRC) at Oak Ridge, are faced with extreme levels of the co-contaminant nitrate. Field-scale bioreduction experiments at the FRC have demonstrated that nitrate concentrations recover soon after cessation of donor addition, such that nitrate removal must be continuous to sustain uranium reduction and immobilization. This project investigates the possibility of stimulating nitrate-indifferent, pH-tolerant organisms to achieve biologically mediated reduction of U(VI), despite nitrate persistence. Enrichments from contaminated sediments were prepared using a variety of electron donors and MOPS/TRIS buffers at pH's ranging from 4.9 to 7. Successful enrichments from pHs 4.9–6.5 containing 10–20 mM methanol or glycerol have demonstrated ~90% reduction of uranium (~10 ppm) with less than 10% loss of nitrate (~850 ppm). Higher pH enrichments demonstrated similar U reduction potential with 5–30% nitrate loss. Bacterial 16S rRNA genes from uranium-reducing enrichments at pH 5.7–6.2 were PCR-amplified and sequenced. Most of the clone sequences were most closely related to *Clostridia* and *Clostridia*-like organisms. Other clone sequences were representative of the aerobic Gram-negative *Aeromonas*, along with members of the phylum Bacteroidetes. T-RFLP analysis of bacterial 16S rRNA genes indicated that the two most dominant groups of organisms present in these enrichments were those most closely related to *Anaerosinus glycerini* and *Desulfotomaculum guttoideum*, strictly anaerobic members of the phylum Firmicutes. Aerobic Proteobacteria and metabolically versatile Bacteroidetes only comprised a very small portion of the bacterial community. There was a great deal of diversity found among the genera of *Clostridia*, and lower pH enrichments with methanol as the electron donor tended to yield overall lower diversity.

In additional experiments, reoxidation of two U(IV) sources was found to correlate with oxygen stress rather than the presence of 850 ppm nitrate or 100 ppm nitrite. U(IV) remained stable in deoxygenated water with or without nitrogen species after 215 days. Current experiments are exploring the reoxidation of U(IV) in the presence of both iron and nitrate. Data suggest that U(VI) release is much more dependent on oxygen stress than anaerobic reactions with iron or nitrogen species.

Coupled Biogeochemical Process Evaluation for Conceptualizing Trichloroethylene Co-Metabolism

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Our research focuses on the coupled biogeochemical processes that dictate the rate of methane-driven co-metabolism of trichloroethylene (TCE) in the Snake River Plain aquifer at the Idaho National Laboratory. Natural attenuation has been accepted as a remediation strategy at this location, and our study seeks to quantify the contribution of methanotrophs to the natural attenuation and to obtain various lines of evidence that indicate that the process is occurring. Aquifer microbial communities and chemistry from within the TCE plume were characterized using water samples from wells and biofilm communities from *in situ* incubation of basalt chips. Methanotrophic microbes, or evidence of their activity, were detected in numerous wells in the "medial zone" of the TCE plume, where TCE concentrations ranged up to 500 ppb. Combined analyses using fluorescent *in situ* hybridization (FISH), enzyme activity probes, Phylochip community characterization and community proteomics targeting the methanotroph-specific soluble methane monooxygenase both detected evidence of methanotrophs in the groundwater and in biomass obtained from basalt chips. Phylochip analyses also indicated the presence of several methanogenic genera in the wells, suggesting that biogenic methane may contribute to methanotroph sustenance in the aquifer. Additionally, the catalytic subunit of soluble methane monooxygenase (*mmo*X) has been detected in concentrated groundwater by conventional PCR. As yet, laboratory incubations of aquifer samples have not determined that co-metabolism of TCE is occurring in any of the samples analyzed; however, based on estimates derived from computational modeling of the rates of natural attenuation, we expect that these rates may be difficult to detect in relatively short-term incubations of aquifer communities. Currently, flow-through *in situ* reactors are incubating at two distinct aquifer flow rates, to determine the affect of hydraulic flow on the microbial communities capable of TCE co-metabolism. Subsequent studies will assess the contribution of methane in TCE co-metabolism carried out by the reactor communities. Determination of the TCE co-metabolism rate, at different methane concentrations and groundwater flow velocities, will yield key modeling parameters for the computational simulations that describe the attenuation, and accordingly improve the predictive capability of the models. Accurate assessment of natural attenuation rates under different aquifer conditions will further justify the use of natural attenuation at the INL and at other DOE sites.

Fluid Flow, Solute Mixing, and Precipitation in Porous Media

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Reactions that lead to the formation of mineral precipitates, colloids, or biofilms in porous media often depend on mixing resulting from molecular diffusion. Solute mixing frequently occurs at the interface between two solutions that each contain one or more soluble reactants, particularly in engineered systems in which chemical transformation or modification of fluid flow are objectives and systems may be far from equilibrium. Dispersion does not necessarily describe the pore-scale molecular-level mixing required for chemical and biological phenomena, such as mineral precipitation and biofilm growth, to occur. Although many of the fundamental component processes involved in the deposition or solubilization of solid phases are reasonably well understood (e.g., precipitation equilibrium and kinetics, fluid flow, and solute transport), the deposition of chemical precipitates, biofilms, and colloidal particles are all coupled to flow and solute transport, and our understanding of such coupled processes is limited. Predicting how such precipitates (and conversely, dissolution or decomposition) are distributed in the subsurface along flow paths with chemical gradients is a complex and challenging problem, particularly in systems that undergo rapid change where equilibrium conditions cannot be assumed. Examples include subsurface systems in which reactants are introduced or generated rapidly *in situ*.

Initial project tasks have involved two- and three-dimensional experiments in packed-sand media where solutions containing calcium and carbonate ions came into contact along a parallel flow boundary and mixed by dispersion and diffusion. Calcium carbonate precipitates are propagated along the solution-solution boundary in the direction of flow. As carbonate precipitates fill the pore space, mixing of the two solutions is restricted, but transverse permeability does not decrease to zero. The spatial distribution of carbonate phases, and the distribution of carbonate species, are dependent upon a complex interaction of precipitation and dissolution kinetics, which are in turn functions of pore-scale saturation indices and solute ratios, nucleation mechanisms (e.g., heterogeneous vs. homogeneous), crystal growth conditions, and changes in porosity and flow.

At the pore scale, we have simulated mineral precipitation, changes in porosity, and reductions in mixing using the Smooth Particle Hydrodynamics method, which allows coupling between pore- and Darcy-scales. The width of the precipitation zone was found to be practically independent of the Peclet number, Pe, but the precipitation rate increased with increasing Pe. Macroscopic simulations have been performed using a finite-element multicomponent reactive transport simulator with various degrees of grid refinement and multiple alternative parameterizations. One of the modeling goals is to use pore-scale simulations to provide the basis for parameterization of macroscopic (more practical) model predictions, by comparing results from the two model scales with our experimental results.

Ongoing and planned activities include: characterization of carbonate precipitation products and kinetics under variable ion ratios (including co-precipitation with strontium); continued 2-D and mesoscale experiments with increasing levels of physical heterogeneity and solution injection sequencing; and continued development of pore-scale and continuum scale modeling simulations with linking between pore-scale and continuum-scale representations of experimental data.

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

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

Experimentally, significant progress was made in establishing the key abiotic and biotic interactions in the *S. algae* plutonium system. Higher-valent plutonium can only persist as the $Pu(V)O_2^+$ species in biologically active systems, owing to reduction of $Pu(VI)O_2^{2+}$ by organic species typically present as transient electron donors in these systems. PuO_2^+ forms weak aqueous complexes and is very bio-available in these systems, leading to high toxicity towards *S. algae* and relatively low (typically less than 10%) adsorption onto biomass. Direct enzymatic reduction of PuO_2^+ by *S. algae* was demonstrated, and the higher-valent plutonium species are reduced under all anaerobic growth conditions investigated. Lastly, the abiotic reduction of higher valent plutonium by aqueous Fe^{2+} and Fe(II/0) phases was established in near-neutral systems. The reactivity of Fe^{2+} towards higher-valent plutonium is also a key reduction pathway for biotic *S. algae* systems when Fe^{3+} is the electron acceptor present. The relative contributions of enzymatic and biogenic Fe^{2+} processes in the overall reduction of higher-valent plutonium are the focus of current experimental efforts. Under the conditions of our batch experiments, the result of bioreduction was the formation of insoluble Pu(IV) precipitates. Future plans are to establish the fate of higher-valent plutonium when iron oxides (rather than aqueous Fe^{3+}) function as the electron acceptor, establish the role and importance of Pu^{3+} relative to Pu^{4+}, and establish the potential for bio-mobilization as a function of the plutonium precipitates present.

Modeling activities have centered on upgrading the CCBATCH biogeochemical model to include Pu and U speciation data. Transforming the model from one allowing aerobic growth (utilizing oxygen as electron acceptor) to anaerobic growth (utilizing Fe^{3+} as electron acceptor) required determining the bio-available form of Fe^{3+}. We upgraded CCBATCH to allow selected complexes of Fe^{3+} as primary electron acceptors and completed the experiments with different ratios of Fe^{3+} and NTA. The bio-available form of Fe^{3+}, as well as of U and Pu, will be determined by matching the simulated results to the experimental one. In addition, we are taking an integrated modeling/experimental approach to estimate the kinetic parameters for Fe, U, and Pu reduction—currently, we are working on upgrading and validating CCBATCH to account for dual electron acceptors and abiotic (chemical) reduction of U or Pu caused by biogenic Fe^{2+}. Future work will involve predicting the conditions under which Pu precipitate phases will prevail and their stability under a long-term, continuous transport system.

Advances in Mechanistic Understanding of Abiotic and Microbial Electron-Transfer Kinetics

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The Environmental Molecular Sciences Laboratory (EMSL) at Pacific Northwest National Laboratory (PNNL) is a partner institution in the Stanford Environmental Molecular Sciences Institute (EMSI) established in 2004. The Stanford EMSI project is broadly focused on developing a molecular-level understanding of chemical and biological interactions at environmentally important mineral-water and mineral-microbe interfaces. The EMSL provides expertise in computational molecular modeling and in microscopic and spectroscopic methods for understanding electron transfer (ET) reactions across interfaces under study in the Stanford EMSI. Our focus includes prediction of U(VI) and Cr(VI) reduction kinetics by Fe(II)-bearing mineral phases, mechanistic interpretation of electron tunneling spectra of bacterial outer-membrane cytochromes, and surface structure determination of hematite surfaces under chemically reducing conditions. This work involves three visiting graduate students periodically making extended visits to the EMSL, from Stony Brook University, the University of Michigan, and Virginia Tech. It also involves a resident post-doc at PNNL.

Our strategy involves primarily the use of computational molecular modeling to estimate the free energies for elementary ET steps, and quantities controlling the ET activation energy such as the reorganization energies and the electronic coupling matrix elements. Abiotic ET mechanisms and kinetics for the reduction of U(VI) by Fe(II) in solution or occurring at surfaces of mixed-valent iron oxides have been modeled (Wander et al., *J. Phys. Chem. A., 110,* 9691–9701, 2006; *Geochem. Trans.*, 2007 [submitted]) and are being experimentally probed. For microbial interfacial ET, calculated values for the reorganization energy are compared directly with values extracted from experimental electron tunneling spectra for the outer-membrane proteins OmcA and MtrC, which are thought to function in a catalytic role for the terminal reduction step in the dissimilatory reduction of Fe(III)-bearing oxide phases by *Shewanella oneidensis*. Scanning tunneling spectroscopy (STM) was used to interrogate the ET properties of individual OmcA and MtrC proteins immobilized by covalent linking to Au (111) substrates (Wigginton et al., *Geochim. Cosmochim. Acta, 71*, 543–555, 2007). Current-voltage spectra for the two proteins are distinctly different, and the differences can be explained using the theory of incoherent multistep ET (Wigginton et al., *J. Am. Chem. Soc.*, 2006 [submitted]). The spectra indicate that MtrC possesses two subgroups of hemes that mediate ET by acting as resonant or off-resonant intermediate sites for the tunneling current. The abiotic and microbial interfacial ET reactions operate on solids having unique electronic properties. Small polaron transport in iron oxides can facilitate the migration of electrons injected during bioreduction, or the migration of holes during metal reduction at the oxide surface. We will report the results of molecular dynamics simulations and quantum mechanical calculations used to compute the Marcus ET quantities for each of these highlighted ET systems, providing a single framework for comparison of the key physical quantities underlying their rates.

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

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

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

One of the challenges of addressing scale-dependent effects of coupled processes in heterogeneous porous media is the problem-specificity of solutions. Much effort has been aimed at developing generalized scaling laws or theories, but these require restrictive assumptions that render them ineffective in many real problems. We propose instead an approach that applies physical and numerical experiments at small scales (specifically the pore scale) to a selected model system, to identify the scaling approach appropriate to that type of problem. Although the results of such studies will generally not be applicable to other broad classes of problems, we believe that this approach (if applied over time to many types of problems) offers greater potential for long-term progress than attempts to discover a universal solution or theory. We are developing and testing this approach using porous media and model reaction systems that can be both experimentally measured and quantitatively simulated at the pore scale, specifically biofilm development and metal reduction in granular porous media.

The general approach we are using in this research involves the following steps:

1. Perform pore-scale characterization of pore geometry and biofilm development in selected porous media systems.
2. Simulate selected reactive transport processes at the pore scale in experimentally measured pore geometries.
3. Validate pore-scale models against laboratory-scale experiments.
4. Perform upscaling to derive continuum-scale (local darcy scale) process descriptions and effective parameters.
5. Use upscaled models and parameters to simulate reactive transport at the continuum scale in a macroscopically heterogeneous medium.

Role of Bacterial Nanowires in Biogeobatteries

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Biological, geochemical, and physical processes in the subsurface produce gradients of electrical potential that can be measured by a multimeter. Voltage gradients can be used to find oil and map the path of groundwater flow. Recent collaborations between geologists, geophysicists, and biologists have yielded a novel application for the classic geobattery model to explain natural electrical field generation (expressed as self potential, SP) in the subsurface in the absence of metallic minerals. Bacteria have been shown to produce electrically conductive appendages called bacterial nanowires (Gorby, PNAS 103, 11358–11363). We hypothesized that bacterial nanowires contribute to the generation of electrical fields in the subsurface. We constructed models of the subsurface by suspending the bacterium *Shewanella oneidensis* MR-1 in columns filled with saturated sands. We observed formation of voltage gradients that corresponded to consumption of the carbon source lactate, and formation of bacterial nanowires (observed by SEM during a preliminary experiment). We hypothesize that submerged bacteria gain access to oxygen through a network of nanowires that extend to the air above the saturated sands. The nanowires might transfer electrons from bacteria in the anaerobic part of the column to bacteria at the surface that have access to oxygen. This transport of electrons through bacterial nanowires might be responsible for the produced electrical potential trends.

These recent findings will significantly contribute to the understanding of the geophysical signatures associated with microbial activity in contaminated soils, substantially improving current monitoring/bioremediation techniques. Additionally, the methods can be used to optimize the bioremediation processes by providing spatial and real-time continuous data on the microbial contaminant processes, thus helping to keep optimal conditions for contaminant remediation.

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

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Our project is motivated by the observations that conventional characterization approaches capture only a fraction of heterogeneity affecting field-scale transport, and that conventional modeling approaches, which use these sparse data, typically do not successfully predict long-term plume behavior with sufficient accuracy to guide remedial strategies. Our working hypothesis is that fine-spatial-scale characterization of hydraulic conductivity and porosity can be achieved through an integration of hydrogeophysical data with knowledge of the hydrogeologic facies configuration. Improvement in prediction of subsurface contaminant migration can then be achieved by incorporating the finer scale heterogeneity in a dual domain transport model. Our objectives are to: (1) develop a facies-based multiscale characterization approach that utilizes log, crosshole, and surface-based characterization information, focused on the parameterization needs of the dual domain modeling; (2) develop a dual domain modeling approach (using the TOUGH2 family of codes) that incorporates the key interactions between mobile and immobile transport regions expected to play a role in long-term plume behavior; and (3) evaluate this methodology by applying it to the prediction of plume behavior at the P-Area at Savannah River.

The first year of our three-year project has focused on field characterization. Three first-round groundwater observation and testing wells were installed at the study site and are being used to collect various downhole hydrological, geochemical, and geophysical measurements. Slug tests have been completed in two of the three observation wells. P-wave and S-wave vertical seismic profiles were acquired using two wells, and crosshole tomographic radar, seismic, and electrical data were collected between all three wells. CPT groundwater samples were acquired at 10 locations and over several depths. These data were used to define the vertical and horizontal extent of the plume. Lithologic logs were obtained at three CPT locations, and dual level piezometers were installed at the same locations. A preliminary surface GPR survey was conducted to evaluate the recoverable data quality. Because of the high clay content in the unsaturated zone, surface ground-penetrating radar is unlikely to image the subsurface at the study site below the water table. Surface electrical resistivity surveys were conducted to map changes in subsurface lithofacies and hydrofacies, and a grid of surface seismic data is currently being collected.

We also have begun development of the framework and models needed to integrate the diverse datasets and accurately simulate transport at the SRS study site. A version of the iTOUGH2 code was modified to enable simulation of dual domain transport in a sedimentary system. To guide the field characterization effort, synthetic studies have been performed using this model to explore the sensitivity of the responses to different features and facies contrasts. A probabilistic multiscale framework is currently being developed, using conditioning of remote datasets, to direct measurements using a Markov chain Monte Carlo approach. Our second year plans are to continue the field characterization and dual domain model development, while beginning the integration of the hydrogeophysical data with hydrogeologic data.

Promoting Uranium Immobilization by the Activities of Microbial Phosphates

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The overall objective of this project is to examine the activity of nonspecific phosphohydrolases present in naturally occurring subsurface microorganisms, for the purpose of promoting the immobilization of radionuclides through the production of uranium [U(VI)] phosphate precipitates. Specifically, we hypothesize that the precipitation of U(VI) phosphate minerals may be promoted through the microbial release and/or accumulation of PO_4^{3-} as a means to detoxify radionuclides and heavy metals. An experimental approach was designed to determine the extent of phosphatase activity in bacteria previously isolated from contaminated subsurface soils collected at the ERSD Field Research Center (FRC) in Oak Ridge, TN. Screening of 135 metal-resistant isolates for phosphatase activity indicated the majority (75 of 135) exhibited a phosphatase-positive phenotype. During this phase of the project, a polymerase chain reaction (PCR)-based approach has also been designed to assay FRC isolates for the presence of one or more classes of the characterized nonspecific acid phophastase (NSAP) genes likely to be involved in promoting U(VI) precipitation. Testing of a subset of Pb resistant (Pb^r) *Arthrobacter*, *Bacillus* and *Rahnella* strains indicated 4 of the 9 Pb^r isolates exhibited phosphatase phenotypes suggestive of the ability to bioprecipitate U(VI). Two FRC strains, a *Rahnella* sp. strain Y9602 and a *Bacillus* sp. strain Y9-2, were further characterized. The *Rahnella* sp. exhibited enhanced phosphatase activity relative to the *Bacillus* sp. Whole-cell enzyme assays identified a pH optimum of 5.5, and inorganic phosphate accumulated in pH 5.5 synthetic groundwater (designed to mimic FRC conditions) incubations of both strains in the presence of a model organophosphorus substrate provided as the sole C and P source. Kinetic experiments showed that these two organisms can grow in the presence of 200 μM dissolved uranium, and that *Rahnella* is much more efficient in precipitating U(VI) than *Bacillus* spp. The precipitation of U(VI) must be mediated by biological activity, as less than 3% soluble U(VI) was removed either from the abiotic or the heat-killed cell controls. Interestingly, the pH has a strong effect on growth and U(VI) biomineralization rates by *Rahnella*. Thermodynamic modeling identifies calcium autunite-type minerals [$Ca(UO_2)_2(PO_4)_2$] as the precipitate likely formed in the synthetic FRC groundwater conditions at all pH investigated. Extended X-ray absorption fine structure measurements have recently confirmed that the precipitate found in these incubations is an autonite autunite and meta-autoniteautunite-type compoundsmineral are formed in these incubations. A kinetic model of U biomineralization at the different pH indicates that hydrolysis of organophosphate can be described using simple Monod kinetics, and that uranium precipitation is accelerated when monohydrogen phosphate is the main orthophosphate species in solution. Overall, these experiments and ongoing soil slurry incubations demonstrate that the biomineralization of U(VI) through the activity of phosphatase enzymes can be expressed in a wide range of geochemical conditions pertaining to the FRC site.

Polymer-Encapsulated Soil as an *In Situ* Methodology to Assess Long-Term Performance of Soil-Immobilized Contaminants

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Any methodology to assess the long-term performance of immobilized contaminants in soil must be able to demonstrate both (1) measurable contaminant immobilization in some unavailable phase, such as a stable mineral or microbial cells, and (2) the permanence of that immobilized phase under prevailing *in situ* geochemical conditions after active manipulation can no longer be sustained. Relatively little attention has been paid to the latter criterion, owing, in part, to the technical difficulty and expense of collecting the large number of soil samples required to decipher statistically significant effects over time, in an all-too-heterogeneous soil environment. In this new FY2007 project, nondestructive assay techniques, using both x-ray fluorescence and gamma spectroscopy, will be applied to soils contained within permeable environmental leaching capsules (PELCAPs) and obtained from the ERSD Field Research Center (FRC) in Oak Ridge, TN (and other areas where either natural attenuation or *in situ* immobilization treatments for U, Tc, Cr, ^{90}Sr, and ^{137}Cs have occurred). Repeated retrieval and replacement of PELCAPs in groundwater will be performed to obtain a long-term time series of contaminant concentrations under *in situ* field conditions. Polyacrylamide, as a soil-encapsulating gel, has been found previously to allow radioisotopes of cesium and strontium to diffuse freely, rapidly, and completely from and into small cylindrical soil capsules during limited field testing. Thus, gel-contained soils now present a novel and powerful tool for nondestructive assay of contaminants in the same soil samples repeatedly, while allowing them to weather *in situ* under ambient physiochemical conditions. The resulting observational time series of U, Tc, Cr, ^{90}Sr, and ^{137}Cs contaminant concentrations will provide the capability to assess *in situ* the long-term performance of immobilized contaminants. The primary application will be to assess U, Tc, Cr, ^{90}Sr, and ^{137}Cs immobilization in treated and untreated or naturally-attenuated soils in uncontaminated groundwater environments at the FRC and ORNL' White Oak Creek watershed under both aerobic and anaerobic conditions. The major hypothesis to be tested is that changes of <1% per year in the total immobilized amounts of U, Tc, Cr, ^{90}Sr, and ^{137}Cs in soil can be detected *in situ* using such encapsulated soil specimens. Such temporal resolution will allow the observation of the asymptotic approach to the final immobilized fraction within each test soil. Precise contaminant retention performance in the field will provide, for the first time, a sensitive methodology to support selections and quantitative comparisons of both natural attenuation or soil contaminant immobilization techniques. Preliminary uptake and release of U and Th from PELCAPs at the FRC will be discussed.

Kinetics of Environmental Hydrogen Gas Production and Consumption in Groundwater

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A methodology is proposed in this new 2007 exploratory research project to simultaneously measure the kinetics of the ambient or unperturbed rates of formation and consumption of molecular hydrogen (H_2) in groundwater. Dissolved H_2 has been found in the ERSD Field Research Center (FRC, Oak Ridge) groundwater at alarmingly high concentrations, approaching 14% by volume of the equilibrium gas phase. Such environmental concentrations are about four orders-of-magnitude larger than those frequently reported for, and commonly assumed to be representative of, anoxic sediments. Although ambient H_2 concentrations in groundwater represent a net equilibrium balance between rates of production and consumption, the perturbed groundwater conditions around the old S3 ponds (low pH, high nitrate and organic carbon, and elevated dissolved sulfate and uranium) may be either inhibiting H_2 consumption or stimulating H_2 production, or both. Identification of the specific reaction rate that is stimulated or inhibited (and results in the elevated H_2) will lead to an understanding of how to manipulate the groundwater to reduce the dissolved H_2 to a less hazardous concentration. More importantly, if it is assumed that the physicochemical environment around the old S3 ponds is not unusual, then such elevated H_2 concentrations in groundwater may be occurring within many DOE and other disposed waste and natural configurations. The difficulties and specialized analytical equipment, previously considered to be required to analyze for H_2 in soils, sediments, and ground and surface waters, has resulted in a salient paucity of needed information on H_2 sources and sinks, and transfer rates throughout the entire global hydrogen cycle. Although our proposed research will be limited to exploratory research and highly focused on the immediate problem of H_2 genesis in the groundwater peripheral to the S3 ponds at the FRC, our kinetic methodology should be quite generally applicable to all “natural” systems, including surface water bodies, bogs, oceans, and geologic formations deep into the earth. Our planned dual labeling of closed systems (i.e., sealed bottles of water, soil, and gas) with deuterium and/or tritium isotopic labels, for both molecular hydrogen and water, will allow simultaneous measurement of H_2 production and consumption within the sealed bottle. Such a methodology will allow sealed bottles of groundwater to be perturbed with changed physicochemical conditions, to determine whether H_2 production or consumption is stimulated or inhibited. Identification of key reaction-rate effects could identify potential key control points on contaminant attenuation in groundwater and lead to further work on new remediation strategies for many contaminants susceptible to oxidative-reductive manipulations.

Scale Dependence of Reaction Rates in Porous Media

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Our research objective is to resolve the discrepancies between laboratory and field rates by improving our understanding of the scale dependence of reaction rates in natural porous media. This involves combining laboratory experiments targeting reactions of environmental and geological importance at a variety of scales, ranging down to the pore scale, with modeling to quantify the coupling of reaction and transport processes

To address the possibility that discrepancies between lab and field rates of mineral dissolution arise as a result of gradients in concentration at the pore scale, we developed a numerical model for a single cylindrical pore and validated it with microfluidic reactive flow experiments. The modeling indicated that discrepancies may develop when rates become scale dependent as a result of comparable rates of reaction and advective transport, and incomplete mixing via molecular diffusion. Analyzing the rates of three important subsurface reactions—calcite dissolution, dissimilatory Fe reduction, and plagioclase dissolution accompanied by clay precipitation—we found that the scale dependence is negligible at the pore scale in most cases of geological and environmental significance. The analysis has now been extended to fractures of much greater length (up to 10 m) to determine the conditions under which a scale dependence arises.

Capillary tubes are being used as pore-scale columns to address scaling effects along a single flow path through porous media. Initial experiments involve capillary tubes measuring 100 μm in diameter packed with glass spheres coated with 2-line ferrihydrite of 75 μm diameter. Subsequent experiments will include smaller spheres (10–15 μm) packed in the same 100 μm capillary tube. The capillary tubes are used in flowthrough mode to obtain steady-state rates where applicable, and to collect effluent for chemical analysis that can be compared with the results of X-ray synchrotron studies of reaction products. Both μ-EXAFS and μ-XRD on Beamline 10.3.2 at the Advanced Light Source have been used for quantification of solid-solid transformation rates. Two abiotic reaction pathways have been addressed to date: (1) injection of $FeSO_4$ solutions (3–20 mM) to determine the rate of conversion to magnetite, and (2) reduction of ferrihydrite by AH_2DS (the reduced form of the electron shuttle AQDS). At 20 mM $FeSO_4$, the conversion to magnetite is essentially complete in 30 hours, with the reaction rate quantified most effectively with μ-XRD. Subsequent experiments will investigate lower Fe(II) concentrations while manipulating the accompanying anion and its concentration, to (1) determine the threshold concentration for magnetite formation and (2) to ascertain whether green rust or ferrous hydroxide act as precursors for magnetite formation. Reaction with AH_2DS is much slower and results in the formation of a discrete zone close to the inlet, where the ferrihydrite is converted to goethite.

Integrating the Molecular Machines of Mercury Detoxification into Host Cell Biology

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Mercury (Hg) is mobile and toxic in all forms. Its geochemical mobility reflects abiotic and biotic processes; bacteria are key in the latter, and the widely found bacterial mercury resistance (*mer*) operon functions in Hg biogeochemistry and bioremediation by converting reactive inorganic [Hg(II)] and organic [RHg(I)] mercurials to relatively inert monoatomic mercury vapor, Hg(0). The key players control gene expression (MerR, MerD, MerOP), Hg uptake (MerT, MerP, and MerC), and demethylation (MerB) and reduction (MerA) of mercurials. We focus on how these components interact with each other and with host cell proteins and membranes to restore function in cells exposed to Hg compounds.

Regulation of mer Operon Expression: To observe MerR's ligand-specific behavior, we made 2-fluorotyrosine-substituted MerR. In the free protein changes in ^{19}F-Tyr, chemical shifts provoked by Hg(II), Cd(II), and Zn(II) were quite similar. However, DNA-bound MerR exposed to Hg(II) undergoes dramatic allosteric changes distinct from those of Cd(II) or Zn(II). Thus, the operator DNA, not just MerR, is a key player in metal recognition. NMR also reveal a 28 Å allosteric trajectory from the metal-binding site to the DNA binding site, including charge and hydrogen-bond interactions. These findings are corroborated by titration calorimetry, which shows a sharp pH-sensitivity, metal-specific thermodynamics and kinetics, and the dramatic effects of operator-DNA on metal binding by MerR. We also now have 4 Å structural data on the Hg-MerR-MerOP complex and are pursuing higher-resolution images. We will apply these techniques to determine how MerD antagonizes MerR's interaction with RNA polymerase at MerOP to turn off transcription, and also broaden our view of metalloregulation to the whole cell by determining the Hg-inducible transcriptome in cells with and without the *mer* operon.

MerA Core Interactions with NmerA: Curiously, the N-terminal metallochaperone domain of MerA, which delivers Hg(II) to the catalytic Core domain, is cleaved from the catalytic Core domain. There are three cleavage sites in a ~30 residue spacer between the NmerA and Core domains, and cleavage is neither autocatalytic nor dependent on any single ATP-dependent protease in *E.coli.* We have mutagenized at least one of these cleavage sites and are sequentially knocking them all out to observe the effect of loss of cleavage on MerA catalysis and Hg resistance. In addition, since our mutation of conserved His17 on NmerA to Asn reduced Hg(II) transfer in both directions between NmerA and MerA core, we have made site directed mutants in other potential interaction partners on the MerA core and are also generating a Hg(II)-crosslinked complex of C14A NmerA and AAAC MerA core mutants for crystallization—to define key contacts enabling this essential interdomain ligand-transfer step.

MerA Core Interactions with MerB: Since functionally important protein/protein interactions become optimized in a co-evolved protein pair, we used the organomercurial resistance operon of pDU1358 to clone the full-length MerA (and its separate catalytic core and NmerA domains), the natural partner for the MerB we are studying. To test for direct Hg(II) transfer, a Hg(II)-MerB complex was used as a substrate for pDU1358 MerA in steady-state assays in the absence of other thiols. We found that NmerA mediates Hg(II) transfer from MerB to the catalytic core. We are now examining transfer of organomercurial substrates from MerB to MerA and whether both proteins fold differently when expressed simultaneously. These studies rely on our high-resolution crystal structure of Hg(II)-product-bound-MerB, which shows that Cys96 and Cys159 are Hg(II)-ligands, as we predicted. The improvements over our earlier NMR structure reveal possible proton donors and a pocket for the substrate organic group, and the active-site-proximal region of MerB most likely to interact with NmerA.

Interactions of NmerA with Hg(II) membrane transporters, MerT and MerC: MerT proteins from three different operons and MerC from the Tn*21* operon have been expressed in good yield for structural and functional characterization in liposomes, including their interactions with cytosolic NmerA and periplasmic MerP.

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

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An extensive knowledge of the composition, dynamics, and metabolic potential of subsurface microbial communities is crucial to the development of scientifically grounded strategies for the reclamation and long-term stewardship of contaminated DOE sites. To date, the complex effect of environmental (both geochemical and biological) parameters on the bioremediative potential of subsurface microbial populations is only partially understood; this is primarily because the majority of microbial ecological studies have focused on a *qualitative* analysis of subsurface microbial diversity, while the impact of *quantitative* changes in microbial communities as a function of environmental factors has not been sufficiently addressed. The newly funded project described here integrates standard molecular phylogenetic analyses, rRNA-targeted fluorescence *in situ* hybridization, and mass spectrometry (MS)-based proteomics to investigate the biological response to experimentally controlled conditions and the concomitant effect on chromate reduction *in situ*. This response will be characterized in terms of microbial community structure (principally, population number and spatial distribution) and community proteome dynamics. Towards this overarching goal, we will (1) construct aerobic and anaerobic laboratory microcosms derived from subsurface soil collected from a Cr(VI)-contaminated DOE site, and introduce Cr(VI)-reducing *Pseudomonas putida* (a facultative anaerobe) and/or *Desulfovibrio desulfuricans* (an obligate anaerobe) into the extant microbial community of each microcosm (Objective 1); (2) determine the qualitative and quantitative effects of organic carbon amendments on microcosm community structure, metabolic activity, and, most importantly, biologically mediated chromate reduction (Objective 2); and (3) investigate the microbial community response from molecular and quantitative perspectives in microcosms challenged with increasing levels of chromate, in order to drive selection of optimal chromate-reducing populations (Objective 3).

This new ERSP project is a collaborative endeavor among scientists from Purdue University and Oak Ridge National Laboratory. A *unique* aspect of this project is the development and application of shotgun MS techniques as molecular ecology tools for metabolically profiling targeted microbial species within the context of complex environmental communities, as well as searching for unique or abundant proteins that might serve as indicators for heavy metal stress and reduction in microsites. Studies such as this—that tackle the challenge of characterizing the proteome dataset of a complex microbial microcosm—will be needed to push the capabilities of current MS methodologies and to develop the next generation of microbial ecology tools for use in assessing, and ultimately enhancing, bioremediation performance. We expect that the experimental protocols and results generated in this project will expand our quantitative and mechanistic understanding of the *in situ* biological contributions to metal contaminant transformation.

Microcantilever Sensors for *In Situ* Subsurface Characterization

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Deployment of inexpensive, miniature, real-time sensors capable of multi-analyte detection with speciation will play a crucial role in characterization, monitoring, and long-term stewardship of subsurface systems. Microfabricated cantilever sensors offer several advantages, including low-power consumption, *in situ* and real-time operation, extreme high sensitivity, and integration into arrays for multi-analyte detection. Analyte molecule adsorption on a cantilever surface results in cantilever bending when adsorption is confined to a single side. Chemical speciation in sensing is achieved by immobilizing a selective self-assembled monolayer on the cantilever surface. Selectivity for certain analytes can be achieved by using electrochemistry, in which the cantilever undergoes bending as a result of charge transfer processes, producing bending as a function of a cyclic voltage ramp similar to an I-V curve in cyclic voltammetry. Though a microcantilever platform has been demonstrated for *in situ* detection of individual analytes, a microcantilever array capable of simultaneous detection of multiple analytes, its integration with wireless telemetry, and field deployment are not yet demonstrated. Ideal candidate chemicals of interest fall into two general groups; heavy metals (lead, chromium(VI), mercury, zinc, beryllium, arsenic, cadmium, and copper), and transuranic elements (uranium, neptunium, and plutonium). Although these two general groups and the members of each group are chemically different and have no general molecular recognition characteristics in common, they do have one characteristic in common, in the sense that many are electrochemically active. Many of the heavy metals (generally RCRA waste) exist in groundwater or in mixed wastes as oxidized ionic species (Pb(II), Cr(VI), Hg(II), Cd(II), and Cu(II)) that can be electroplated on gold, platinum, or nickel surfaces. Therefore, SAM coatings, together with electrochemical cantilevers, offer a simple method of achieving selectivity as well as pre-concentration.

We have developed SAM coatings that can be used for selective detection of Cs, Hg(II), methyl-Hg(II), Cu(II), Cr(VI), and Cd(II). These SAMs were immobilized by gold-coated silicon cantilevers using thiol chemistry. Initial responses using these SAM layers show extreme high selectivity and robustness. Electrochemical cantilevers have been demonstrated for Cr(VI) and Pb(II). Preliminary results were obtained using optical beam deflection as a signal transduction method. We have designed and developed piezoresistive cantilevers in which cantilever bending results in electrical resistance variation. These silicon piezoresistive cantilevers are electrically insulated with a thin coating of silicon nitride, so that they can be used in aqueous media. The insulated piezoresistive cantilevers will be employed in an array format for simultaneous detection of multiple analytes in mixtures. Cantilevers will also be externally coated with a gold layer for immobilization of SAM. In addition, we have also designed and developed a readout electronic chip that can monitor the response from ten cantilevers simultaneously. We plan to integrate the cantilever platform, readout electronic, and wireless telemetry into a miniature device that can selectively and sensitively detect multiple analytes simultaneously, using battery power.

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

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Molecular microbial ecology methods, physiological studies, and microscopy analysis were used to study the microbial communities involved in the subsurface sediment bioremediation at the Oak Ridge Field Research Center (FRC), including the physiology of *Desulfitobacterium hafniense* DCB-2 when reducing uranium and other relevant metals. Six 16S rRNA gene clone libraries from sediments within the reducing zone in Area 3 were constructed to analyze the microbial community diversity and structure. Bacteria belonging to known uranium-, iron-, sulfate- and nitrate- reducing genera were detected, including *Geobacter, Desulfovibrio, Acidovorax,* and *Anaeromyxobacter*. Whole community comparisons across different wells showed pronounced heterogeneity between communities, suggesting that the well communities are quite different from each other. In spite of community differences, uranium in groundwater dropped to below drinking water standards across the treated zone. Differences in the structure and the diversity-based clustering were better explained when considering the groundwater hydrologic flow depicted by tracer studies. The geology of the site is likely to influence the structure and function of microbial communities by defining gradients of electron donors and acceptors, and should be considered when evaluating the present and future performance of the communities.

Physiological studies of the Gram-positive, *Desulfitobacterium hafniense* DCB-2 are also under way to understand its abilities to reduce heavy metals and uranium. This anaerobic, spore-forming motile rod has been found in contaminated soils at Oak Ridge and was previously isolated for its ability to dehalogenate. Previous studies focused on the abilities of *D. hafniense* to reduce the heavy metals iron and selenium. Recent observations focus around the capabilities of *D. hafniense* to reduce uranium. The response of *D. hafniense* to uranyl is of particular interest, because of its ability to not only tolerate, but to reduce, uranyl acetate at concentrations up to 750 μm. Under these toxic conditions, growth was relatively rapid, and viable cells were found to proliferate on uranium precipitates. These results portray *D. hafniense* as a versatile physiologically robust organism and a good Gram-positive model for bioremediation of metal contamination including uranium.

To understand how contaminants affect microbial community structure, we sequenced the microbial community from an FRC groundwater sample contaminated with very high levels of nitrate, uranium, and other heavy metals and pH ~3.7. As with trends expected in stressed ecosystems, the metagenome reveals a community of low species and strain diversity dominated by a single *Frateuria*-like γ-proteobacteria, with other γ- and β-proteobacteria present at low proportions. Single nucleotide polymorphism (SNP) analysis revealed a low level of polymorphism, with the overwhelming majority of SNP representing unique changes within the assembled reads, suggesting that the strains in the sample are largely clonal. Metabolic reconstruction reveals specific adaptations to the geochemical conditions of FW106, including genes encoding metal resistance (*czcABC*, *czcD*, *cadA*, *merA*, *arsB*), denitrification, and solvent resistance (1,2-dichloroethene, acetone, butanol). Certain resistance genes appear to be overrepresented in the metagenome, including genes for nitrate/nitrite transport (*narK*) and metal translocation (*czcABC*, *czcD*, *cadA*). This accumulation of genes appears to have resulted from a combination of gene duplication and lateral gene transfer. A genome-wide positive selection screen shows that most of these transporters appear to be under strong negative selection, suggesting that in the short term, the overabundance of these transporters provides a positive fitness benefit to the cell, by increasing the baseline rate of ion transport.

Mesoscale Biotransformation of Uranium

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Remediation and long-term stewardship of uranium-contaminated sediments and groundwaters are critical problems at a number of DOE facilities and mining sites. Some remediation strategies based on *in situ* bioreduction of U are potentially effective in significantly decreasing U concentrations in groundwaters. However, a number of basic processes require understanding in order to identify environments where reduction-based U stabilization is more likely to succeed. Our current research targets several of these issues, including: (1) effects of organic carbon (OC) forms and supply rates on stability of bioreduced U, (2) the roles of Fe- and Mn-oxides as potential U oxidants in sediments, and (3) microbial community changes in relation to U redox changes. Most of our studies are being conducted on historically U-contaminated sediments from Area 2 of the ERSD Field Research Center (FRC), Oak Ridge National Laboratory, in flow-through columns.

The rate of OC supply is a critical factor in U reduction, not only in determining the rate of electron donor supply, but also in determining the resulting concentration of aqueous (bi)carbonate generated by microbial respiration. As shown in our previous ERSP research, increased (bi)carbonate concentrations drive aqueous U(VI) concentrations to higher levels through formation of stable U(VI) carbonato complexes, including $Ca_2UO_2(CO_3)_3(aq)$. Thus, the influence of OC supply rate on U reduction is more complex than previously assumed. Redox transformations of U are being tested in new FRC2 sediment columns supplied with OC at rates ranging from 0 to 580 mM (kg sediment)$^{-1}$ year^{-1}. These columns are being supplied with either lactate or acetate, and have been running for over 400 days. The effluent U concentrations do in fact show complex but very reproducible, nonlinear dependence on the OC supply rate, consistent with OC oxidation having the dual impact of driving the reduction of U and the formation of U(VI)-carbonato complexes. This portion of our study also shows that lactate and acetate have the same geochemical impact on effluent U concentrations (and all other measured chemical species), when compared on the basis of supply rate of C.

We identified several factors that point to a residual reactive Fe(III) fraction in these sediments that likely serves as the terminal electron acceptor for U reoxidation. We are conducting even longer-term column incubations targeted at completely reducing the reactive Fe(III) fraction in sediments, micro- X-ray absorption spectroscopy for determining distributions of Mn, Fe, and U oxidation states in sediments at various stages of OC-stimulated bioreduction, and use of chemical methods for determining concentrations of Fe(II) and Fe(III) in sediments and pore waters.

We analyzed the structure of the stimulated microbial communities in columns receiving ten different OC supply treatments at two time points; during a phase of net U-reduction and during a later phase of U-reoxidation and remobilization. Community analysis using a high-density 16S microarray (16S Phylochip) indicates that OC supply rate is the primary determinant of the bacterial community composition, and that significant shifts in community dynamics occur between the U-reduction and remobilization phases.

The Role and Regulation of Melanin Production by *Shewanella oneidensis* MR-1 in Relation to Metal and Radionuclide Reduction and Immobilization

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Humic compounds (byproducts of the natural degradation of organic matter) are known to accelerate the process by which microorganisms transfer electrons to (i.e., reduce) toxic metals, thereby decreasing metal toxicity and mobility. The pigment pyomelanin is a particularly important humic compound in this process. It is produced by bacteria in the genus *Shewanella*, which occur in subsurface soil. Pyomelanin associated with the bacterial surface increases hydrous ferric oxide reduction rates by as much as tenfold (*FEMS Microbiol. Lett. 220*, 99–104, 2003). This is accomplished because, under anaerobic conditions, pyomelanin serves as a terminal electron acceptor and soluble electron shuttle to iron minerals (*Appl. Environ. Microbiol. 68*, 2436–2444, 2002). The capacity for some species of bacteria to reduce metals offers a strategy for bioremediation of metal- and radionuclide-contaminated environments. One of the most challenging forms of metal contamination is that which occurs when the contaminant metal is associated with solid-phase minerals. The overall hypothesis of this investigation is that Pyomelanin production in the genus *Shewanella* is related to external tyrosine concentrations and plays a significant role as a mechanism of electron transfer to solid-phase metals, resulting in immobilization of these inorganic contaminants.

An understanding of the role of pyomelanin in metal reduction may lead to technologies for accelerated remediation rates of solid-phase metal and radionuclide contamination in the environment. We have confirmed the role of tyrosine in pyomelanin production and demonstrated increased rates of metal oxide reduction by a pyomelanin-overproducing mutant compared to a pyomelanin-deficient mutant. Based on electrochemical studies of whole cells, the presence of bacterial-produced pyomelanin increased the rate and amount of electrons transferred from the cell surface to a solid electrode. The primary focus of our continued investigation is on the role of pyomelanin on solid-phase metal and radionuclide reduction. We expect to demonstrate that pyomelanin-based electron shuttling and its associated metal chelation properties contribute significantly to biogeochemical activity at the microbe-mineral interface.

Coupled Hydrological-Geochemical Studies of Hanford Vadose Zone Uranium Plumes

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Massive quantities (exceeding 85 tons) of U currently reside in the Hanford vadose zone, and will threaten groundwater and the accessible environment for generations to come. Understanding the status and mobility of the contaminant U in the vadose zone is extremely difficult, because of the insufficient historical records, sediment heterogeneity, and complexity of the strongly coupled hydrological and geochemical processes. The objectives of this research are to identify the dominant geochemical reactions and transport processes between waste streams and sediments occurring during seepage and upon aging. This knowledge is important because the major reactions and transport processes largely determine the current spatial distribution, speciation, and mobility of U within the Hanford plumes.

To achieve these goals, we constructed a field process-based column profiling method. Using this laboratory method, we simulated the Tank BX-102 over-filling event of 1951 (the largest single accidental release of radioactive waste at the Hanford Site, which left 7–8 tons of hexavalent uranium in the vadose zone, some of which has reached groundwater). We synthesized the historical tank waste solution containing extremely high levels of U (0.11 M U in alkaline brine), and conducted infiltration experiments under well-defined conditions as relevant to the field release event as possible. Flow rate and aging time were chosen as the main variables in these experiments. Analyses of aqueous and solid phases from these experiments were used to obtain profiles of U concentrations and speciation along the plume paths.

In recent results, several processes demonstrated in these laboratory experiments have shown direct relevance to U-contaminated sediments such as those at the Hanford 200 Area. Dramatic pH reducing occurred at the plume fronts. The pH within a U plume was shown to vary from its waste solution value (pH 10.4) at the point of discharge, down to pH~7.0 at the moving plume front. Large peak values of Ca^{2+} and Mg^{2+} concentrations were found within plume profiles, resulting from rapid displacement of cation exchange sites by high concentrations of Na^{+}. Maxima in concentrations of these divalent cations, along with pH minima, are indicators of the distance of waste-plume migration. Accelerated U transport was revealed through measured U concentration maxima at plume fronts that exceeded their source levels by up to 5-fold. Thus, peaks in U concentrations in contaminated sediments can be expected from fast U transport and accumulation at plume fronts. Kinetic limitations on sorption and precipitation permitted practically unretarded U transport at higher flow rates. Transport of U in waste plumes was strongly dependent on flow rate.

In general, this laboratory study shows that the rate of waste infiltration must have been a primary factor behind the observed deep transport of U in the Hanford vadose zone, and that predictions based on equilibrium K_d partitioning of U would greatly underestimate the extent of U migration.

Molecular-Level Investigations of Nucleation Mechanisms and Kinetics of Formation of Environmental Nanoparticles: Results from Silicate on Hematite and Iron Oxide on Quartz

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Environmental nanoparticles are often poorly crystalline or metastable structures, whose kinetics of formation and growth are poorly understood. Further, the sorption or growth of nanoparticles on mineral surfaces may control the mineral surface's reactivity and modify its ability to influence contaminant transport. Because of the characteristic length scale, a holistic understanding of the nucleation mechanisms and kinetics of nanoparticle formation on mineral surfaces is difficult to achieve with traditional methodology. In this work, our intent is to determine the molecular nature of nucleation on surfaces, the kinetics of surface nucleation and growth, and the effect of crystal surface topology using new synchrotron-based techniques.

We have approached these objectives by: (1) combining state-of-the-art crystal-truncation rod diffraction (CTR) and grazing incidence X-ray absorption fine structure spectroscopy (GIXAS) techniques to investigate the three-dimensional molecular-scale geometry of silicate monomer sorption on the r-plane of hematite; and (2) developing a new grazing-incidence small angle x-ray scattering (GISAXS) setup at SSRL ($0.08\ nm^{-1} < q < 8\ nm^{-1}$) to explore the initial development of environmental nanoparticles on various mineral surfaces. This study also includes complementary techniques such as atomic force microscopy (AFM), bulk SAXS, dynamic light scattering (DLS), XRD, and TEM.

Recent results show that, from both nonspecular and specular CTR data, the silicate adsorption geometry on hematite surfaces can be obtained in three-dimensions. CTR intensity changes resulting from silicate adsorption are evident in the ($00L_s$), ($02L_s$), ($10L_s$), and ($11L_s$) rods when compared to those from a clean surface, thus demonstrating ordering of silicate sorbates on the hematite surface. The preliminary best-fit model is a monodentate mononuclear complex, perhaps analogous to silicate-containing natural mineral structures. Complementing this work, the first Si K-edge GIXAS data were successfully collected and are being analyzed.

In the other part of our study, our initial GISAXS work with iron oxide nanoparticles on quartz constitutes the first environmental application of GISAXS, and demonstrates our ability to detect low concentrations of sorbed 7.2 ± 0.6 nm diameter nanoparticles. Our results agree with simulations of nanoparticle GISAXS on substrates, and is further confirmed via DLS, bulk SAXS, and AFM. Most recently, using *in situ* GISAXS, we have been able to observe the nucleation and growth of iron oxide nanoparticles on quartz surfaces. This technique can provide statistically improved morphological information of environmental nanoparticles compared with AFM and SEM, and allow real-time geochemical kinetics analysis of nanoparticle growth and reactions.

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

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

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

We prepared a batch of NAPL and wastewater representative of material disposed of at the Hanford site. We called this combination MIX1. Two additional mixtures are planned (MIX2, MIX3), as well as experiments to age these mixtures. The organic phase of MIX1 includes carbon tetrachloride, DBBP, TBP, and lard oil. The wastewater phase contains nitric acid, various nitrate salts, and sodium hydroxide. Interfacial tension and contact angle on quartz glass slides were measured for MIX1, and compared to results from a pure carbon tetrachloride/nanopure water mixture. Interfacial tension of MIX1 is markedly reduced (5x) compared to the pure mixture case. Similarly, the contact angle of MIX1 is reduced. Results with individual components of MIX1 indicate that lard oil and wastewater components only marginally affect results; the additive DBBP is primarily causes changes in wettability.

During this coming year, we will prepare the additional NAPL-wastewater mixtures (MIX2, MIX3). For all three NAPL-wastewater mixtures (MIX1, MIX2, MIX3) we will use both amended nanapure water (that has no microbial activity) and amended Hanford groundwater (that has microbial activity). We will complete measurements of interfacial tension and contact angles for all fresh NAPL-wastewater mixtures, and we will begin to measure these parameters for aged mixtures. This summer, we will measure capillary-pressure saturation profiles of all fresh NAPL-wastewater mixtures at PNNL. We will also complete fabrication of the homogeneous micromodel and run mass-transfer experiments using the fresh NAPL-wastewater mixtures, as well as perform transient column experiments using the fresh NAPL-wastewater mixtures. Finally, we will complete modification of STOMP to include phase partitioning and interphase mass transfer of multicomponent NAPLs.

Microscopic Controls on the Desorption/Dissolution of Sorbed U(VI) and Their Influence on Reactive Transport

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This project was first initiated in FY 2003. Over its course, eight manuscripts have been published on the speciation of U(VI) in two different Hanford waste sites and the desorption/dissolution behavior of sorbed U(VI) from contaminated vadose zone sediments. Project scope was revised in light of the CY 2005 EMSP call to which a successful renewal proposal was submitted. The new research that began in FY 2006 will investigate the kinetics of U(VI) dissolution and desorption and the scaling of reaction rates, using a unique suite of U(VI)-contaminated sediments from the Hanford 300 A whose speciation was studied in the first project. Shallow sediments from this location contain co-precipitated U(VI) with calcite, intermediate depth sediments contain precipitated U(VI) as metatorbernite, while the deepest sediments contain an adsorbed U(VI) species. Project focus is to understand how the chemical/physical state of "sorbed" U(VI) in long-term contaminated sediments controls future plume migration.

The research will (1) identify physical (e.g., diffusion) and geochemical controls (e.g., molecular speciation) on U(VI) reaction kinetics at the microscopic scale, (2) parameterize microscopic rate laws of controlling geochemical reactions and mass-transfer rates, and (3) evaluate how the complex, derived microscopic rate laws may be scaled to U(VI) reactive transport in meter-length columns with coarse, field-textured sediment. Detailed characterization measurements on the sediments using state-of-science microscopies and spectroscopies, and batch and column experimentation, will parameterize a rigorous, reaction-based, subgrid model that will be imbedded in a dual continuum, reactive transport model. Additional experimentation will explore the coupling of kinetic geochemical processes and water advection using columns of increasingly coarse sediment. Iterative comparisons of model simulations with experimental results of large column studies will allow the evaluation of a central project hypothesis on the scaling of mass-transfer rates.

At the 2007 ERSP program meeting, we will describe speciation measurements performed on select U-contaminated 300 A sediments using bulk, extended X-ray adsorption fine structure (EXAFS), micro-EXAFS, and x-ray microprobe, and cryogenic laser-induced fluorescence spectroscopy (CLIFS). These speciation measurements are used to interpret wet-chemical results of batch desorption experiments with different size fractions of the sediments, and column dissolution/desorption experiments with the <2.0 mm sediment fraction, which reveal complex kinetic behavior controlled by either mass-transfer or chemical-kinetic limitations. Lastly, issues of reaction network "scale-up" are highlighted by presenting the results of a large column experiment in which the long-term desorption of contaminant U(VI) was investigated in field-textured materials dominated by coarse river cobble. Several reactive transport models are applied to describe the data.

GeoChip: Development and Applications for Microbial Community Analysis

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Microarray technology provides the opportunity to identify thousands of microbial genes or populations simultaneously. Recently, a comprehensive functional gene array, called GeoChip, has been developed, evaluated, and applied for characterizing microbial communities in natural systems. GeoChip 2.0 contains 24,243 oligonucleotide (50mer) probes and covers >10,000 genes in >150 functional groups involved in nitrogen, carbon, sulfur, and phosphorus cycling, metal reduction and resistance, and organic contaminant degradation. It is a powerful generic tool and can be used for: (1) profiling various environmental samples, such as soil, groundwater, sediments, oil fields, deep sea, animal guts, etc; (2) studying biogeochemical processes and functional activities of microbial communities important to human health, agriculture, energy, global climate change, ecosystem management, and environmental cleanup and restoration; (3) exploring direct linkages of microbial genes/populations to ecosystem processes and functions; and (4) detecting functional genes and/or organisms in a particular environment. Here, we present two application examples on the dynamics and stability of microbial genes and associated communities during a bioremediation period at the ERSD Field Research Center (FRC) at Oak Ridge and the Hanford site. GeoChip 2.0 was first used to track the dynamics of metal-reducing bacteria and associated communities for an *in situ* bioremediation at the FRC site. Samples were taken from different wells after ethanol injections (after Day 166). During the uranium reduction period, both FeRB and sulfur-reducing bacteria (SRB) populations reached their highest levels at Day 212, followed by a gradual decrease over 500 days. Consequently, the uranium in groundwater and sediments was reduced, and the uranium concentrations in the groundwater were significantly correlated with the total abundance of *c*-type cytochrome genes from *Geobacter*-type FeRB and *Desulfovibrio*-type SRB. Mantel tests also indicated that there was significant correlation between the differences of uranium concentrations and those of total c-cytochrome gene abundance or *dsrAB* gene abundance. These results suggested that *Geobacter*-type FeRB and SRB played significant roles in reducing uranium to a level below the drinking standard (<30 μg/L). GeoChip 2.0 was also used to evaluate functional communities at a lactate-fed chromium reduction system at the Hanford site. Extraction well samples showed higher numbers of functional genes than the injection well at the same depth. Within the extraction well, abundance decreased with depth. However, the relative abundance of chromium resistant genes increased with depth in this same well. All results demonstrate that GeoChip is a useful tool for analysis of microbial communities in natural systems.

GeoChip 2.0 is the most comprehensive functional gene array currently available for environmental studies, but because of exponential increases in the number of genes and sequences for each gene, a new generation of this array (GeoChip 3.0) is in development. GeoChip 3.0 is expected to have the following new features: (1) it is more comprehensive and representative, and covers >37,700 gene sequences of 290 gene families, including the phylogenetic marker, *gyrB*; (2) the homology of automatically retrieved sequences by key words is verified by HUMMER, using seed sequences so that unrelated sequences are removed; (3) a software package (including databases) has been developed for sequence retrieval, probe and array design, probe verification, array construction, array data analysis, information storage, and automatic update, greatly facilitating management of such a complicated array, especially for future updates; and (4) it includes GeoChip 2.0 probes, and those probes are checked against with new databases.

A New Method for Estimating Soil Hydraulic Parameter Uncertainty, and Heterogeneity Using Bayesian Updating and Neural Network Methods

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This project involves extending a Bayesian updating method, developing innovative artificial neural networks (ANN), postulating a new Bayesian geostatistical inference method, and combining these three methods to estimate heterogeneous soil hydraulic parameter fields. Specific objectives are to:

(1) Extend a Bayesian updating method to estimate DOE's Hanford site-specific probability distributions and associated statistics of hydraulic parameters for uncertainty analysis. The extension will eliminate the assumption that the parameters follow normal distributions.

(2) Develop innovative ANN-based pedo-transfer functions (PTFs) to estimate soil hydraulic parameters using "soft" data (e.g., particle size distribution and soil texture and bulk density). The PTFs will incorporate parameter distributions obtained from the extended Bayesian updating method and eliminate the artificial correlation of output hydraulic parameters.

(3) Propose a new Bayesian geostatistical inference method to estimate spatial correlation scale of inputs to the developed neural network models. Heterogeneous input fields will be generated and fed to the neural network models to result in heterogeneous fields of soil hydraulic parameters that can be used for numerical simulation and uncertainty analyses.

Seventy-point data sets ("hard" and "soft" data) have been gathered and compiled for the Hanford site. Using cokriging and the ANN approaches, site-specific ANN-based PTFs have been developed in conjunction with a Bayesian method. The ANN uses the cokriged heterogeneous fields of pedotransfer variables as input to generate heterogeneous fields of the soil hydraulic parameters. Based on the collected data, we have conducted preliminary studies to identify probability distributions for the parameters and to estimate posterior distributions of correlation scales. To further incorporate the parameter statistical distributions and correlations into the PTFs, we have formulated synthetic case studies by generating synthetic hydraulic parameter fields from existing regression PTFs in the literature. A few examples of adding the parameter means and variances into the ANN's objective functions have been investigated and evaluated. Results indicate that adding parameter statistics into objective functions improves ANN's performance.

Bayesian updating algorithm will be used to update the parameter distributions without the restriction that they follow normal distributions. The estimation of posterior distributions of the parameter correlation scales will be continued. After the synthetic case testing and evaluations, we will then use the parameter data sets from the available field measurements for extensive ANN training and validation. The ANN training and validation will be carried out in two scenarios, similar to the synthetic cases considered earlier, i.e., incorporating hydraulic parameter distributions as well as both hydraulic parameter distributions and correlations into the ANN objective functions. Using the updated parameter distributions and the estimated posterior distributions of correlation scales, in combination with the newly developed ANN-based PTFS, we will be able to generate spatially heterogeneous random hydraulic parameter fields.

Integrated Field-Scale Subsurface Research ChallengeS (IFC)

Multiscale Investigations on the Rates and Mechanisms of Targeted Immobilization and Natural Attenuation of Metal, Radionuclides, and Co-Contaminants in the Subsurface

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The ERSD Field Research Center (FRC), located on the Oak Ridge Reservation (ORR) in Tennessee, is the site of an integrated research project that seeks to improve the scientific understanding of contaminant fate and transport at multiple temporal and spatial scales and produce practical predictive tools applicable to numerous DOE sites. The primary objective is to advance the understanding and predictive capability of coupled hydrological, geochemical, and microbiological processes that control *in situ* transport, remediation and natural attenuation of metals, radionuclides, and co-contaminants across multiple scales, ranging from molecular to watershed levels. The proposed research focuses on determining the key coupled hydrobiogeochemical factors that control the fate and transport of uranium, technetium, and co-contaminant nitrate within spatially distributed source zones and groundwater plumes at the FRC. Factors of primary investigation include pH, electron donor utilization, and redox conditions along hydrologic pathways and within specific transition zones. The specific objectives are to (1) quantify recharge and other hydraulic drivers for groundwater flow and dilution of contaminants along flow pathways and determine how they change temporally and spatially during episodic events, seasonally, and long term; (2) determine the rates and mechanisms of coupled hydrological, geochemical, and microbiological processes that control the natural attenuation of contaminants in highly diverse subsurface environments, over scales ranging from molecular to watersheds; (3) explore novel strategies for enhancing the subsurface stability of immobilized metals and radionuclides; (4) understand the long-term impact of geochemical and hydrologic heterogeneity on the remobilization of immobilized radionuclides; and (5) improve our ability to predict the long-term effectiveness of remedial activities and natural attenuation processes that control subsurface contaminant behavior across a variety of scales. Experimental efforts will use a combination of geophysical, chemical, microbial, and hydrological strategies to interrogate contaminant pathways and quantify active attenuation or transition zones to obtain *in situ* attenuation rates and mechanisms. This information will be coupled with multiprocess numerical models to predict the influence of natural attenuation processes on long-term site performance, and to determine if active manipulation is required to control contaminant plumes. Several assisted attenuation or manipulation strategies will be tested at the pilot scale within the complex contaminant plumes and will include (1) sustained *in situ* bioreduction for U/Tc immobilization, (2) adjustment of groundwater pH for enhanced removal of U/Tc via enhanced microbial metabolism and mineral co-precipitation, and (3) organophosphate and oleate amendments to enhance U/Tc precipitation processes. Groundwater recharge investigations will also be conducted in an effort to alter and decrease groundwater and associated contaminant fluxes along various contaminant pathways at the FRC. These research findings will be analyzed using a multiscale computer model that will enable evaluation of field-scale remediation performance and its uncertainty for alternative strategies for the highly contaminated groundwater plumes at the Oak Ridge Y-12 site and for other complex DOE-managed sites. Active participation in the DOE Oak Ridge Reservation (ORR) Groundwater Technical Core Team by the FRC manager and principal investigators will assure remediation planning needs are addressed, and technical insights are transferred into DOE ORR remediation efforts, in a timely and constructive manner.

Microbiological, Geochemical, and Hydrologic Processes Controlling Uranium Mobility: An Integrated Field-Scale Subsurface Research Challenge Site at Rifle, Colorado

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The U.S. Department of Energy faces the challenge of cleaning up and/or monitoring large, dilute plumes contaminated by metals, such as U and Cr, whose mobility and solubility change with redox status. At the Uranium Mill Tailings Site in Rifle, CO, field-scale experiments with acetate as the electron donor have stimulated metal reducing bacteria to effectively remove uranium [U(VI)] from groundwater. The shallow depth to groundwater (3–4 m), thin saturated zone (~2.5 m), and well-defined groundwater flow system at the Rifle site facilitated the monitoring of microbial and geochemical processes, which led to two important findings: the transition from iron reduction to sulfate reduction significantly decreased the U(VI) bioreduction rate, and U(VI) removal from groundwater continued for 18 months, actually increasing after acetate amendment was terminated. Understanding these behaviors in the context of site-specific hydrologic, geochemical, and biological processes and conditions is critical to the design of optimal biostimulation strategies for prolonging uranium bioremediation.

The objective of the research planned for the Rifle site is to gain a comprehensive and mechanistic understanding of the microbial factors and associated geochemistry controlling uranium mobility. By doing so, DOE can more confidently remediate uranium plumes as well as support long-term stewardship of uranium-contaminated sites. Specifically, we propose to test four hypotheses that address knowledge gaps in the following areas: (1) geochemical and microbial controls on stimulated U(VI) bioreduction by iron-reducers, (2) U(VI) sorption under Fe-reducing conditions, (3) post-biostimulation U(VI) stability and removal, and (4) rates of natural bioreduction of U(VI). The approach specifically targets new knowledge that can be translated into scientifically defensible flow and reactive transport process models of microbially mediated and abiotic reactions, taking a major step toward ERSP's long-term goal to "…incorporate coupled biological, chemical and physical processes into decision making for environmental remediation." Hypotheses will be tested with a focused set of field and lab experiments that use the recently developed sciences of proteogenomics and stable isotope probing to track microbial metabolic status and specific organisms responding to acetate amendment. We will directly relate this information to changes in Fe redox status and sulfide minerals, with field-scale changes detected by noninvasive hydrogeophysics, including 3-D complex resistivity tomography. A key facet of the planned research is the linkage of microbial gene and protein expression with geochemical and geophysical changes during *in situ* biostimulation. This linkage will enable optimization of controllable factors such as electron donor concentration and identification of nutrient limitations, which, if relieved, could further enhance long-term bioremediation of redox-sensitive metals.

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

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The Pacific Northwest National Laboratory and a group of collaborators will use the Hanford 300 Area uranium (U) plume in waste management area 300-FF-5 as a site for an Integrated Field-Scale Subsurface Research Challenge (IFC). Multiscale mass-transfer processes are our scientific theme. A series of forefront science questions on mass transfer are posed for research, questions which relate to the effect of spatial heterogeneities; the importance of scale; coupled interactions between biogeochemical, hydrologic, and mass-transfer processes; and measurements/approaches needed to characterize and model a mass-transfer-dominated contaminant system. Three site-specific hypotheses will be evaluated that take advantage of the interesting contaminant geochemistry and hydrogeologic attributes of the site. The hypotheses will focus on multiscale mass-transfer processes in the vadose zone and saturated zone, their influence on field-scale U(VI) biogeochemistry and transport, and their implications for natural attenuation and remediation.

An innovative experimental site has been designed that represents a transect from waste sources in the vadose zone, through contaminated aquifer regions, to the nearby Columbia River. It will be rigorously characterized through borehole sampling and geophysical measurements. The site experiences seasonal changes in groundwater flow direction and chemical composition, allowing the study of chemical and mixing gradients, as well as flow paths of different trajectory. Adsorption and desorption processes of U(VI) within the contaminated aquifer show strong kinetic behavior as a result of phenomenology that will be studied by the project. Science and experimental collaborations, as well as leveraged facilities use and sharing, are planned with an EM-22 project that evaluates the feasibility of polyphosphate-induced autunite precipitation to mitigate U(VI) discharge from this plume to the Columbia River. The IFC will proactively publish results in high-impact scientific journals, support collaborations with external ERSD investigators, and transfer data, knowledge, and coupled models to the Hanford site during and after the term of the project. Funding for the Hanford IFC will begin in the second half of FY 2007.

STUDENT PRESENTATIONS

Determination of the Metabolically Active Microbial Groups in Contaminated Subsurface Sediments of the ERSD Field Research Center in Oak Ridge, Using Stable Isotope Probing (SIP)

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In co-contaminated subsurface sediments of the ERSD Field Research Center (FRC) at Oak Ridge, metal- and nitrate-reducing bacteria often mediate electron flow, thereby controlling the fate and transport of radionuclides. However, there is no consensus on the composition and distribution of active community members in the FRC subsurface that are likely to effect bioremediation potential. Ethanol has been shown in laboratory- and field-based bioremediation studies to promote the biological reduction and immobilization of U(VI) at the FRC. Therefore, the goal of this study was to employ stable isotope probing (SIP) techniques to directly link the phylogenetic structure and function of subsurface microbial communities that utilize ethanol as a carbon substrate/ electron donor. To characterize the metabolically active microbial community, we amended FRC Area 2 sediment microcosms with ^{13}C-labeled ethanol and monitored electron acceptor (NO_3^-, Fe(III), and U(VI)) and donor utilization. At selected time points corresponding to phases of terminal electron accepting processes, ^{13}C incorporation into community DNA was examined by density gradient centrifugation, along with PCR amplification and terminal restriction fragment length polymorphism analysis (TRFLP). Incorporation of the ^{13}C-labeled substrate into microbial biomass was detected by Day 3, which corresponded to the onset of nitrate reduction. With the depletion of NO_3^-, metal reduction commenced with U(VI)-reduction preceding Fe(III) reduction. The predominant and active denitrifying microbial groups were identified as members of the *Betaproteobacteria* (*Dechloromonas, Azoarcus, Alcaligenes, Ralstonia*, and *Diaphorobacter*) as they assimilated ^{13}C-ethanol during the nitrate-reduction phase of our incubations. Members of the phyla *Actinobacteria, Firmicutes* and *Bacteroidetes* were also shown to be active during various phases of the incubations. Statistical analysis of TRFLP profiles revealed differences between the microbial communities present during nitrate and metal reduction. These results indicate that SIP methods can be used successfully to couple the composition and function of microbial communities in FRC subsurface sediments. Our data also suggest that members of the *Betaproteobacteria* and *Actinobacteria* may play an important role in nitrate removal and subsequent metal reduction, although further work is needed to elucidate the biogeochemical processes mediated by the microbial groups that incorporated ^{13}C-labeled ethanol.

Role of Speciation on U(VI) Biomineralization in Acidic and Aerobic Conditions

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The bioremediation of uranium in a contaminated subsurface, such as at the ERSD Field Research Center (FRC) at Oak Ridge, is made especially difficult by environmental conditions. High concentrations of nitrate and calcium in the subsurface, along with aerobic and acidic conditions, can hinder the bioreduction of U(VI) to the insoluble uraninite. Additionally, uraninite may be reoxidized to U(VI) by the introduction of oxygen to the system or through abiotic interactions with Fe(III) and nitrite. Biomineralization, or the precipitation of U(VI) minerals facilitated by the enzymatic activities of microorganisms in oxic conditions, offers an alternative to the more extensively studied bioreduction. Our research has demonstrated that two aerobic heterotrophic bacterial isolates from the FRC can hydrolyze an organophosphate substrate to produce sufficient orthophosphate and precipitate uranyl phosphate. Several factors, including pH, organic ligand concentration, and sorption to mineral surfaces, can affect uranyl speciation and subsequently uranyl precipitation. The objective of this study is to examine the effects of U(VI) speciation on the biomineralization of uranium in aerobic conditions.

Below circumneutral pH in oxic conditions, the speciation of U(VI) in natural systems is affected by hydrolysis, precipitation/dissolution, and sorption/desorption, as well as complexation with soil organic matter. Under these conditions uranyl exists primarily as positively charged aquocomplexes, which are capable of complexing with negatively charged orthophosphate. Uranyl-sulfate and -nitrate species may also be important in groundwater with high concentrations of sulfate and nitrate, such as at the FRC. However, if phosphate and cations—such as Ca, K, Mg, and Na—are available, autunite/meta-autunite group minerals are formed, as shown through EXAFS spectra and predicted by thermodynamic calculations. Carbonates, which are prevalent in natural systems, form highly soluble complexes with uranyl; however, below pH 7 they do not affect the precipitation of uranium phosphate. The adsorption of U(VI) to mineral surfaces, such as iron oxides, is minimal below pH 6. However, in the presence of phosphate, uranyl adsorption increases via ternary surface complexes. U(VI) complexation with soil organic matter may also inhibit uranyl phosphate precipitation. Our experimental results indicate that the organic ligands glycerol-3-phosphate (G3P) and inositol-6-phosphate (IP6), containing one and six phosphate moieties, respectively, complex with the uranyl ion in a mole-to-mole ratio at pH 5.5. Uranyl precipitates with the ligand at U:L ratios > 1:5, but is soluble at U:L ratios < 1:5. These results indicate that at high U:L ratios, the precipitation of a uranyl-ligand compound should prevent the precipitation of a more insoluble mineral, such as autunite.

The rate of uranyl phosphate precipitation is proportional to pH. Uranyl phosphate precipitates approximately 4 times faster at pH 7.0 compared to pH 4.5 in the presence of comparable orthophosphate concentrations. EXAFS spectra indicate that the mineral formed at and below pH 5.5 as an autunite/meta autunite group mineral, while a mixture of uranyl hydroxide and autunite/meta-autunite is identified at pH 7.0. Both mineral formations are confirmed by thermodynamic modeling. These experiments demonstrate that uranyl complexation may play a significant role in the biomineralization of U(VI).

Effects of Chromium(VI) and Chromium(III) on *Desulfovibrio vulgaris* Cells

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Desulfovibrio vulgaris ATCC 29579 is a well-studied sulfate reducer that has known the capability to reduce heavy metals and radionuclides, like chromium and uranium. Cultures grown in a defined medium (i.e., LS4D) had a lag period of approximately 40 hours when exposed to 50 μM of Cr(VI). Substrate analysis revealed that although chromium is reduced within the first 5 hours, growth does not resume for another 35 hours. During this time, small amounts of lactate are still utilized, but the reduction of sulfate does not occur. Sulfate reduction occurs concurrently with the accumulation of acetate approximately 40 hours after inoculation, when growth resumes. Similar amounts of hydrogen are produced during this time compared to hydrogen production by cells not exposed to Cr(VI); therefore, an accumulation of hydrogen cannot account for the utilization of lactate. There is a significant decrease in the carbohydrate to protein ratio at approximately 25 hours, and this result indicated that lactate is not converted to glycogen. Most-probable-number analysis indicated that cell viability decreased steadily after inoculation and reached approximately 6×10^4 cells/mL 20 hours postchromium exposure. Regeneration of reducing conditions during chromium exposure does not induce growth and in fact may make the growth conditions even more unfavorable. This result suggested that an increase in E_h was not solely responsible for the decline in viability. Cell pellets collected 10 hours after chromium-exposure were unable to resume growth when suspended into fresh medium. Supernatants from these pellets were able to support cell growth upon re-inoculation. *D. vulgaris* cells treated with a non-dose-dependent addition of ascorbate at the same time of Cr(VI) addition did not enter a lag period. Ascorbate added 3 hours post-Cr(VI) exposure did not prevent the growth lag. These results indicated that *Desulfovibrio* utilized lactate to reduce Cr(VI) without the reduction of sulfate, that the decline in cell viability and cell growth was most likely a consequence of Cr(III), and that an organic ligand could protect *D. vulgaris* cells from Cr(III) toxicity. Lactate consumption decoupled from sulfate reduction in the presence of Cr(VI) could provide organic carbon for organo-Cr(III) complexes.

Influence of Electron-Donor Form and Supply Rate on Dynamics of Bacterial Populations Associated with Uranium Reduction and Remobilization

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Enhanced reductive precipitation of U(VI) through stimulation of indigenous microorganisms is an attractive, low-cost strategy for *in situ* remediation of contaminated groundwaters and sediments. However, our previous long-term column studies demonstrated that after an initial period of U(VI) reduction and immobilization, reoxidation of U(IV) and remobilization of U(VI) occurred. The rate of organic carbon (OC) supply determines not only the amount of electron donor available for bioreduction of U(VI), but also affects the resulting concentration of aqueous (bi)carbonate generated by microbial respiration. Increased (bi)carbonate concentrations drive aqueous U(VI) concentrations to higher levels through formation of stable U(VI) carbonato complexes, including $Ca_2UO_2(CO_3)_3(aq)$, and make U(IV) oxidation under reducing conditions favorable. We are currently investigating how various OC forms and supply rates affect the long-term stability of bioreduced U and the dynamics of the resulting microbial communities in relation to U redox changes. These current studies are being conducted using historically U-contaminated sediments from Area 2 of the ERSD Field Research Center, Oak Ridge, Tennessee, in flow-through columns.

Microbial communities were stimulated by the addition of OC supplied as either acetate or lactate at four different concentrations (100 mM, 30 mM, 10 mM, 3 mM OC equivalents). Columns were sampled at two time points; during a phase of net U-reduction and during a later phase of U(IV) reoxidation and U(VI) remobilization. We employed a high-density 16S DNA microarray (Phylochip) to determine the structure and dynamics of the microbial communities. Comparison of the resulting communities indicates that OC supply rate is the primary determinant of the bacterial community composition. Additionally, significant shifts in population dynamics occurred in all communities between the U-reduction and U-reoxidation/remobilization phases. Phylochip analysis showed an increase in the number of operational taxonomic units detected between the U-reduction and U-remobilization phases in all columns except those receiving high OC lactate supply (100 mM and 30 mM OC equivalents). Known U-reducing bacteria such as *Anaeromyxobacter dehalogenans*, *Desulfovibrio desulfuricans*, *Geobacter metallireducens*, and *Pseudomonas putida* were detected in all columns by Phylochip analysis. Although the dynamics of these U-reducing bacteria differed, depending on the form of OC supplied and the supply rate, our data indicate that U-reducing bacteria were present in each column during the phase of U(IV) reoxidation and U(VI) remobilization, and in some cases showed an increase in relative abundance.

The results of our research indicate that the form of OC substrate provided to promote bioreduction of U(VI) has less of an effect on microbial community structure than the OC supply rate, and that different forms of OC supplied at equivalent rates have a similar geochemical impact on effluent U concentrations.

Integrated Geophysical Characterization and Monitoring of Key Properties and Processes Associated with Subsurface Transport, Remediation, and Attenuation at the ERSD Field Research Center at Oak Ridge

(Component of the IFC project "Multiscale Investigations on the Rates and Mechanics of Targeted Immobilization and Natural Attenuation of Metal, Radionuclides, and Co-Contaminants in the Subsurface," Philip M. Jardine, PI)

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Near-surface geophysical investigations will be performed at the ERSD Field Research Center (FRC) at Oak Ridge prior to, concurrent with, and subsequent to subsurface manipulation, to quantify the *in situ* transport, remediation, and attenuation of contaminants associated with both natural and anthropogenic perturbations and as a function of hydrogeological heterogeneity. Surface time-lapse data (i.e., electrical resistivity tomography, seismic refraction tomography) and wellbore time-lapse data (i.e., crosshole tomographic radar, seismic, complex resistivity tomography) will be collected on day, week, and month time scales over a minimum 3-year period at and along key locations in the recharge, transition, and manipulated zones. The proposed geophysical research, which is a component of a multidisciplinary investigation of watershed-scale contaminant issues, is designed to monitor both temporal and spatial variations in geophysical attributes (e.g., seismic velocity, electrical resistivity and phase, dielectric constant, SP). Through long-term monitoring of multiple variables and the integration of a variety of data sets, the proposed research will further elucidate the relationship between hydrogeological and biogeochemical variables to geophysical attributes, as well as provide the basis for the characterization and geophysical modeling of contaminant flow in the FRC watershed. A particularly important component will be to use the advanced, integrated characterization and monitoring datasets to explore the impact of natural perturbations (such as recharge) and anthropogenic manipulations (such as biostimulation) on subsurface transformations at the FRC.

Changes in Microbial Community Structure During Biostimulation for Uranium Reduction at Different Levels of Resolution

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Former radionuclide waste ponds at the ERSD Field Research Center in Oak Ridge, TN, pose several challenges for uranium bioremediation. The site is marked by acidic conditions, high concentrations of nitrate, chlorinated solvents, and heavy metals. Aboveground treatment of groundwater, including nitrate removal via a denitrifying fluidized bed reactor (FBR), pre-conditions the groundwater for subsurface uranium immobilization. A series of recirculating wells serve to create a subsurface bioreactor to stimulate microbial growth for *in situ* U(VI) immobilization. Well FW-104 is the injection well for the electron donor (i.e., ethanol); Well FW-026 is the extraction well for the recirculation loop; Well FW-101 and FW-102 are the inner zones of biostimulation; and FW-024 and FW-103 are upstream and downstream wells, respectively, which are the outer protective zones. Bacterial community composition and structure of groundwater from the wells were analyzed via clonal libraries of partial SSU rRNA gene. Both qualitative and quantitative methods were used to analyze the changes in bacterial diversity and distribution. LIBSHUFF analysis was used for the comparison of bacterial community population between the different clonal libraries. Bacterial community from the denitrifying FBR was different from the groundwater bacterial community, which indicated that different bacterial communities were stimulated in the two separate systems. The clonal libraries of the recirculating wells showed that over each phase of manipulation for uranium immobilzation, the bacterial communities of the inner zones of biostimulation were more similar to each other and than those of the outer protective zones. The outer protective zones were more similar to the injection well. Clonal libraries from FW-104 (injection), FW-101 and FW-102 showed that bacterial communities of the three wells were initially similar, but diverged over time. FW-101 and FW-102 bacterial communities developed changes in parallel, while those of FW-104 showed gradual change. These results were further compared to data generated from Unifrac analysis. Preliminary results with Unifrac analyses showed that the bacterial community in each of the wells changed over the bioremediation process, and the changes could be attributed to variations along temporal, spatial, and geochemical scales. Diversity indices showed that bacterial diversity tended to increase during the initial phase of uranium bioreduction and decreased toward the end of uranium bioreduction (i.e., low U(VI) levels). As uranium levels declined, increasing *Desulfovibrio* and *Geobacter*-like sequences were detected from the clonal libraries, and the *Desulfovibrio*-like sequences predominated over time. These results were confirmed via qPCR; they also correlated with OTU distributions for *Desulfovibrio*. Furthermore, the results indicated that the bacterial community composition and structure changed upon stimulating for uranium bioreduction conditions, and that sequences representative of sulfate-reducers and metal-reducers were detected in wells that displayed a decline in U(VI). Further analysis is under way to determine the relationships between different functional groups and site geochemistry.

Synthesis of Goethite-Coated Sand and Analysis of Its Interactions with Uranium(VI)

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Iron(III) oxide coating on soils/sediments is reported to be the most important factor controlling sorption of radioactive metals in groundwater systems. Various forms of iron(III) oxides occur in nature, which exist in both crystalline and noncrystalline (amorphous) forms. Our review indicated that out of about fifteen different forms of natural iron(III) oxides, goethite (α-FeOOH) is the most ubiquitous form of iron oxide commonly encountered in the weathered sediments present at various contaminated sites. Therefore, our goal was to investigate the reactive chemistry of uranium(VI) with goethite-coated sand (GCS). Since our eventual goal was to develop scalable formulations to predict uranium transport, we chose to study uranium transport using a well-characterized, synthetic, goethite-coated sand. The goethite was synthesized in our laboratory using two suspension methods, homogeneous and heterogeneous. For the homogeneous method, iron(II) serves as the iron source that under rigorous oxidation results in a goethite coating on pure quartz sand. Synthesis of GCS using the heterogeneous suspension method was a two-step process, wherein pure goethite was synthesized first by the rapid hydrolysis of Fe(III), followed by heating. In the second step, the pure goethite was coated onto the quartz at pH 7.0 and ionic strength of 0.01M.

The GCS obtained from both methods was characterized using scanning electron microscopy (SEM), which involved energy-dispersive spectra and elemental mapping analysis. The SEM of the precipitate obtained from the homogeneous suspension reaction showed acicular crystals confirming the goethite coating on quartz. Powder X-ray diffraction and Fourier transform infrared spectra confirmed pure goethite formation from the heterogeneous suspension reaction. Standard iron extraction methods, which included ammonium oxalate in the dark, dithionate-citrate-bicarbonate extraction, and acid digestion, were used to quantify the crystalline and amorphous forms of iron. A comparison of iron content indicated that the homogeneous suspension method resulted in higher iron coating than the heterogeneous suspension method. However, the crystallinity of iron was higher in the heterogeneous suspension method. Batch adsorption experiments at fixed solid-to-solution ratios were performed using the goethite-coated sand at a pH of 4.4±0.1 and ionic strength of 0.1 M. Although the iron content of the GCS varied between 0.04 % and 0.5%, similar isotherms were obtained when uranium sorption was normalized to the iron content.

Constraints on Microbial Reduction of Uranium within Soils and Sediments

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Uranium is a redox active contaminant of concern to both human health and ecological preservation. In soils and sediments subject to anaerobic conditions, the more mobile, oxidized form of uranium (UO_2^{2+}) may be reduced by dissimilatory metal-reducing bacteria. Despite rapid reduction in controlled systems, various factors within soils may limit biological reduction of the uranyl ion, inclusive of competing electron acceptors such as nitrate and alterations in uranyl speciation. Here, we elucidate the impact of uranyl speciation on the extent and rate of reduction with a focus on the formation of the ternary calcium-uranyl-carbonato species. Calcium decreases the rate of microbial uranyl reduction by limiting the accessibility of U(VI), we propose, through changes in the conformation of the electron accepting moiety. The impact of calcium concentration on U(VI) reduction is compared between systems containing ferrihydrite, a short-range order iron (hydr)oxide, goethite, and hematite.

Observed pseudo first-order rate constants for reduction of uranyl by *S. putrefaciens* in the presence of 0 to 1 mM Ca (and 3 mM HCO_3^-) range from $12 \pm 0.6 \times 10^{-3}$ h^{-1}, for 0 mM Ca in the presence of goethite to $2.0 \pm 0.10 \times 10^{-3}$ h^{-1} for 0.8 mM Ca in the presence of hematite. Dissolved Ca at concentrations from 0.2 to 0.8 mM decreasesthe extent of U(VI) reduction by 25% after 528 hours relative to rates with no Ca in solution. Decreased uranyl reduction rates in the presence of calcium suggest that longer residence times are required in flow systems to reduce uranyl compared to systems without Ca. Additionally, the presence of iron (hydr)oxides can have contrasting impacts on uranyl reduction, serving to further diminish rates in certain cases while mitigating the effects of calcium in others. Goethite and hematite, for example, decrease the dissolved concentration of calcium through adsorption, thus diminishing the effect of calcium on uranium reduction. Ferrihydrite, in contrast, acts as a competitive electron acceptor and thus, like Ca, tends to decrease uranium reduction. However, while ferrihydrite decreases U(VI) reduction in solutions without Ca, with increasing Ca concentrations U(VI) reduction is enhanced in the presence of ferrihydrite. Uranium(VI) reduction appears to become almost independent of Ca concentration at higher Ca levels in ferrihydrite-bearing systems.

These results demonstrate that the presence of Ca in solution imposes limitations on dissimilatory uranium reduction that are reflected in decreased biotic reduction rates. There are several possible mechanisms by which Ca may diminish U reduction: Ca may render the reduction less thermodynamically favorable, it may have a toxic effect on the microorganisms, or it may kinetically limit reduction by yielding a complex with poor orbital overlap with the U reductase or hindering site accessibility. Previous studies have eliminated the possibility of toxic effects, while a thermodynamic analysis included in this study illustrates that there is less than 10% difference in energy gained between the reduction of uranyl associated with carbonate and uranyl associated with calcium and carbonate. This suggests kinetic limitation is most likely the governing mechanism of inhibition in these systems. The quantitative framework provided could be implemented in remediation strategies to better understand and predict how reduction rates will be impacted given a known Ca concentration.

NMR and EXAFS Studies of the Impact of Mineral Transformation and Sorbate Aging on Contaminant Speciation and Mobility

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The work of our collaborative research team is aimed at developing a predictive-mechanistic understanding of the coupling between mineral weathering from caustic waste release and contaminant (Cs, Sr, I) fate and transport in waste-impacted sediments, across space, time, and geochemical gradients that encompass the process-level heterogeneity observed at the Hanford DOE site. In these studies, we have examined sets of samples ranging from homogeneous precipitates to Hanford sediments, each reacted with simulated tank waste leachate (STWL) containing varying contaminant concentrations of Sr, Cs, and/or I in batch laboratory experiments from 1 d to 2 yrs.

Here we highlight synergistic results arising from the use of two of our main analytical research tools: solid-state nuclear magnetic resonance (NMR) and extended X-ray fine structure (EXAFS) spectroscopies, along with supporting information from electron microscopy and vibrational spectroscopies. Initial studies focused on the identification and quantification of neophases formed in a kaolinite/STWL system as a function of reaction time and varying contaminant concentration. In this work, variable-field ^{27}Al magic-angle spinning (MAS) NMR provided kinetic data for the destruction of octahedral aluminum species and the formation of tetrahedral aluminum-containing neophases. Recent studies comparing homogenous precipitation in controlled model systems and more complex Hanford sediment samples lend new insight into the molecular structures and kinetics of neoformed phases that sequester contaminants. New NMR data have been acquired at high magnetic field strengths at the Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by the Department of Energy's Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory (PNNL). Our studies follow the transformation of aluminum-containing phases during reaction with STWL. Neophase formation is rapid in homogeneous systems (hours to days), with product phases influenced by the Si to Al ratio and by the concentration and type of contaminant cation (Cs^+ or Sr^{2+}). In Hanford sediments, the rate of neophase formation is slower (days to months). Aluminosilicate neophases formed within one month are poorly crystalline and easily extracted, with Sr EXAFS indicating incorporation into cation sites in feldspathoid-type phases. Additional information regarding the coordination and identification of aluminum atoms in neophase systems could also been accessed via multiple-quantum magic-angle spinning (MQMAS) NMR experiments, and implementation of these methods is demonstrated in studies of cesium siting in aluminum-containing phases.

Finally, we will present details of preliminary progress toward the measurement of reactive surface area on complex oxides in the environment. Probe molecules containing fluorine are selectively attached to reactive sites on the surfaces of oxide materials, and quantitative ^{19}F MAS NMR measurements have demonstrated linear scaling of reactive surface sites with surface dissolution rates. Our measurements on aluminosilicate glasses and clay materials will be presented, and possible applications of these methods to more complex sediment samples (both pristine and reacted) will be discussed.

SYNCHROTRON STUDIES

Environmental Science Program at Synchrotron Light Sources

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The Environmental Science Program at synchrotron light sources was established to provide support to ERSP principal investigators (PIs). The mission of this program is to enable a deeper knowledge of the fundamental chemical, biological, and physical factors that govern the reactivity and cycling of contaminants in the environment. Based on expressed and documented ERSP PI research needs, this program offers advice and direct support across a suite of beamlines suitable for environmental research at all four of the Department of Energy's national user facility synchrotrons.

At the Lawrence Berkeley Laboratory's Advanced Light Source (ALS), the capabilities of the facility include infrared spectromicroscopy on beamline 1.4.3, microtomography on beamline 8.3.2, micro-X-ray absorption spectroscopy (XAS) and microdiffraction on beamline 10.3.2, and scanning transmission X-ray microscopy (STXM), X-ray photoelectron spectroscopy, and near-edge X-ray absorption fine structure (NEXAFS) spectroscopy on beamline 11.0.2. Experimental time at the ALS is awarded through the general user proposal system. The program is available to work with ERSP PIs on experiment planning and proposal development, as well as on data collection and interpretation.

At Brookhaven National Laboratory's National Synchrotron Light Source (NSLS), the EnviroSuite program supports ERSD researchers through direct and General User Proposal access. Beamlines for environmental science applications include hard X-ray microprobes at X26A and X27A (for fluorescence, spectroscopy, diffraction and tomography at ~10-micron resolution), bulk absorption spectroscopy at X11A and B (over 5-35 keV energy range), low-energy bulk spectroscopy at X15B (1-5 keV, e.g. for phosphorus and sulfur K edges and uranium M5 edge), soft X-ray STXM and NEXAFS at X1A, and IR microspectroscopy at U10B.

Argonne National Laboratory's Advanced Photon Source (APS) supports ERSD research at many beamlines. Four are dedicated to XAS, while three others offer X-ray microprobes combining imaging with fluorescence and absorption spectroscopies and diffraction with sub-10 micron resolution. A fourth microprobe offers imaging with 150 nm resolution, small enough to resolve metal distribution in individual microbes. Other beamlines offer small and wide angle scattering with instruments suitable for environmental science research.

The Stanford Synchrotron Radiation Laboratory (SSRL) Environmental Remediation Science program provides hands-on assistance at all stages of the experimental process to ERSP researchers conducting XAS and *in situ* x-ray scattering measurements. Highlights from the past year include user commissioning of a hard X-ray microprobe (2 μm spot) that can access U, Np, Pu, and Tc edges, the release of a major new software package for analyzing microprobe image data, and growth of an X-ray scattering user program to measure (bio-)mineral transformation dynamics.

We will present examples of synchrotron-based research and discuss the mechanisms for accessing experimental facilities at the four synchrotrons. More information can be found at: ALS: http://esd.lbl.gov/ALS_environmental/, NSLS: http://www.bnl.gov/envirosuite/, APS: http://www.aps.anl.gov/, SSRL: http://www-ssrl.slac.stanford.edu/mes/remedi/.

ENVIRONMENTAL MOLECULAR SCIENCE INSTITUTES (EMSI)

The Center for Environmental Kinetics Analysis: An NSF- and DOE-funded Environmental Molecular Science Institute (EMSI) at Penn State

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Physicochemical and microbiological processes taking place at environmental interfaces influence natural processes as well as the transport and fate of environmental contaminants, the remediation of toxic chemicals, and the sequestration of anthropogenic CO_2. A team of scientists and engineers has been assembled to develop and apply new experimental and computational techniques to expand our knowledge of environmental kinetics. We are also training a cohort of talented and diverse students, to work on these complex problems at multiple length scales and to compile and synthesize the kinetic data. Development of the human resources capable of translating molecular-scale information into parameters that are applicable in real world, field-scale problems of environmental kinetics is a major and relatively unique objective of the Institute's efforts. The EMSI team is a partnership among 10 faculty at The Pennsylvania State University (funded by the National Science Foundation Divisions of Chemistry and Earth Sciences), one faculty member at Juniata College, and four researchers drawn from Los Alamos National Laboratory, Pacific Northwest National Laboratory, and Lawrence Berkeley National Laboratory (funded by the Department of Energy Division of Environmental Remediation Sciences). Interactions among the applied and academic scientists drive research approaches aimed toward solving important problems of national interest.

The Institute is organized into three interest groups (IGs) focusing on the processes of dissolution, precipitation, and microbial reactions at surfaces. The IGs interact with each other as each interest group studies reactions across the molecular, microscopic, mesoscopic and, in most cases, field scales. For example, abiotic dissolution and precipitation reactions of Fe oxides, as studied in the Dissolution IG, provides the baseline for kinetic behavior as the third IG researches the interaction of micro-organisms with these same minerals. The attachment of bacteria and redox chemistry that occurs between micro-organisms and minerals are critical factors in maintaining groundwater quality and remediation of many toxic waste sites, and is one of the main thrusts of research within our EMSI. The IGs also participate in using visualization tools to promote greater understanding of complex environmental data.

Uranium Speciation in Contaminated Sediments in the Hanford Vadose Zone

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Long-term sequestration of uranium at sites within the DOE complex is a significant problem, one that requires molecular-level information on the speciation, phase association, and spatial distribution of uranium. As an NSF-DOE funded Environmental Molecular Science Institute (EMSI), we have conducted molecular-level studies of the speciation and phase association of uranium in the vadose zones of Hanford Area 200 and Area 300 sites, as well as in model systems designed to understand how U(VI) adsorbs to mineral surfaces. Contamination of vadose zone sediments under Tank BX-102 at the Hanford site in Washington resulted from the accidental release, in 1951, of 7 to 8 metric tons of uranium dissolved in caustic aqueous sludge. We have applied synchrotron-based X-ray spectroscopic and diffraction techniques to characterize the speciation of uranium in samples of these contaminated sediments. U L_{III}-edge X-ray absorption fine structure (XAFS) spectroscopic studies demonstrate that uranium occurs predominantly as a U(VI)-silicate from the uranophane group of minerals. XAFS cannot distinguish between the members of this mineral group because of the near-identical local coordination environments of uranium in these phases. However, these phases differ crystallographically, and can be distinguished using X-ray diffraction (XRD) methods. Since the concentration of uranium is too low for conventional XRD to detect these phases, X-ray microdiffraction (μXRD) was used to collect diffraction patterns on ~20 μm diameter areas of localized high uranium concentration (found using microscanning x-ray fluorescence [μSXRF]). Only sodium-boltwoodite, $Na(UO_2)(SiO_3OH)\cdot 1.5H_2O$, was observed; no other uranophane group minerals were present. Sodium-boltwoodite formation has effectively sequestered uranium in these sediments under the current geochemical and hydrologic conditions. Attempts to remediate the uranium contamination will likely face significant difficulties because of the speciation and distribution of uranium in the sediments. The disposal of basic sodium-aluminate and acidic U(VI)-Cu(II) wastes into the now-dry North and South 300 A Process Ponds at the Hanford site resulted in a U(VI) groundwater plume.

To gain insight into the geochemical processes that occurred during waste disposal and that will affect the future fate and transport of this uranium plume, the solid-phase speciation of uranium, in a depth sequence from the base of the North Process Pond through the vadose zone to the water table, was investigated using electron microprobe measurements, μ-XRF, μ-XRD, and XAFS spectroscopy. Uranium in sediments from the base of the pond was predominantly co-precipitated with calcite. From ~2 m below the pond base to the water table, uranium occurred predominantly in a sorbed form, likely on the surface aluminosilicate clay minerals. The presence of a U(VI)-phosphate phase was also observed in this region, but it only occurred as a major uranium species at one depth. Also present are U(VI)-Cu(II) phosphate and silicate phases, including metatobernite and cuprosklodoswkite. Initial sequestration of U(VI) in these sediments likely occurred through co-precipitation with calcite, because conditions did not favor adsorption. Since the calcite-bearing pond sediments have been removed as part of a remediation effort, future uranium fate and transport will likely be controlled primarily by adsorption/ desorption phenomena and dissolution of the U(VI)-Cu(II) silicates.

Center for Environmental Molecular Science (CEMS)—A Collaborative Environmental Molecular Science Institute (EMSI) at Stony Brook University and Brookhaven National Laboratory

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The Center for Environmental Molecular Science (CEMS) is a multidisciplinary research and training center supported by funding from NSF and DOE. CEMS is one of six NSF-DOE funded Environmental Molecular Science Institutes (EMSI) in the U.S. CEMS research, outreach, education, and training activities—focused on environmental chemistry and geochemistry—are jointly conducted by faculty, scientists, staff, and students at Stony Brook University and Brookhaven National Laboratory (BNL), with two additional investigators at Penn State University and Temple University.

CEMS research areas include: (1) Interfacial Processes and Sorption Mechanisms, (2) Role of Inorganic and Organic Ligands on Sequestration, (3) Biological and Microbially Mediated Processes, (4) Engineered Porous and Layered Materials, and (5) Technique Development in Environmental Molecular Science. Collaborative teams of faculty/scientists, graduate students, and postdocs join forces to work on focused research projects within these areas. Research projects are not limited to, but significant attention is given to, DOE priority contaminants. Laboratory facilities at Stony Brook, Brookhaven, Penn State, and Temple are made available for research and graduate training. Investment in beamlines at the National Synchrotron Light Source has provided enhanced capability and access to essential techniques. Frequent meetings involving all CEMS personnel allow regular evaluation of research progress, exchange of ideas, and planning for future research.

CEMS activities include graduate student training and education/outreach programs. An emphasis is placed on providing graduate students with opportunities for multidisciplinary research, reflecting the view that future scientists will benefit from training in more than one field. This enhanced training is accomplished by encouraging students to have co-advisors in related departments, by conducting research in labs with related faculty and/or at other institutions, and by presentation of results beyond home departments. CEMS also offers a summer REU program, seminar series, technique training workshops, and education/outreach programs focused at underrepresented groups.

CEMS website: http://www.cems.stonybrook.edu/