DOE-NABIR PI WORKSHOP:
Abstracts

March 17–19, 2003
Warrenton, Virginia

Natural and Accelerated Bioremediation Research Program

(NABIR)

Table of Contents

Introduction
DOE–NABIR PI Workshop

March 17–19, 2003

The mission of the NABIR program is to provide the fundamental science that will serve as the basis for the development of cost-effective bioremediation and long-term stewardship of radionuclides and metals in the subsurface at DOE sites. The focus of the program is on strategies leading to long-term immobilization of contaminants in situ to reduce the risk to humans and the environment. Contaminants of special interest are uranium, technetium, plutonium, chromium, and mercury. The focus of the NABIR program is on the bioremediation of these contaminants in the subsurface below the root zone, including both vadose and saturated zones.

The program consists of four interrelated Science Elements (Biotransformation, Community Dynamics/Microbial Ecology, Biomolecular Science and Engineering, and Biogeochemistry). The program also has a cross-cutting Assessment Element that supports development of innovative approaches and technologies to support the science elements. An element called Bioremediation and its Societal Implications and Concerns (BASIC) addresses potential societal issues of implementing NABIR scientific findings. The material presented at this year's workshop focuses on approximately 60 research projects funded in FY 2000–2003 by the Environmental Remediation Sciences Division in DOE's Office of Biological and Environmental Research (BER) in the Office of Science. Abstracts of NABIR research projects are provided in this book.

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Agenda
NABIR PI Meeting

Airlie Conference Center
Warrenton, VA
March 17–19, 2003

Monday, March 17

8:30–8:45 AM Welcome—Anna Palmisano

8:45–9:00 Ari Patrinos, Associate Director, Biological and Environmental Research

9:00–9:15 Teresa Fryberger, Director, Environmental Remediation Sciences Division, BER

9:15–9:30 Dave Geiser, Office of Environmental Management (invited)

9:30–10:00 Biogeochemistry (Bruce Honeyman, Colo. School of Mines)

10:00–10:30 Biogeochemistry (Larry Hersman, LANL)

10:30–11:00 BREAK

11:00–11:30 Biogeochemistry—Student presentation (John Senko, U. of Oklahoma)

11:30–12:00 Biotransformation (Yuri Gorby, PNNL)

12:00–12:30 Biotransformation (A.J. Francis, BNL)

LUNCH

Afternoon Session			
2:00–5:00	Posters	Breakout: Research at UMTRA Sites (Phil Long, PNNL)	Breakout: Lateral Gene Transfer (Tamar Barkay, Rutgers)
5:00–6:00	Free time		
6:00–7:00	Dinner		
7:00–9:00	Poster session: Community Dynamics/Microbial Ecology, BASIC, Biomolecular Science and Engineering. Authors must be at posters.		

Tuesday, March 18

8:30–9:00 AM Community Dynamics (Darrell Chandler, ANL)

9:00–9:30 Biomolecular Science and Engineering (Judy Wall, U. of Missouri)

9:30–10:00 Biomolecular Science and Engineering (Michael Daly, USUHS)

10:00–10:30 BREAK

10:30–11:00 Proteomics (Mary Lipton, PNNL)

11:00–11:30 DOE Genomes to Life Program (Marv Frazier, Director, Life Science Division, BER)

11:30–11:50 BASIC (Rob Anex, U. of Oklahoma)

11:50–12:15 How is NABIR Enhancing EM's goals? (Caroline Purdy, EM)

LUNCH

Afternoon Session			
2:00–5:00	Posters	Breakout: Functional Biodiversity (Allan Konopka, Purdue)	Breakout: Numerical Modeling in NABIR (Peter Kitanidis, Stanford)
5:00–6:00	Free time		
6:00–7:00	Dinner		
7:00–9:00	Poster Session: Biogeochemistry, Biotransformation, FRC projects; NABIR–EM projects. Authors must be at posters.		

Wednesday, March 19 (ALL FRC RESULTS)

8:30–8:45 AM Introduction and Strategic Planning for the NABIR FRC at Oak Ridge (Paul Bayer)

8:45–9:00 Update on NABIR FRC activities (David Watson, ORNL)

9:00–10:00 Factors controlling in situ U and Tc bioreduction and reoxidation (Jack Istok, Oregon State University)

10:00–10:30 BREAK

10:30–11:30 Field-scale evaluation of biostimulation for remediation of U-contaminated groundwater (Craig Criddle, Stanford)

11:30–12:00 In situ immobilization of U in structured porous media (Tim Scheibe, PNNL)

12:00–12:15 Wrap-up; Meeting adjourns

12:15–1:15 PM Lunch

ABSTRACTS

PROGRAM ELEMENT 1
Biotransformation

The Kinetics of Direct Enzymatic Reduction of Uranium(VI): Effects of Ligand Complexation and U(VI) Speciation

Calvin C. Ainsworth,[1] Zheming Wang,[1] Kevin M. Rosso,[1] and James K. Fredrickson.

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[2]Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA

The kinetics of Fe(III) and U(VI) reduction by AH_2DS is being examined in a systematic manner, through the selection of specific ligands under specific conditions (pH, pCO_2, temp., etc.), to elucidate the effects of complexation on U(VI) bioreduction. Marcus theory is being utilized to develop a fundamental basis and understanding of the electron-transfer reaction in these systems and to develop a predictive structure-reactivity relationship based on first principle calculations and experimental data.

An electron transfer model was developed to determine fundamental rate controls inherent in one-electron, self-exchange reactions in the AH_2DS system and their reduction cross-reactions with Fe(III) complexes. Computed rates for the six one-electron self-exchange reactions in the 9-membered AH_2DS system are all of similar magnitude (10^8 M^{-1} s^{-1}) and in excellent agreement with measured rates (10^6–10^8 M^{-1} s^{-1}). Predicted reduction cross-reaction rates between AH_2DS and the Fe(III)–aquo, –hydroxo, and –organic ligand complexes are in near-quantitative agreement with our measured rates, generally validating an outer-sphere reaction mechanism. The calculations suggest that the observed rate differences are primarily due to differences in the electron transfer distances, the charge of the Fe(III) complexes, and the driving force for electron transfer, while the reorganization energies remain remarkably independent of ligand size. Thus, the main effect of ligand size is a steric one, which directly impacts the electron transfer distance and the "exposure" of the Fe(III) electron-accepting center. Ligand structure directly influences the electron affinity of the Fe(III) center, which in turn also affects the electron transfer rate.

Ongoing experimental studies of U(VI) reduction by AH_2DS have shown that the aqueous uranyl ion and its hydrolysis products are reduced at a significantly slower rate compared with Fe(III). While reduction rates of U(VI)–organic chelate complexes vary with the speciation of uranyl, rates are dependent on solution pH even if the speciation remains the same. While uranyl fluorescence spectra at cryogenic temperatures suggested energy transfer from AH_2DS/AQDS to uranyl ion, which is evidence of encounter complex formation, the reaction mechanism appears far more complicated compared to Fe(III). Work is currently under way to delineate the photophysical effect of uranyl and AH_2DS on the redox kinetics.

Current modeling efforts involve adapting and applying the model for the reduction of U(VI)–aquo, –carbonato, and –hydroxamate complexes. Recent experimental work has shown that UO_2^{2+} reduction rates vary significantly as a function of complexation, but simpler interpretations in terms of ligand structure, reorganization energy, or driving force do not appear to be possible. In addition, the internal reorganization energy may be small, and less dependent on the presence of equatorial ligands in the inner coordination sphere. Therefore, it is anticipated that the current modeling efforts will bring significant insight to bear on the physical quantities that control the reduction rate.

A New Generation of Reagents for Immunosensors: Recombinant Antibodies that Recognize Metal–Chelate Complexes

James B. Delehanty, R. Mark Jones, Haini Yu, and Diane A. Blake (PI)

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A subgoal of our present project is to develop new protein reagents that will provide superior performance in the hand-held immunosensor. In support of this specific aim, we have expressed two antibodies, 5B2 and E5, as recombinant $F(ab)'_2$ fragments and subsequently used these constructs to identify residues important in the recognition of metal–chelate complexes. Antibody 5B2 was raised to a Pb(II)-loaded isothiocyanatobenzyl-diethylenetriamine pentaacetic acid (DTPA)-protein conjugate. The native antibody bound to complexes of Pb(II)-*p*-aminobenzyl-DTPA with an affinity of 4.6×10^{-9} M. A monovalent Fab fragment prepared from the native protein and the bivalent recombinant fragment exhibited comparable affinities for the same Pb(II)–chelate complex, approximately six times lower than that of the intact antibody. Covalent modification of lysine residues and molecular modeling predicted that Lys^{58} in the heavy chain contacted the Pb(II)–chelate ligand. Mutational analysis supported a role for Lys^{58} in ion pair or hydrogen bond formation with the carboxylate groups on the chelate. Antibody E5 was directed toward an isothiocyanatobenzyl-ethylenediamine tetraacetic acid (EDTA)-protein conjugate that was loaded with ionic Cd(II). The native immunoglobulin recognized Cd(II)-*p*-aminobenzyl-EDTA with an affinity of 8.2×10^{-12} M. A proteolytically derived $F(ab)'_2$ fragment and the bivalent recombinant fragment bound to the same Cd(II)–chelate complex, with affinities that were comparable to that of the native antibody. Homology modeling and mutagenesis identified three residues (Trp^{52} and His^{96} in the heavy chain and Arg^{96} in the light chain) that were important for Cd(II)–chelate recognition. His^{96} likely mediates a direct ligation to the Cd(II) ion, and Trp^{52} appears to be involved in hydrophobic stacking with the benzyl moiety of the chelator. Arg^{96} appeared to mediate an electrostatic or hydrogen bond to the chelate portion of the complex. These modeling/mutagenesis studies provide insight into the molecular interactions most important in antigen recognition and will ultimately lead to engineered antibodies specifically designed to provide superior performance in immunosensor applications.

Biodegradation of PuEDTA and Impacts on Pu Mobility

Harvey Bolton, Jr.[1] (PI), Dhanpat Rai,[1] and Luying Xun[2]

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The contamination of many DOE sites by Pu presents a long-term problem because of its long half-life (240,000 yr) and the low drinking water standard ($<10^{-12}$ M). EDTA was co-disposed with radionuclides (e.g., Pu, ^{60}Co), formed strong complexes, and enhanced radionuclide transport at several DOE sites. Biodegradation of EDTA should decrease Pu mobility. One objective of this project was to determine the biodegradation of EDTA in the presence of PuEDTA complexes. The aqueous system investigated at pH 7 (10^{-4} M EDTA and 10^{-6} M Pu) contained predominantly $Pu(OH)_2EDTA^{2-}$. The EDTA was degraded at a faster rate in the presence of Pu. As the total concentration of both EDTA and PuEDTA decreased (i.e., 10^{-5} M EDTA and 10^{-7} M PuEDTA), the presence of Pu decreased the biodegradation rate of the EDTA. It is currently unclear why the concentration of Pu directly affects the increase/decrease in rate of EDTA biodegradation. The soluble Pu concentration decreased, in agreement with thermodynamic predictions, as the EDTA was biodegraded, indicating that biodegradation of EDTA will decrease Pu mobility when the Pu is initially present as Pu(IV)EDTA. A second objective was to investigate how the presence of a second metal will influence the speciation and biodegradation of Pu(IV)EDTA. Preliminary results indicate that the presence of Fe(III) out competes the Pu(IV) for the EDTA complex. This indicates that Pu(IV) will not form stable complexes with EDTA for enhanced transport of Pu in Fe(III)-dominated subsurface systems. A third objective is to investigate the genes and enzymes involved in EDTA biodegradation. BNC1 can use EDTA and another synthetic chelating agent, nitrilotriacetate (NTA), as sole carbon and nitrogen sources. The same catabolic enzymes are responsible for both EDTA and NTA degradation, except that additional enzymes are required for EDTA degradation. When the catabolic genes were cloned and sequenced, the gene cluster also contained genes encoding a hypothetical ABC-type transporter. RT-PCR analysis showed that the transporter genes and EDTA monooxygenase gene (emoA) are co-transcribed. EppA is one of the transporter genes, and it codes for a periplasmic binding protein responsible for binding to the substrate before transport across the membrane can occur. EppA was cloned, expressed, and purified in *Escherichia coli* and found to bind MgEDTA, CaEDTA, Fe(III)EDTA, MgNTA, CaNTA, and Fe(III)NTA. Our data also suggest that BNC1 uses the same ABC-type transporter for both EDTA and NTA uptake. Results from these studies can provide mechanistic understanding and approaches to assist in the bioremediation of PuEDTA and other radionuclide-EDTA complexes at DOE sites.

Natural Organic Matter Inhibits Biological Uranium Reduction by *Shewanella putrefaciens* CN32

William D. Burgos[1] (PI), Brian A. Dempsey,[1] Gour-Tsyh (George) Yeh,[2] and Eric E. Roden[3]

[1]Pennsylvania State University; [2]University of Central Florida; [3]University of Alabama

The broad objective of our NABIR research project is to conduct experiments to generate/expand a kinetic/thermodynamic database for use in modeling the concomitant biological reduction of iron(III) and uranium(VI) in field sediments amended with natural organic matter (NOM). Reaction-based reactive transport models will be validated with laboratory experiments, and the validated models will be used for eventual field-scale predictions. The specific objectives of our poster-presented research were to examine the effect of several different NOMs on U(VI) bioreduction, resolve the mechanism by which the NOM affects this process, and collect kinetic data for rate formulation/parameterization purposes. Experiments were performed with the dissimilatory metal-reducing bacterium (DMRB) *Shewanella putrefaciens* CN32 in a $NaHCO_3$-buffered solution under a N_2-CO_2 atmosphere with 5 mM lactate as the sole electron donor and 100 μM uranyl acetate as the sole electron acceptor. A preliminary experiment conducted with cell densities of 10^7 and 10^8 cells mL^{-1} revealed that U(VI) was bioreduced to a lesser extent with the higher cell density, possibly due to greater biosorption of U(VI). U(VI) was measured as dissolved (0.2 μm filter) and carbonate-extractable by kinetic phosphorescence analysis (KPA). Precipitated solids retained on the 0.2 μm filter were analyzed by x-ray photoelectron spectroscopy (XPS) to confirm the presence of U(IV). Preliminary experiments were also conducted at "high" and "low" HCO_3^- concentrations (30 and 1 mM, respectively) such that two different inorganic U(VI) complexes were the dominant species, and revealed no difference in the rate and extent of bioreduction.

Five different NOMs were tested: (1) Field Research Center (FRC) forest soil humic acid, (2) FRC groundwater NOM, (3) IHSS Suwannee River humic acid, (4) IHSS Suwannee River NOM, and (5) IHSS soil humic acid. The electron shuttling compound anthraquinone-2,6-disulfonate (AQDS) was also tested. For all experiments, a biotic no-amendment control was always prepared. Results from the biotic no-amendment controls were highly reproducible and yielded a zero-order reduction rate of 14.4 ± 0.4 (n = 6) μmol U(VI) d^{-1} (with 10^7 cells mL^{-1} and $[U(VI)]_{initial}$ = 100 μM). The FRC groundwater NOM had no effect on the rate and extent of U(VI) bioreduction. However, with the other four NOMs, no measurable U(VI) bioreduction occurred after five days. AQDS increased the rate of U(VI) bioreduction. Two mechanisms are proposed to explain the inhibition of U(VI) bioreduction by NOM. First, NOM may complex U(VI), making it biologically unavailable or less available. Second, NOM may act as a competitive electron acceptor and not as an electron shuttle. In the second case, it is proposed that the reduction of U(VI) by NOM is either thermodynamically unfavorable or extremely slow. Additional work to improve our interpretation of these results include: U(VI)–NOM dialysis experiments to confirm that complexation can occur under these experimental conditions; U(VI) bioreduction with low concentrations of NOM to examine the competitive electron acceptor hypothesis; U(VI) bioreduction experiments with excess citrate to compare NOM results to conditions of known complexation/speciation.

Bio-oxidation of Fe(II) and Radionuclide Immobilization by *Dechlorosoma suillum*

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Previous studies on microbial attenuation of heavy metals and radionuclide (HMR) contamination have demonstrated the potential of nitrate-dependent Fe(II) oxidation for the permanent immobilization of HMR in the environment. Geochemical profiles of sediment cores collected from Campus Lake, Southern Illinois University, revealed the co-existence of both nitrate and Fe(II), indicating that in situ chemical conditions for anaerobic microbial nitrate-dependent Fe(II) oxidation are present. Furthermore, the potential for anaerobic Fe(II) bio-oxidation is supported by MPN enumeration studies that revealed a nitrate-dependent Fe(II)-oxidizing population as high as 1.47×10^5 cells g^{-1} sediment. Furthermore a novel autotrophic nitrate-dependent Fe(II)-oxidizing isolate (*Cosmobacter millennium* strain KWA2) with a 16S rDNA sequence similarity of 94% with *Chromobacterium violaceum* in the beta subclass of the Proteobacteria was obtained from the MPN series. Independently, two other nitrate-dependent Fe(II) oxidizers were also obtained from enrichment cultures, one of which was closely related to *Dechlorosoma suillum* strain PS, which was previously demonstrated as a ubiquitous nitrate-dependent Fe(II) oxidizer in the environment. Nitrate-dependent metal oxidation by *D. suillum* was specific for Fe(II). In an effort to identify proteins associated with Fe(II) oxidation in *D. suillum*, we investigated the expressed protein content of cells grown under NO_3-reducing conditions in the presence and absence of Fe(II). Electrophoresis of the total protein content of cell lysates prepared from these cultures did not reveal the selective expression of any proteins associated with the presence of Fe(II). Similar results were obtained when electrophoresed proteins were stained with o-Dianisidine-based heme dyes to identify cytochromes specifically associated with this metabolism.

Further investigation of the biogenic iron mineral phases produced by *D. suillum* in the presence of 100 μM U(VI) by density gradient centrifugation resulted in the recovery of five distinct fractions. Differential solubility analysis of 0.5M HCl extractable Fe and 3M HCl extractable Fe of each of these fractions indicated the existence of a positive correlation between fraction density and crystallinity. The percentage of 0.5M HCl-extractable Fe decreased from 100% to 3%, with an increase in sucrose concentration (20% to 60%), indicating the separation of crystalline Fe compounds. Analysis of the total iron content in each fraction revealed that most of the iron (>86%) was present in the more dense crystalline phases. Analysis of the U(VI) content revealed that the majority (~80%) of the U(VI) was also associated with the densest crystalline phase (3% 0.5M HCl extractable). The ratio of 3M HCl-extractable Fe(II) to total Fe (0.68) was also highest in this fraction, suggesting crystalline mixed Fe(II)/Fe(III) phases. The formation of a mixed-phase crystalline Fe compound sequestering a significant proportion of U(VI) is ideal for a remediation strategy, as the U(VI) would not be easily remobilized as a result of reductive dissolution of Fe.

These results demonstrate that biogenic crystalline Fe compounds can sequester significant proportions of HMR [U(VI)]. Furthermore, existing in situ geochemical conditions for microbial anaerobic Fe(II) oxidation and the presence of microorganisms capable of this metabolism support the applicability of this remediation strategy for the in situ sequestration of HMR on a long-term basis.

Reductive Precipitation and Stabilization of Uranium Complexed with Organic Ligands by Anaerobic Bacteria

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This research addresses the principal mechanisms of microbial alteration of organic–radionuclide complexes and the resultant impacts on radionuclide solubility and stability under anaerobic conditions.

Synthesis and Characterization of Uranium–Organic Complexes. Equimolar and excess ligand complexes of uranium with the naturally occurring organics ketogluconic acid, malic acid, citric acid, protocatechuic acid, catechol, salicylic acid, and fulvic acid were prepared at pH 3.5 and 6.0. Potentiometric titrations were performed to confirm the presence of the complexes. Structural characterization of the complexes was performed by EXAFS analysis and synchrotron-based FTIR at the NSLS. Ketogluconic acid formed a mononuclear complex with uranium involving the carboxylate group while malic acid, citric acid, and catechol formed binuclear complexes with U. The catechol was bonded to uranium through the two hydroxyl groups, whereas the hydroxycarboxylic acids were bonded in tridentate fashion to uranium through two carboxylate and the hydroxyl groups.

Biotransformation of Uranium Complexed with Organic Ligands by Anaerobic Bacteria. The equimolar U:citric acid complex was not metabolized by *Shewanella putrefaciens, Clostridium* sp., or *C. sphenoides*. However, in the presence of excess citrate, U(VI) bound to citric acid was reduced to U(IV) and the reduced uranium remained in solution as the U(IV)–citrate complex. EXAFS analysis of the reduced complex showed that the structure is a mononuclear bi-ligand citrate complex. The uranium exhibited an eight-fold coordination with oxygen and possible tridentate coordination to citric acid. These results show that complexed uranium is readily accessible for microorganisms as an electron acceptor, despite their inability to metabolize the organic ligand complexed to the actinide. The U(VI)–malate complex was reduced to U(IV)–malate at a rate and extent similar to that of the U–citrate complex. Addition of bacterial cells to the U(VI)–ketogluconate complex resulted in the dissociation of the complex with reduction of U(VI) to U(IV).

Uranium Speciation in Field Research Center (FRC) Samples. Groundwater and soil core samples from the FRC were characterized by X-ray absorption spectroscopy. XANES and EXAFS analyses of the freeze-dried groundwater (FW024/026) at pH 3.7 indicated the uranium was bonded to chloride ions as the uranyl chloride species. EXAFS measurements of pH adjusted water samples showed U was coprecipitated with Al hydroxide as the hydrated uranyl ion. XANES spectra of soil core samples (FWB105, FW 16, FW 33) showed the presence of U(VI).

Reduction of Uranium in FRC Groundwater by *Clostridium* sp. We examined the reductive precipitation of uranium in groundwater at acidic pH by *Clostridium* sp. Addition of varying concentrations of FRC groundwater (FW024) to a growing culture (18 h, pH 3.5) resulted in the reduction of U(VI) to U(IV), and the Al in the groundwater had no effect on uranium reduction. Addition of uranyl nitrate to the growing culture also confirmed reduction of uranium at pH 3.2. These results suggest that *Clostridium* sp is capable of uranium reduction at acidic pH.

Production of Extracellular Polymeric Substances by the Facultative Iron-Reducing Bacterium *Shewanella oneidensis* Strain MR-1: Implications for Metal Reduction and Sequestration under Aerobic Conditions

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The dissimilatory iron-reducing bacterium *Shewanella oneidensis* strain MR-1 formed cell aggregates (flocs) when cultivated in a chemically defined medium with dissolved oxygen concentrations ranging from 10 to 100% of air saturation. These flocs, with diameters between ten to several hundred microns, were composed of hundreds of cells bound in a matrix of extracellular polymeric substances (EPS). The EPS contained polysaccharides and proteins, including c-type cytochromes, and exhibited an abundant cation sorption capacity. Colloidal gold particles (1.4 nm), which were coated with positively charged polyamines, served as contrasting agents for transmission electron microscopy, and clearly revealed the homogeneous distribution of charged groups throughout the EPS matrix. When ferrous iron solutions were added to anaerobic suspensions of flocs, iron precipitates formed throughout the EPS matrix, as visualized by electron microscopy. Identification of these precipitates is pending low temperature Mossbauer spectroscopy. However, the greenish color of pelleted flocs and associated precipitates coupled with electron microscopic evidence suggest the formation of a poorly crystalline green rust similar to that which forms on surfaces of MR-1 grown under anaerobic conditions.

Aerobic suspensions of flocculated MR-1 reduced Co(III)–EDTA and Tc(VIII). Although the rates of reduction were significantly slower than observed from anaerobic suspensions, reduction of Tc(VIII) resulted in the formation of black Tc(IV) precipitates. In contrast, no reduction was detected in aerobic suspensions of cells that were prevented from flocculating by omitting Ca^{2+}, a divalent cation that is involved in assembly of the EPS matrix, or in aerobic cultures that were deflocculated by the addition of EDTA. These results are consistent with our hypothesis that cell aggregates establish an oxygen gradient that decreases with depth. Hence, cells located toward the center of the floc are oxygen-limited and reduce metals under suboxic or anaerobic conditions that are not represented by the aerobic bulk aqueous phase. These results present new evidence that iron (metal) reduction can occur in biofilm communities present within aerobic media, and that the fate of the reduction products (cations) is controlled by charged groups within the EPS.

Investigation of the Spatial Distributions and Concentrations of Biologically and Environmentally Relevant Elements at the Mineral–Microbe Interface

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Understanding the fate of heavy-metal contaminants in the environment is of fundamental importance in the development and evaluation of effective remediation and sequestration strategies. Bacteria and the extracellular material associated with them are thought to play a key role in determining a contaminant's speciation and thus its mobility in the environment. Additionally, the metabolism and surface properties of bacteria can be quite different depending upon whether the bacteria exhibit a planktonic (free-floating) or biofilm (surface-adhered) habit. The microenvironment at and adjacent to actively metabolizing cells also can be significantly different from the bulk environment. Thus, to understand the microscopic physical, geological, chemical, and biological interfaces that determine a contaminant's macroscopic fate, the spatial distribution and chemical speciation of contaminants and elements that are key to biological processes must be characterized at micron and submicron length scales for bacteria in both planktonic and adhered states. Hard x-ray microimaging is a powerful technique for the element-specific investigation of complex environmental samples at the needed micron and submicron resolution. An important advantage of these techniques results from the large penetration depth of hard x-rays in water. This advantage minimizes the requirements for sample preparation and allows the detailed study of hydrated samples. The objectives of the studies presented here are (1) to determine the spatial distribution, concentration, and chemical speciation of metals at, in, and near bacteria and bacteria-geosurface interfaces; (2) to use this information to identify the metabolic processes occurring within the microbes; and (3) to identify the interactions occurring near these interfaces among the metals, mineral surfaces, and bacterially produced extracellular materials under a variety of conditions.

We have used x-ray fluorescence microscopy to investigate the spatial distribution of 3*d* elements in single *Shewanella oneidensis* cells grown with oxygen and fumarate as electron acceptors. Measurements were made on subsamples of cells taken at varying times during a five-day growth period. Cells analyzed were either in a surface-adhered or planktonic state. The zone plate used in these microscopy experiments produced a focused beam with a cross section (and hence spatial resolution) of 0.15–0.30 μm. The samples (both planktonic and biofilm) were all grown in a consistent manner in a defined minimal salts medium.

Results from x-ray fluorescence imaging experiments indicate that the distribution of P, S, Cl, Ca, Fe, Ni, Cu, and Zn can define the location of the microbe. Additionally, quantitative elemental analysis of individual microbes identified significant changes in concentration of 3*d* transition elements depending on the age of the culture and the type of electron acceptor presented to the microbes. These results and a discussion of the use of this technique for identifying metabolic states of individual microbes within communities will be presented.

Impacts of Mineralogy and Competing Microbial Respiration Pathways on the Fate of Uranium in Contaminated Groundwater

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[1]Department of Oceanography and the College of Medicine, Florida State University, Tallahassee, Florida;
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This is a field-oriented project designed to elucidate the microbiological and geochemical factors controlling U(VI) reduction/immobilization in subsurface environments at the Field Research Center (FRC). Since the majority of U(VI) contamination in the FRC subsurface is associated with acidic sediments where the groundwater contains extremely high nitrate concentrations, we have focused our efforts on these acidic sediments. Our objectives are to (1) determine the dominant Fe minerals likely to limit U speciation, (2) directly quantify reaction rates and pathways of terminal-electron-accepting processes that control subsurface sediment chemistry, and (3) characterize the anaerobic microbial consortia—Fe(III)-reducing bacteria (FeRB)—likely to catalyze U reduction.

FeRB were enumerated and characterized from most probable number (MPN) enrichment cultures. Phylogenetic analysis of the 16S rRNA gene sequences extracted from positive MPN enrichments revealed that the only FeRB cultivated at acidic pH were gram-positive organisms not closely related to any previously cultured FeRB. In contrast, members of the family *Geobacteraceae* predominated at neutral pH in enrichment cultures from background sediments. Geochemical parameters likely to limit the activities of FeRB in situ were studied in parallel with freshly collected samples of all of the subsurface sediments studied. Chemical analyses in groundwater included nitrate, ammonium, U(VI), and pH. Solid phase characterization included porosity, density, organic matter content, sorbed nitrate, and sediment pH. Using wet chemical extractions and Mossbauer spectroscopy, aluminosilicates (66%) and Al-substituted goethite were the predominant Fe minerals observed. A small but substantial percentage (<5%) of the Fe minerals present were associated with poorly crystalline minerals such as ferrihydrite. Iron mineralogy did not appear to vary between sites or with sediment pH. Carbon metabolism (as CO_2 accumulation) and available electron acceptors were monitored at regular intervals in sediment microcosms. Sulfate concentrations in microcosms were generally very low, and little or no Fe(III) reduction activity was observed, whereas millimolar amounts of nitrate were depleted over time. Little or no methane accumulated in these incubations, while millimolar amounts of carbon dioxide accumulated in conjunction with the observed nitrate depletion and in response to carbon substrate addition. High denitrification rates, monitored using the acetylene block technique, were observed but only in neutral pH microcosms.

Our results indicate that the contaminated FRC subsurface is a heterogeneous "extreme environment" where the metabolism of FeRB is likely to be controlled by acid tolerance and competition with nitrate as an electron acceptor. Results also point to nitrate removal and neutralization of pH for establishment of conditions conducive to U(VI) reduction/immobilization by FeRB. Currently, the approaches of our multidisciplinary team (mineral characterization, rate measurements, microbial community analysis) are being combined to determine the interactions between Fe mineral transformation and U(VI) solubility during biostimulation in sediment microcosms.

Potential for In Situ Bioremediation of Uranium and Vanadium Contamination in the Subsurface

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A field test was conducted for the first time to further evaluate the potential for in situ bioremediation of uranium-contaminated groundwater. A test plot consisting of a 20-well injection gallery, 15 downgradient monitoring wells, and 3 upgradient control wells was installed at the Old Rifle UMTRA site in Colorado. Acetate (1–3 mM) was injected into the aquifer over a four-month period in order to stimulate the activity of dissimilatory metal-reducing microorganisms. Loss of U(VI) began almost immediately after initiation of acetate injection. This was accompanied by production of Fe(II), resulting from Fe(III) reduction. After 50 days, soluble U(VI) approached or fell below the UMTRA MCL limit (0.18 nM). There was no significant removal of sulfate from the groundwater during this period, and pH remained constant. Loss of U(VI) was coincident with an enrichment of *Geobacteraceae*, with *Geobacteraceae* accounting for over 70% of the microbial community at the height of the Fe(III) and U(VI) reduction phase. These results are consistent with previous laboratory studies with uranium-contaminated subsurface sediments, which demonstrated that the addition of acetate promoted the growth of *Geobacteraceae*, which grew primarily by Fe(III) reduction, but which also reduced U(VI) concurrently. Beyond 50 days of acetate injection in the field experiment, sulfate concentrations started to decrease. This was accompanied by a decrease in *Geobacteraceae* and an increase in sulfate-reducing microorganisms. These results suggest that, as Fe(III) became depleted at the point of injection, Fe(III) reducers could no longer out compete sulfate reducers and thus sulfate reducers began consuming the acetate. As sulfate reducers became predominant, U(VI) concentrations increased, suggesting that sulfate reducers were not as effective in reducing U(VI) as the *Geobacteraceae*. This suggests that it is important to maintain Fe(III)-reducing conditions in order to effectively remove U(VI) from the groundwater. The results demonstrate that promoting the growth and activity of *Geobacteraceae* in the subsurface can be a very effective mechanism for immobilizing uranium in contaminated aquifers.

Although not currently a priority contaminant of focus for the NABIR program, vanadium is a prevalent contaminant in the groundwater at a number of UMTRA sites. Vanadium toxicity and solubility vary considerably with the nature of the compound, but in general, both decrease as the valence state is reduced. Thus, promoting the reduction of V(V) may be a remediation strategy. In order to determine if *Geobacteraceae* microorganisms were capable of reducing V(V), *Geobacter metallireducens* was inoculated into a medium in which acetate was the electron donor and V(V) was the sole electron acceptor. The yellow color of the medium, due to the presence of V(VI), changed to blue, attributable to the presence of V(IV), which subsequently precipitated. Reduction of V(V) was associated with cell growth, and there was no V(V) reduction in heat-killed or abiotic controls. These results demonstrate that *G. metallireducens* has the potential to convert vanadium from a soluble form to an insoluble form and suggest that vanadium may be removed from contaminated waters by stimulating the growth of *Geobacteraceae* in a manner similar to that successfully employed for in situ bioremediation of uranium-contaminated groundwater.

New Catalytic DNA Biosensors for Radionuclides and Metal Ions

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We aim to develop new DNA biosensors for simultaneous detection and quantification of bioavailable radionuclides, such as Sr, U, Tc, and Pu, and metal contaminants, such as Pb, Cr, and Hg.[1,2] The sensors will be highly sensitive and selective, not only for different metal ions, but also for different oxidation states of the same metal ion. They will be applied to on-site, real-time assessment of concentration, speciation, and stability of radionuclides and metal contaminants during and after bioremediation. To achieve this, we have employed a combinatorial method called "*in vitro* selection" to search for catalytic DNA molecules that are highly specific for radionuclides or other metal ions.[3] Comprehensive biochemical and biophysical studies have been performed on the selected DNA molecules. The findings from these studies allow the elucidation of metal-binding sites in DNA and thus facilitation of the design of improved metal sensors. The DNA has been labeled with fluorescent donor/acceptor pairs to investigate and to signal the structural changes upon metal ion binding. Once a collection of individual DNA sensors is identified, each specific for a particular metal ion at a certain concentration range, the sensors will be assembled into a DNA microarray for simultaneous detection and quantification of radionuclides and metal contaminants.

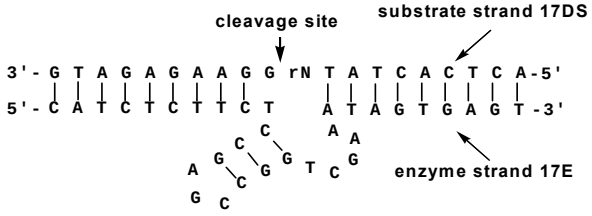

Figure 1. An example of catalytic DNA sensor.

We have successfully used this methodology to develop a highly sensitive and selective DNA biosensor for Pb^{2+}, with a quantifiable detection range from 10 nM to 4 μM.[4] Even in the presence of other metal ions and under simulated physiological conditions, this biosensor displays a remarkable sensitivity and selectivity. To further improve the metal ion selectivity, we developed a "negative" selection strategy that can significantly improve metal-binding selectivity in the selection process.[5] To improve metal-binding affinity, we have developed a new synthetic strategy for making phosphoroselenote DNA/RNA that is capable of binding different metal ions.[6] To provide insight into the metal-binding sites in DNA and allow the design of metal ion sensors and chelators from first principle, we also carried out a detailed biochemical[7] and biophysical[8] study of the DNA lead sensors obtained in the lab. More importantly, we made a breakthrough by converting the catalytic DNA into highly sensitive and selective colorimetric metal sensors, making on-site, real-time detection more affordable and achievable, as no equipment is needed .[9] We accomplished this by attaching gold nanoparticles to the catalytic DNA. Finally, we are making progress in applying the strategy developed in the lab to making sensors for radionuclides such as uranium ions. The latest results will be presented.

1. Y. Lu, *Chem. A Eur. J. 8*, 4588–4596 (2002); 2. Y. Lu, et al., *Biosensors & Bioelectronics* (in press); 3. J. Li, et al., *Nucleic Acids Res. 28*, 481–488 (2000); 4. J. Li & Y. Lu, *J. Am. Chem. Soc.,122,* 10466–10467 (2000); 5. P.J. Bruesehoff, et al., *Chem. High Throughput Screening, 5,* 327–355 (2002); 6. G.A. Holloway et al., *ChemBioChem. 3*, 1061–1065 (2002); 7. A.K. Brown, et al., *Biochemistry* (submitted); 8. J. Liu, and Y. Lu, *J. Am. Chem. Soc.* (in press); 8. J. Liu & Y. Lu, *Nature Biotechnology* (submitted).

Environmental Actinide Mobility: Plutonium and Uranium Interactions with Naturally Occurring Microorganisms

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Objectives. Our overall goal is to understand the interactions of actinides and bacteria that can stabilize or destabilize actinides in the subsurface environment with respect to mobility. Our objectives toward achieving this goal include the following: (1) characterize the chemical toxicity of actinides to bacteria that could stabilize actinides using biostimulation or bioaugmentation, (2) determine and characterize the binding of actinides to extracellular polymers and whole cells, and show how that binding will affect environmental speciation and distribution, (3) examine and quantify the siderophore-mediated redox, speciation, and membrane translocation of actinides, and (4) investigate the redox influence of bacteria on actinides as a function of initial form.

Chemical toxicity. When considering the bioremediation of sites contaminated with radioactive materials, microbial tolerance to the metal contaminant must be addressed. We have determined the toxicity of a variety of metals, radionuclides, and organic chelators to several bacteria. We found that actinides are less toxic than most other metals [e.g., Ni(II), Cd(II), Pb(II), Al(III])], inhibiting growth only at concentrations in the micromolar to millimolar concentration range, far above contamination levels reported in the U.S. Also, by testing the toxicity of isotopes with differing specific activity ($^{238, 239, 242}$Pu), we found that actinide toxicity is primarily chemical, not radiological, in nature.

Siderophore speciation and membrane translocation. We continue to investigate the redox properties of a range of Pu–chelator and Pu–siderophore complexes, including expanding our studies to include EDTA, pyoverdin, and acetohydroxamic acid. Pyoverdin, isolated and purified from *P. putida*, forms a 1:1 complex with Pu(IV), which has a reduction potential of approximately –0.400 V (versus NHE). Having previously demonstrated and quantified the microbial uptake of Pu-hydroxamate siderophore complexes, we seek to determine if this phenomenon is general. That is, are other types of actinide–siderophore complexes translocated across the cell membrane? Our initial results suggest that, at conditions under which Fe(III)–pyoverdin is accumulated by metabolically active cells of *P. putida*, the Pu(IV)–pyoverdin complex does not appear to be taken up.

Reduction by dissimilatory iron-reducing bacteria. With respect to reduction, experiments done previously by others have shown that iron-reducing bacteria (i.e., *Shewanella* sp. and *Geobacter* sp.) are capable of radionuclide reduction—primarily U(VI)—for growth. We have now shown that whole-cell suspensions of *Shewanella oneidensis* rapidly reduce concentrations (up to 10 mM) of Pu(VI) to minimal concentrations of Pu(IV), with either formate or lactate as the electron donor. More recently, experimental results have indicated that in growth cultures in the presence of varying Pu(VI) concentrations, a toxicity threshold less than the 10 mM Pu(VI) done in cell suspension exists that may limit cell growth. Once this factor has been determined, we will proceed with growth cultures utilizing Pu(VI) as the sole electron acceptor, and determine the redox potential range accessible to c-type cytochromes, possible important factors in regard to geochemical cycling and microbial metabolism in contaminated areas.

Mesoscale Coupled Transport and Biogeochemical Effects on Reduction of U(VI) and NO_3^- as Co-contaminants in Natural Sediments and Soils

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As fluids in subsurface environments are not well mixed, even at relatively small scales, the subsurface can be categorized into advective and diffusion-limited domains. Information is lacking concerning local, diffusion-limited processes that arise from small-scale variations in permeability, preferential flow paths, and soil/sediment structure. The primary objective is to study the coupled transport and biogeochemistry of U(VI) and NO_3^-. The redox reactions of these co-occurring contaminants are investigated in realistically heterogeneous model systems. We emphasize direct measurements within diffusion-limited domains at the mesoscale (defined as the typical diffusion-limited scale, ranging from about 10^{-4} to 10^{-1} m). It is within these diffusion-limited domains that large gradients in microbial activity, chemical potentials, reaction rates, and transport rates can coexist. Thus, dynamics at this mesoscale can control redox-dependent biotransformations in nature.

Part 1. Effects of pH and carbon on bioreduction of NO_3^- and U(VI) (FRC Area 3 sediment and groundwater high in nitrate). Rates of NO_3^- and U(VI) reduction in response to organic carbon amendments are under investigation. Due to the carbon concentration required to complete denitrification, we are investigating the efficacy of a commercially available slow-releasing polylactate form of carbon (Regenesis HRC) as well as a rapid release version of the same product (Regenesis HRC Primer). We are following microbial activity through analysis of gas headspace CO_2, CH_4, N_2O, and H_2. Upon complete reduction of NO_3^- and U(VI) we will destructively sample sediment/solution and extract DNA to determine changes in microbial community composition in response to each treatment.

Part 2. Diffusion-limited U(VI) biogeochemical transformation (FRC background area sediments). Measurements include U oxidation state determination by micro-XANES spectroscopy, soluble U (kinetic phosphorimetry) redox potential profiling, microbial activity, and microbial community analysis. Column experiments of 2 types are in progress. The first examines the U contamination process, with U(VI) diffusion into soil columns at different levels of initial organic carbon and microbial activity. Strong retardation of the U(VI) diffusion front in initially reducing sediments has been found to persist even after nearly 1 year without resupply of organic carbon. The second set of experiments focus on bioremediation of previously contaminated sediments, with organic carbon solutions diffusing into columns already containing U(VI). Significant U(VI) reduction has occurred only in columns supplied with high levels of organic carbon.

Part 3. U(IV) reoxidation in subsurface environments that revert to oxygenated conditions (FRC Area 2 sediments). These experiments will evaluate long-term stability of previously bioreduced U. In step 1, FRC Area 2 sediments containing 200 ppm U, initially primarily as U(VI), were packed into columns and infused with lactate solutions to accelerate reduction. Supporting measurements include analyses of U concentrations in effluents, in situ determination of sediment U(VI):U(IV), redox potential profiling, and pore-water chemical analyses. This step is near completion. In subsequent steps, columns will be exposed to oxygenated waters under flow-through or diffusion conditions, with sediment and solution U(VI) concentrations monitored to determine reoxidation rates.

PROGRAM ELEMENT 2

Community Dynamics/ Microbial Ecology

Artificial Neural Network Tools for the Analysis of Microbial Community Data

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A major challenge in the implementation of bioremediation technologies is to understand the composition of the indigenous microbial community and how this composition is affected by environmental conditions. Because microbial communities contain a large number of species that cannot be identified by traditional microbiological methods, molecular methods are used to characterize the community structure. However, these methods often generate complex but sparse data sets that are not easily analyzed by conventional statistical methods. The objective of our NABIR project is to develop artificial neural network (ANN) tools for analyzing such data sets. An ANN is an analysis tool capable of approximating continuous nonlinear functions using a computational paradigm that simulates the parallel and distributed processing mechanisms of the brain.

As an example, we trained an ANN to use geochemical measurements to predict the frequencies of nitrite reductase (*nir*K and *nir*S) genes in groundwater samples collected at the NABIR Field Research Center. Reducing the model complexity was critical in working with this data set. We used principal components analysis to reduce the six geochemical factors to two components. The first component represented contamination level (except uranium), and the second component was negatively correlated with uranium. The *nir*K and *nir*S data were grouped phylogenetically into five classes each to reduce the dimensionality of the community data. The ANN model explained nearly all of the variation in the observed *nir*S frequencies for the five classes (92.3%), whereas the generalized linear model (logistic) accounted for only 33.9% of the observed variation. For *nir*K, the ANN and logistic models explained 65.1% and 53.2% of the variation respectively. These results indicated that at least some phylogenetically related sequences could be associated with particular environmental conditions, and that specific predictions and trends could be obtained from ANN analysis. Understanding these relationships may be helpful in predicting the response of microbial communities under differing geochemical conditions.

We are also developing a Web-based tool kit that incorporates several innovative analyses for applying ANNs to biological data sets with a small number of samples. A variety of tools will be provided, including input data transformations, selecting network architecture, various learning algorithms, regularization techniques, and cross-validation methodologies. In addition, several standard linear statistical tools will be available, such as principal components analysis and multiple linear regression. A Web-based interface will be an integral component of the system that links all of the tools.

Integrated Particle-Handling Methods for Multiplexed Microbial Identification and Characterization in Sediments

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The objective of this project is to develop integrated microbial and nucleic acid detection methods and instrumentation for monitoring metal-reducing microbial communities in subsurface sediments before and after biostimulation. This objective is being met by coalescing recent developments in 16S rRNA microarrays, microfluidic systems, microparticle chemistries, renewable surface techniques and suspension array technology. In FY02, we developed the instrumentation, software, and analytical methods for automated capture and release of 16S rRNA on color-coded 5 μm particles. We investigated whether tunable surface-bead chemistry and peptide nucleic acids (PNA) could enhance the recovery and detection of intact rRNA in both test tube and automated suspension array hybridization formats. Intact rRNA was easily captured and detected on PNA-coated beads from 0.1 ng total RNA with a 15 minute hybridization in pH 7 buffer, representing 1.7×10^3 cell equivalents of total RNA. DNA-conjugated beads in pH 5 hybridization buffer required an overnight hybridization to achieve a detectable signal at 0.1 ng target RNA. Standard DNA hybridization conditions (pH 7) were one order of magnitude less sensitive than the tunable surface (pH 5) condition. The PNA-conjugated particles were 100 times more sensitive than the tunable surface DNA particles in the automated format, with a detection limit of 0.1 ng total RNA. The detection limits for total RNA on PNA-conjugated microparticles is immediately conducive to the detection and characterization of microorganisms in low biomass environments without using PCR. We also studied the physical spacing and nucleotide mismatch tolerance between capture and proximal chaperone detector probes that are required to achieve species-specific 16S rRNA detection on planar arrays. Species-specific rRNA detection was achieved using a 22 nt capture probe and a 15 nt detector probe separated by 10–14 nt along the primary sequence. Chaperone detector probes with up to 3 mismatched nucleotides still resulted in species-specific capture of 16S rRNAs. There was no obvious relationship between position or number of chaperone mismatches and within- or between-genus hybridization specificity. Interestingly, chaperone probes were not required to directly capture and detect 16S rRNA on suspension array particles. From these results, we conclude that relieving secondary structure is of principle concern for the successful capture and detection of 16S rRNA on planar surfaces, but that the sequence of the capture probe is more important than relieving secondary structure for achieving specific hybridization. By the spring meeting, we anticipate comparing the automated capture efficiency of DNA and PNA probes in amended environmental samples (e.g., FRC, UMTRA sediment extracts from NABIR investigators) to understand the relationship between soluble environmental constituents and fluorescent detection/reporting systems. Results from these studies will determine if our single-step capture/detection strategy is viable, or if more complex sample processing steps will be required for continued development of an integrated, environmental, microbial monitoring system.

Ecological Interactions Between Metals and Microbes Impacting Bioremediation

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The project objectives are to determine whether spatial heterogeneity in metal distribution is a major determinant of microbial community activity and diversity in contaminated soils; the effects of increased metal bioavailability and mobility upon microbial community activity and diversity; the role of metal-resistant bacteria in microbial communities that contain many metal-sensitive members (these microbes might function as either "bioprotectants" through their physiological activity, or reservoirs of transferable resistance genes); and the interactions among multiple toxicants upon microbial activity in mixed-waste sites. Distinct microbial communities were found in contaminated soils that varied in their concentrations of Pb, Cr, and aromatic compounds. Contamination with metals and hydrocarbons is correlated in these soils. So it is difficult to distinguish between their effects on community structure and activity. Microcosms were constructed in which either Pb^{+2} or CrO_4^{-2} was added at levels that produced acute modest or severe effects (50 or 90% reduction) on community activity. Either glucose or xylene was added as a C source, as substrates broadly used by microbes or restricted in their catabolism. Activity was monitored via carbon dioxide evolution, and total biomass changes by analyses of phospholipid phosphate. Heavy metal additions had more severe effects on xylene catabolism than on glucose. Temporal changes in bacterial community composition were determined by DGGE analysis of 16S rDNA genes. Addition of an organic energy source selected for a relatively small number of phylotypes. The addition of Pb or Cr(VI) modulated community response to the added organic substrate. Some phylotypes selected in the absence of metal additions were still selected at modest or severe metal stress, but there were also instances where different phylotypes were selected. Nucleotide sequencing of these phylotypes is in progress.

Xylene was used in these studies, as published reports maintain aromatic degradation was severely inhibited in metal-contaminated soils and metal-resistant aromatic degrading could not be cultured from these habitats. Real-time PCR was used to quantify copy numbers of aromatic dioxygenase genes in the microcosms. There were 10-fold increases in biphenyl dioxygenase and phenol hydroxylase genes in microcosms receiving xylene. However, 4 other dioxygenase genes were not detectable. Bacteria were isolated from microcosms that received xylene and Cr(VI). Initial attempts to isolate bacteria on media with both xylene and Cr(VI) were unsuccessful. Isolates were then obtained on complex media with Cr(VI)—all were *Arthrobacter* sp. unable to use xylene. When bacteria were selected on xylene as sole C source, 3 phylogenetic groups were isolated. Many of the *Arthrobacter* sp. selected in this way were resistant to high concentrations (>20 mM) of Cr(VI), but only if they were pregrown at 1 mM Cr(VI). The correlation between microbial activity, community structure, and metal level was analyzed on 150 mg of soil collected at spatial scales of <1, 5, 15, and 50 cm. There was no correlation between metal content and activity level. Soils <1 cm apart could differ in activity 10-fold and extractable Pb and Cr 7-fold. DGGE analyses indicated substantial differences at the <1 cm scale. The nucleotide sequence of phylotypes in samples with high metal concentrations is being investigated. The characteristics of bacteria isolated from these habitats were also investigated. *Arthrobacter* VN23-1 is Pb-resistant. Pb resistance can be transferred to a Pb-sensitive *Arthrobacter* strain by conjugation, although at a relatively low frequency. Pb resistance appears to be inducible, and the genetic trait is associated with Cd but not Zn resistance. A cloned gene has homology to P type ATPases and is related (but not identical) to a Pb-resistance determinant from the gram-negative bacterium *Ralstonia metallidurans*.

Distribution and Activity of Dissimilatory Metal-Reducing Microorganisms in Contaminated Subsurface Environments

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Investigations into the diversity and ecology of dissimilatory metal-reducing microorganisms in uranium-contaminated subsurface environments were conducted with a combination of culturing, molecular ecology, and genomic techniques. Culturing studies yielded several novel isolates capable of dissimilatory metal reduction that have unique physiological properties that could be useful for engineered bioremediation of metal-contaminated environments. Molecular analyses of microbial communities associated with in situ metal remediation were carried out at the FRC and UMTRA sites.

For example, as part of a field experiment evaluating the potential for in situ microbial U(VI) reduction to remove U(VI) from contaminated groundwater, the microbial community associated with U(VI) removal was evaluated via analysis of 16S rDNA sequences. When dissimilatory metal reduction was stimulated with the addition of acetate, microorganisms in the family *Geobacteraceae* became the predominant organisms, accounting for well over half the microbial community when the rates of Fe(III) and U(VI) reduction were the highest. These results are in accordance with a number of studies that have demonstrated that *Geobacteraceae* are the predominant dissimilatory metal-reducing microorganisms in a wide variety of environments in which dissimilatory metal reduction is an important process.

Given the predominance of *Geobacteraceae* in subsurface environments in which dissimilatory metal reduction is important, studies on the genetic potential of *Geobacteraceae* living in these sediments were initiated. Genomic DNA extracted from uranium-contaminated subsurface sediments was used to make a large-insert BAC library, and the library was screened to identify genomic DNA from *Geobacteraceae*. One of the most interesting findings was that an "as-yet-uncultured" *Geobacter* species living in sediments from the Old Rifle field experiment site was found to contain an important gene duplication event that has recently been studied in *Geobacter sulfurreducens*. This gene duplication includes a gene for an 89 kDa *c*-type cytochrome shown to be essential for Fe(III) reduction in *G. sulfurreducens*. This and other results from sequencing environmental genomic DNA suggest that the *Geobacteraceae* living in uranium-contaminated subsurface sediments have important genetic similarities with *Geobacteraceae* currently being intensively studied in pure culture.

In order to assess the activity and metabolic state of the *Geobacteraceae* predominating in these environments, techniques to extract mRNA from subsurface sediments were optimized, and analysis of gene expression in *Geobacter* species under various growth conditions helped identify key genes for monitoring metabolism of *Geobacteraceae* in the subsurface. Results of ongoing sediment studies using the mRNA approach will be presented.

Isolation and Characterization of Osmotolerant Strains from the Bear Creek Field Research Site

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Our overall objective is to provide information on the structure of the microbial community relevant to bioremediation efforts at the FRC Bear Creek site. To that end, the work reported herein focused on osmotolerant strains isolated from impacted sediments. Aquifer sediment at the FRC site has experienced dramatic changes in solute concentrations and hence water activity over the past 50 years. The large quantities of nitric acid have resulted in plumes of nitrate, aluminum and, undoubtedly, other solutes. With that in mind, we isolated osmotolerant strains from an impacted sediment to characterize the survivors of this extreme high-solute, low-water-activity environment. Sediment samples were washed in R2A broth and allowed to settle. Supernatants were serially diluted in R2A broth and plated directly onto R2A agar containing one of four osmo-stressors (40% sucrose; 20% glycerol; 20% polyethylene glycol [PEG]; 10% glycerol and 10% PEG). Thirty five isolates were clonally purified, archived, and characterized from the four isolation media. Twenty six isolates representative of the detected colony/cell diversity were selected for rRNA sequencing. Phylogenetic analysis of the rRNA sequence revealed that the majority of the isolates were Gram positives from the *Arthrobacter* group. Other isolates were closest to *Taxeobacter*, *Rhodanobacter*, *Cellulomonas*, and *Bacillus*. Additional work will focus on a comparison of the osmotolerant diversity detected at the FRC site with that detected in unimpacted soil. These FRC isolates will be screened for novel biodegradative activities.

Competitiveness Among Alternative Electron-Accepting Populations

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Denitrifying and sulfate-reducing molecular diversity was examined at the FRC to obtain gene probes for constructing a Functional Gene Array (FGA) to evaluate community dynamics at the site. Optimized conditions distinguished sequences >85% similar. A total of 539 *amo*A clones and 26 *pmo*A clones were screened by RFLP. Rarefaction analyses indicated that the majority of recovered diversity was sampled within 10 to 20 analyzed clones. The sites were dominated by two dominant *amo*A clones, which accounted for 56 to 88% of the libraries from any site. The *pmo*A sequences were only observed in samples FW-005, FW-300, and TPB-16, and the nitrate levels at these sites are relatively low compared to the others ($\leq$ 200 ppm vs. $\geq$ 1,000 ppm). The *amo*A diversity indices were only slightly higher at the circum neutral pH sites. The two dominant *amo*A nucleic acid sequences were between 93 and 98% similar to the *amo*A of *Nitrospira* species. A total of 958 *nir*K and 1,162 *nir*S clones were screened by RFLP, and 48 and 143 distinct *nir*K and *nir*S clones, respectively, were obtained. A single dominant *nir*K restriction pattern was observed for all six samples, and was 83% identical to the *Hyphomicrobium zavarzinii nir*K gene. A dominant *nir*S pattern was observed for four of the samples, including the background sample, and was 95% identical to the *nir*S of *Alcaligenes faecalis*. Principal component analysis (PCA) of the sites based on geochemistry grouped the samples by low, moderate, and high nitrate, but PCA of the unique operational taxonomic unit (OTU) distributions grouped the samples differently. The results indicated that the contaminated groundwater contained novel *nir*K and *nir*S sequences, functional diversity of both genes changed in relation to the contaminant gradient, but the *nir*K and *nir*S functional diversity was affected differently.

Six different FRC groundwater-sampling wells were selected for molecular investigation of subsurface sulfate-reducing assemblages. Three high contaminant load sites (FW-010, FW-005, FW-015) were within the immediate perimeter of the S3 source ponds, two moderate load sites (FW-003, TPB-16) were selected down strike by more than 275 m from the source ponds, and a single background site (FW-300) was selected for comparison. RFLP analysis of groundwater-derived SO_4-reducing gene libraries revealed 20 to 30% genetic overlap between all sampling wells, except TPB-16. TPB-16 differed markedly, having no shared patterns with any other site. A reactive barrier installed at this well prior to this study is the most likely explanation for the observed differences. PCA weighted nitrate and technetium with high statistical loading factors for system variability. These findings highlight that mixed legacy waste, while having a substantial impact on subsurface geochemistry, has not dampened the extent of sulfate-reducer genetic diversity. Some taxa appear to be highly resilient across the full range of environments sampled, while gradients in environmental selectors have encouraged the emergence of presumptive new genetic patterns that differ drastically from others described to date.

Geobacter, Geothrix, Shewanella, and *Desulfitobacterium* are considered the most likely genera to be involved in metal reduction, but their competitive dynamics under different conditions and over time have not been defined. We developed quantitative PCR probes to evaluate both among and within species competitiveness in microcosms with different matrices.

New Methods to Monitor Microbial Community Dynamics During In Situ Biostimulation

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A key to demonstrating the effectiveness of biological treatment strategies at a contaminated site is establishing that the desired bioprocesses are occurring or are likely to occur. In the last year we applied the use of simple in situ microbial sampling devices (biotraps) and expanded the analysis of lipid and DNA biomarkers to monitor UMTRA and FRC biostimulation experiments. At the Old Rifle UMTRA site, injection of highly bioavailable acetate increased viable microbial biomass, and decreased the starvation biomarker at both the shallow and deep wells immediately downgradient from the point of injection. Acetate addition promoted anaerobic conditions and stimulated the activity of Fe and U reducers that contained lipid biomarkers characteristic of the *Geobacteraceae* and a much smaller complement of the of low-G+C gram-positive *Clostridium*, and resulted in reduction of soluble U(VI) to insoluble U(IV). The microbial community change associated with the acetate addition was readily detected by DNA and lipids extracted from the traps. Specific DNA analysis of ribosomal RNA genes identified sequences of known uranium and sulfate reducers.

At the FRC site, traps were suspended in wells undergoing push-pull tests using a variety of electron donors. Results to date show that biotraps acted as sensitive in situ monitors of the microbial-community response to the addition of electron donors. Microbial biomass (total PLFA) levels increased in the stimulated wells, lipid biomarkers characteristic of anaerobes increased, and relative stress levels decreased as compared to the controls. Sequence analysis on bands recovered from 16S rDNA-DGGE gels identified bacteria involved in nitrate and metal reduction. The newly developed "rapid extraction and detection" techniques are now being routinely applied to samples from FRC and UMTRA sites. Additional assays (quinones, polar lipids, and diglycerides) as well as the PLFAs are being shown to be rapid, reproducible, and cost effective in monitoring of microbial communities involved in bioprocesses related to remediation.

Development of Microarray-Based Genomic Technology for Environmental Studies

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Development of novel chemistry immobilizing oligonucleotides and proteins on microarrays. Appropriate attachment of nucleic acids and proteins to an array surface is critical to microarray-based biomolecule analysis and detection. A novel three-dimensional surface chemistry was developed and evaluated for immobilizing oligonucleotides and proteins. Unmodified oligonucleotide probes and proteins were immobilized on glass slides coated with multilayered nanofilms through electrostatic adsorption and entrapment within the porous structure of the three-dimensional nanofilm. The binding capacity of oligonucleotides on the nanofilm slide was 2–3 times higher than that on the epoxy-activated and aldehyde-modified slides, and the nanofilm coated slides could differentiate single nucleotide differences. In addition, specific antibody-antigen interactions were obtained with a prototype of the nanofilm protein microarrays. Our results indicated that the nanofilm-based oligonucleotide and protein arrays had increased sensitivity and decreased background when compared to commercially available slides.

Microbial community diversity at FRC. Clonal libraries of the SSU rRNA gene were constructed from different groundwater samples at the FRC, and these sites differed with respect to geochemical characteristics. Inserts from the clones were amplified, partial nucleotide sequences were determined for 1,600 clones, and the sequences compared with cluster analysis. The results indicated that the microbial community composition at contaminated sites was altered compared to background. All unique clones from the six samples were selected (approximately n=325), and full-length sequences determined for the selection of distinctive oligonucleotides.

SSU rRNA Gene Oligonucleotide Microarray. Oligonucleotide microarrays were developed for the assessment of microbial communities based on conserved SSU rRNA gene sequences, and to determine whether single mismatch discrimination could be achieved. Our results indicate that the position of the mismatch, the type of mismatch, and the concentration of hybridization additives, such as formamide and tetramethylammonium chloride (TAMCl), significantly affected the specificity and signal intensity. The hybridization signal intensity of the probes with a single-base mismatch was decreased approximately 3- to 10-fold, depending on the oligonucleotide sequence. These results indicate that single-base discrimination for SSU rRNA genes can be achieved with array-based hybridization. The effects of probe length and GC content on microarray hybridization were also determined.

Bioremediation Gene Array (BGA). A bioremediation gene array (BGA) was also constructed based on more than 2,000 available sequences involved in biodegradation of pollutants and metal resistance. We have optimized the conditions for the discrimination of probes (50-mer) with up to 85% similarity without a significant reduction in sensitivity. Probes of less than 85% similarity showed less than 10% hybridization signal intensity with perfect match probes. For assessment of BGA, we used several reference strains that could degrade different pollutants. The results indicated that BGAs could be successfully used for the determination of metabolic pathways and biochemical activity.

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PROGRAM ELEMENT 3
Biomolecular Sciences and Engineering

Lateral Transfer of Metal Homeostasis Genes: A Comparison between Surface Bacteria and Isolates from the Deep Subsurface

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P_{IB}-type ATPases are a family of metal homeostasis proteins that utilize the energy generated by ATP hydrolysis for the transport of metals across cell membranes. These proteins are prevalent in nature, and they have been found in nearly all of the currently completed genome sequences in each of the three domains of life. The ubiquity of P_{IB}-type ATPase genes in bacteria, and their ability to confer resistances to Co, Ni, Cd, Pb, Zn, Ag, and/or Cu, are of relevance to bioremediation in metal and mixed waste contaminated sites, especially in deep subsurface soils and groundwater aquifers that are difficult to treat by conventional methods. We examined the role of lateral gene transfer in the evolution and spread of P_{IB}-type ATPases in the deep subsurface by focusing on bacterial isolates from the Subsurface Microbial Culture Collection (Tallahassee, Fla.). We developed a nested PCR approach to collect sequence data from over thirty strains belonging to the beta and gamma proteobacteria and high %mol G+C gram-positive bacterial groups. Phylogenetic analysis of these sequences revealed that three of the thirty metal homeostasis genes detected were incongruent with the 16S phylogeny, indicating that evolution by lateral gene transfer among subsurface bacteria has occurred, although it appears to be rare. As a comparison with surface strains, over 120 P_{IB}-type ATPase sequences were obtained from genome and DNA databases and examined for phylogenetic congruency with the corresponding 16S trees. Like the subsurface strains, lateral gene transfer of metal homeostasis genes among bacteria from the surface appears to be rare. Interestingly, nearly all of the putative transfer events among surface strains involved the movement of genes to or from gamma proteobacteria, indicating that their competence with diverse ecological niches may favor their participation in recent gene-transfer events.

Facilitated Chromate Reduction by *Deinococcus radiodurans* Engineered for Fuel Hydrocarbon Catabolism, and the Development of a Genetic System of Transformation for *Deinococcus geothermalis*

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The end of the Cold War in the early 1990s ushered in a shift in emphasis from nuclear weapons production to environmental cleanup. This enormous remediation challenge has driven the development of microbiological approaches to detoxify the immense volumes of ground that have been, or have the potential to be, contaminated by leaking radioactive storage tanks. However, the lethal effects of radiation have constrained consideration of microbial treatment of environments adjacent to highly radioactive tanks, where intervention could begin before contaminants become widely dispersed. Among the classes of toxic organic compounds identified in stored radioactive wastes, the fuel hydrocarbon toluene is one of the most prevalent, frequently occurring together with Cr(VI) and other toxic metals. We have engineered the extremely radiation-resistant bacterium *Deinococcus radiodurans* (DEIRA) to derive energy from complete toluene mineralization to support Cr(VI) detoxification and immobilization in natural sediment and groundwater analogues of U.S. Department of Energy (DOE)-contaminated environments. In addition, the engineered DEIRA utilizes carbon/energy from toluene oxidation for cellular biosynthesis and survival. During early operation, short-lived radionuclide heating of tanks raised their temperatures as high as 150°C, and in many cases contributed to their rupture and ongoing release of contaminants into the environment. Temperatures in some contaminated environments remain as high as 70°C because of long-lived radionuclide decay, and there is a need to develop radiation-resistant bioremediating organisms that function at elevated temperatures. *Deinococcus geothermalis* (DEIGEO) is thermophilic and closely related to the mesophilic DEIRA. We have shown that DEIGEO is naturally transformable with autonomously replicating and integration vectors derived from DEIRA, yielding a Hg(II)-resistant DEIGEO strain capable of reducing Hg(II) at elevated temperatures and in the presence of 60 Gy/hour. Additionally, like DEIRA, DEIGEO is capable of reducing Fe(III)–NTA, U(VI), and Cr(VI). These characteristics support the prospective development of engineered DEIGEO for bioremediation of radioactive mixed waste environments with temperatures as high as 55°C.

Uranium Reduction by *Shewanella oneidensis* MR-1 Requires *nrfA*, a Homolog of the *Escherichia coli* Nitrite Reductase Structural Gene

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Shewanella oneidensis MR-1 is capable of anaerobic respiration with uranium [U(VI)] as terminal electron acceptor. The genes and gene products required for anaerobic respiration on U(VI) are poorly understood. A U(VI) reduction-deficient mutant-screening technique was recently developed and used to identify anaerobic respiratory (Urr) mutants unable to respire with U(VI) as electron acceptor. The majority of Urr mutants displayed multiple respiratory deficiencies when grown with oxygen, nitrate, nitrite, sulfite, thiosulfate, Fe(III) oxide, Mn(IV) oxide, fumarate, or trimethylamine-*N*-oxide as electron acceptor. Urr mutant U14 was deficient in anaerobic growth on U(VI) and NO_2^-, yet retained the ability to grow on all other electron acceptors. An *Escherichia coli* nitrite reductase (*nrfA*) homolog, identified by BLAST analysis of the *S. oneidensis* genome, was insertionally inactivated by disruptive integration with broad-host-range suicide vector pKNOCK-Gm. The resulting *nrfA*::pKNOCK-Gm insertional mutant was unable to respire on NO_2^- or U(VI) as sole terminal electron acceptor. These results suggest that a structural component of nitrite reductase is involved in anaerobic U(VI) reduction by *S. oneidensis* MR-1.

Construction of Whole Genome Microarrays for *Desulfovibrio vulgaris* and Expression Analysis of Cells Grown Under Uranium-Reducing Conditions

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Microbial bioremediation is of great scientific and practical interest. The genetic and physiological capability of microorganisms to reduce and transform environmentally toxic metals and radionuclides is evident in nature; however, little is known regarding the molecular mechanisms and regulatory networks controlling these processes. Access to the genomic contents of metal- and radionuclide-reducing bacteria, combined with microarray technologies, provides an opportunity to elucidate metal/radionuclide respiratory pathways, thereby providing avenues for predictable and effective bioremediation practices. *Desulfovibrio vulgaris* Hildenborough has been the focus of biochemical and physiological studies in the laboratory, and the metabolic versatility of this organism has been largely recognized. *D. vulgaris* is capable of coupling the oxidization of a variety of electron donors (e.g., lactate, pyruvate, succinate, ethanol) to the reduction of many different electron acceptors (e.g., sulfate, fumarate, iron, uranium, chromium, potentially O_2). The capacity of this bacterium to reduce different metals and radionuclides enzymatically has been demonstrated, and the focus of the proposed work is to identify and characterize the cellular mechanisms for these reductions.

Desulfovibrio vulgaris Hildenborough is a δ-proteobacterium with a genome of 3.6 Mb that is 65% G+C. The genome sequence is complete, and the gaps have been closed (http://www.tigr.org/tigr-scripts/ufmg/ReleaseDate.pl). *Desulfovibrio* spp. are relatively easy to culture, and significant amounts of biomass can be harvested when grown in the presence of sulfate and other metals. This versatility clearly facilitates laboratory growth, maintenance, and manipulation of *D. vulgaris*. We are currently conducting experiments to compare the utility of cDNA-based and oligonucleotide-based microarrays. Once the comparison is complete, whole genome microarrays for *D. vulgaris* will be constructed. Our primary goals are the: (1) construction of a whole-genome microarray for *Desulfovibrio vulgaris* and (2) use of the microarray to identify cellular responses to uranium. Preliminary data suggest that *D. vulgaris* has unique periplasmic polypeptides when exposed to uranium, as visualized with one-dimensional SDS-PAGE.

Gene and Protein Expression in Wild-Type and Mutant *Shewanella oneidensis* MR-1

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Shewanella oneidensis MR-1 is a facultative anaerobe that can readily adapt metabolically to environmental changes. To better understand the metabolic transitions that occur in *S. oneidensis* cells in response to the growth environment, we are comparing the gene and protein expression in wild-type and mutant MR-1 cells grown under different conditions. Regulation of gene expression occurs at the DNA level, whereas protein expression can be altered at the DNA, mRNA, or even the protein level (i.e., changes in the rate of protein degradation). Therefore, parallel analysis of both gene and protein expression provides a more complete picture of cellular response to the environment than analysis of either component alone. In our project, a microarray representing the MR-1 genome has been used to screen the mRNA population, while two-dimensional gel electrophoresis coupled with peptide mass spectrometry has been used to monitor the protein component. Comparison of wild-type cells grown with different electron acceptors revealed numerous changes in both mRNA and protein expression, including a protein annotated as a conserved hypothetical protein. Analysis of cells in which the gene encoding this conserved hypothetical protein has been deleted revealed numerous gene and protein expression changes relative to wild-type expression. We are also working on the identification of numerous gene and protein changes that occur in cells lacking the OxyR gene. The identification of these proteins, and the comparison of expression levels at the mRNA and protein levels, will reveal which metabolic pathways are involved in the observed phenotypic changes and will provide insight into the mechanisms of their regulation.

Metabolic Engineering of Microorganisms for Actinide and Heavy-Metal Precipitation

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Heavy metals and actinides are significant problems at a number of DOE sites and industrial locations in the U.S. Many of these sites contain heavy metals, actinides, and organics. Due to the costs associated with excavating, transporting, and remediating contaminated sediments at remote locations, an economically viable solution is to mineralize the organic contaminants in situ and immobilize the metals and actinides to prevent movement to other locations. There are few reports in the literature of organisms capable of all of these functions. Besides their potential use in situ, these organisms should find use in treating waste tanks at sites such as Hanford that contain mixed organics, metals, and actinides.

During the previous grant period, we isolated (from a deep-sea hydrothermal vent) and characterized a novel strain of *Pseudomonas aeruginosa* capable of removing high levels of cadmium from solution by reducing thiosulfate to sulfide and precipitating cadmium as cadmium sulfide on the cell wall. To improve upon this system, we successfully engineered *Escherichia coli*, *Pseudomonas aeruginosa*, and *Pseudomonas putida* to remove heavy metals and actinides from solution and immobilize them on the cell wall. For precipitation of cadmium, zinc, lead, and other metals that form strong sulfide complexes, we developed two systems for aerobic sulfide production: (1) expression of serine acetyl transferase and cysteine desulfhydrase in *E. coli* for overproduction of cysteine and subsequent conversion to sulfide; and (2) expression of thiosulfate reductase in *E. coli* and *P. putida* for reduction of thiosulfate to sulfide. The *P. putida* system was shown to allow simultaneous heavy metal precipitation and organics degradation. For precipitation of actinides as complexes of phosphate, we overexpressed polyphosphate kinase in *E. coli* and *P. aeruginosa* to enable these organisms to accumulate high levels of polyphosphate during phosphate excess and high levels of exopolyphosphatase for polyphosphate degradation and concomitant secretion of phosphate from the cell. All of these systems were shown to be capable of removing relatively high levels of metals from solution and have the potential to remove metals and actinides from contaminated waste streams or to immobilize these elements in situ.

The goal of our work is to engineer heavy metal and actinide precipitation in two microorganisms that will be relevant for treatment of DOE sites contaminated with heavy metals, actinides, and/or organics: *Pseudomonas aeruginosa* and *Deinococcus radiodurans*. Specifically, we propose (1) to engineer polyphosphate synthesis and degradation into *Deinococcus radiodurans* and *P. putida* for removal of uranium(VI) and plutonium(VI, V); (2) to engineer aerobic sulfide production into *D. radiodurans* for removal of cadmium, zinc, and lead; and (3) to test removal of actinides; actinides and heavy metals; and actinides, heavy metals, and organics using the engineered organisms.

Signature-tagged Mutagenesis for Identification of Genes Required for Survival of Sulfate-Reducing Bacteria in Sediments

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Sulfate-reducing bacteria (SRB) are widely distributed in aquatic and terrestrial sediments, and they play a key role in the bioremediation of metals and hydrocarbons in the environment. We have proposed that sediment-dwelling microorganisms have unique functions, encoded at the genetic level, that are manifest only during exposure to conditions in their natural environments. Signature-tagged mutagenesis (STM) is a powerful technique that can be used to identify these genes. Several strains of *Desulfovibrio* have been tested to identify a strain suitable for mutagenesis experiments. *Desulfovibrio desulfuricans* strain G20 is amenable to introduction of foreign DNA through conjugation and electroporation. We have also tested many transposon-containing plasmids in an effort to develop a mutagenesis system capable of generating random mutants at high efficiency in strain G20. Several Tn5, Tn7, and mariner-transposon-containing vectors were transformed into strain G20, but none produced a random set of mutants. We have recently identified pBSL180 containing a modified Tn10 transposon that will efficiently transform strain G20 and produce stable transformants. The transformation frequency reached about 10^{-5}, with at least 33 unique insertion patterns identified by southern blot analysis out of 42 randomly picked transformants (>78%).

STM requires the presence of oligonucleotide tags within the transposon ultimately used to identify mutants. These tags are comprised of an internal 40-base-pair variable region flanked by 20-base-pair constant arms used for amplification of tags. Unique tags (96) have been cloned into the multiple-cloning site within the Tn10 transposon. Microarray technology has been adapted to screen mutants. Variable-region targets are attached to slides and hybridized with amplified DNA from mutants. Tags have been tested for cross-hybridization, and 60 out of 96 tagged transposon-containing vectors showing no cross-hybridization were chosen to perform subsequent experiments. Mutants will be incubated in sediments to identify those containing mutations required for survival. Growth of *Desulfovibrio* transformants in sediments has been studied and shown to peak after a 7–9 day incubation. During this time period, populations of *Desulfovibrio* grow to reach 10- to 50-fold of their initial concentration. This is optimal for selection of mutants.

Recent experiments were also completed using another technique to identify important genes for microbial processes of environmental significance. With differential display techniques, we have identified genes expressed by *Desulfovibrio vulgaris* on exposure to metals. *D. vulgaris* was cultivated in media containing 50 μM Cu(II) or Hg(II), and total RNA was extracted during the early log phase of growth. The RNA extracts were analyzed using random arbitrarily primed PCR (RAP-PCR). Products were compared to mRNAs from cells grown without metals, and differentially expressed cDNAs were isolated and sequenced. The genes for an ATP binding protein (ORF 2004) and an ATPase (ORF 856) were upregulated (4- to 6-fold with Hg and 1.4- to 3-fold with Cu) in metal-treated cultures. These results suggest that *D. vulgaris* uses an ATP-dependent detoxification mechanism for living with toxic metals in the environment.

AdnA-Regulated Biofilm Formation in *Pseudomonas fluorescens* Pf0-1

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Pseudomonas fluorescens is a soil bacterium with potential applications in bioremediation and biocontrol. Motility and adhesion are thought to be indispensable for both applications. Previously, a nonadherent, nonmotile mutant of *P. fluorescens*, Pf0-1, was isolated and shown to be defective in soil persistence. Consistent with the hypothesized importance of motility for soil persistence, the mutation was found to lie in a gene (*adnA*) with similarity to *fleQ,* a master regulator of flagella gene expression in *P. aeruginosa*, and a member of the NtrC family of transcriptional regulators. Genetic analysis was used to show that the AdnA regulon is composed of at least 7 genes/operons (termed *aba* genes) in *P. fluorescens*, two of which have no sequence similarity with previously described genes. Interestingly, while the AdnA mutant is unable to form biofilms on abiotic surfaces, four of the *aba* mutants were able to suppress the requirement for AdnA in biofilm formation. To further investigate this phenotype, Pf0-1 and the *aba* mutants were tagged with GFP, and biofilms growing on glass slides were examined using fluorescence microscopy. Mutants *aba18* and *aba51* developed biofilms that were structurally similar to the wild type, but appeared to consist of fewer cells and more extracellular material. Another mutant, *aba203*, was able to adhere to the glass surface but could not form microcolonies or develop into a mature biofilm, supporting an operational distinction between surface adhesion and biofilm development. Members of the NtrC family of regulators require the alternate sigma factor σ^{54}, which may facilitate environmental responsiveness. To further understand AdnA and its regulon, we examined the dependence of *aba* genes on σ^{54}. Surprisingly, the *aba* genes have differing requirements for σ^{54}; some genes seemingly have no requirement for σ^{54}, and some have a partial dependence, while others appear to be totally dependent on σ^{54}. We speculate that expression of *aba* genes in the environment is fine-tuned by the level of dependence upon AdnA, σ^{54}, and as yet unidentified factors, allowing rapid responses to environmental perturbations.

Mechanisms for the Reduction of Actinides and Tc(VII) in *Geobacter sulfurreducens*

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Uranium and technetium are the primary radioactive metals contaminating subsurface environments at Department of Energy (DOE) sites. Dissimilatory Fe(III)-reducing microorganisms can control the mobility of these contaminants through the enzymatic reduction of highly soluble U(VI) and Tc(VII) to insoluble tetravalent forms that will precipitate from groundwater and be immobilized in the subsurface. The objective of the proposed research is to characterize the mechanisms of U(VI) and Tc(VII) reduction in the dissimilatory Fe(III)-reducing bacterium *Geobacter sulfurreducens*. In a previous two-year NABIR-funded study, we purified and characterized a periplasmic cytochrome c_7 that reduced a range of electron acceptors in vitro, including U(VI), Fe(III), and humics. Confirmation that the protein was required for the reduction of these electron acceptors in vivo was obtained using a deletion mutant lacking the structural gene for the cytochrome. The mechanism of enzymatic Tc(VII) reduction was also characterized in whole cells. The oxidation of hydrogen was coupled to the reduction of Tc(VII) leading to the precipitation of Tc(IV) in the periplasm. The involvement of a periplasmic Ni/Fe hydrogenase was implicated by CO profiling.

The aims of this new study are to use the tools of biochemistry and molecular biology to confirm the identity of the genes encoding the relevant U(VI) and Tc(VII) reductases in *G. sulfurreducens*, and to elucidate the detailed mechanisms of U(VI) and Tc(VII) reduction by the corresponding enzymes. Furthermore, we propose to explore the range of other metals and radionuclides reduced by *Geobacter sulfurreducens*—including Np(V), Pu(IV) and Hg(II)—and identify the roles of the U(VI) and Tc(VII) reductases in the reduction of these other priority pollutants.

The specific hypotheses that will guide our research are:

(1) Cytochrome c_7 functions as the U(VI) reductase of *G. sulfurreducens* in vivo, and is also capable of reducing and modifying the solubility of other actinide species [including Np(V) and complexed Pu(IV)] and toxic metals [including Co(III) and Hg(II)] via a mechanism that is distinct to that catalyzing the transfer of electrons to insoluble Fe(III) oxides.

(2) Key amino-acid residues can be mutated to identify regions of cytochrome c_7 that are required for the reduction of metals and radionuclides.

(3) A periplasmic Ni/Fe hydrogenase is the Tc(VII) reductase of *G. sulfurreducens*. This enzyme is also able to reduce a range of other electron acceptors, including U(VI) directly when hydrogen is supplied as the electron donor.

(4) Additional genes may be required for U(VI) reduction in *G. sulfurreducens*, and can be identified by transposon mutagenesis.

This project is in collaboration with co-PIs Dr. Francis Livens and Dr. Iain May of the Radiochemistry Centre, Manchester, and we will also continue successful collaborations with experts in protein crystallography (Dr. Marianne Schiffer of the Argonne National Laboratory) and the molecular biology of *Geobacter* (Dr. Derek Lovley of the University of Massachusetts).

Biomolecular Mechanisms for Microbe–Fe(III) Oxide Interactions in *Geobacter* species

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The most promising strategy for in situ bioremediation of radioactive groundwater contaminants identified by the NABIR program to date is to stimulate the activity of dissimilatory metal-reducing microorganisms to reductively precipitate uranium, technetium, and radioactive cobalt. Previous studies with a variety of subsurface sediments, including those at uranium mine tailings (UMTRA) sites, have indicated that *Geobacteraceae* are the primary agents for metal reduction and that, even when uranium levels are high, electron transfer to Fe(III) reduction accounts for ca. 99% of the growth of the *Geobacteraceae*. These results suggest that, in order to understand the factors controlling the growth and activity of the predominant U(VI)-reducing microorganisms during subsurface bioremediation, it is important to understand how *Geobacteraceae* interact with the Fe(III) oxides. Preliminary studies have demonstrated that *Geobacteraceae* specifically produce pili and flagella when growing with insoluble Fe(III) oxide as the electron acceptor and that these appendages are important in aiding *Geobacter* species in accessing insoluble Fe(III) oxides. Therefore, the objective of this research is to investigate the outer surface of *Geobacter* species and to determine what outer surface structures these organisms use to access insoluble Fe(III) oxides. In the proposed research we will: (1) evaluate with novel proteomic approaches which proteins in *Geobacter* species, other than pili and flagella, are exposed to the extracellular environment; (2) determine which of these proteins are specifically expressed during growth on Fe(III) oxide; (3) determine with immunological techniques if, as hypothesized, these proteins are localized on one side of the cell; (4) examine the role of these proteins in cell–Fe(III) oxide interactions with genetic techniques and biological force microscopy; (5) determine the lipid composition of the cell membranes and potential changes in membrane composition during growth on Fe(III) oxide; and (6) use state-of-the-art electron microscopy procedures to examine the structure of the outer surface of the cell during growth on soluble electron acceptors and insoluble Fe(III) oxide.

These studies combine expertise in the physiology of *Geobacteraceae* (U. Mass.) with expertise in the analysis of microbial surface structure and cell-metal interactions (Terry Beveridge, U. Guelph), as well as expertise in novel proteomics approaches (Mary Lipton, PNNL). This project, which has only just started, is expected to provide insights into the factors controlling the growth and metabolism of *Geobacteraceae* during in situ bioremediation of uranium, and to identify molecular targets that can be used to assess the activity of *Geobacteraceae* in the subsurface. Progress that has already been made in identifying some outer membrane proteins and their functions will be presented.

Molecular Biology and Biology of Bacterial Chromate Reduction

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Chromate is a serious environmental pollutant that is amenable to bacterial bioremediation. Biomolecular and genetic engineering approaches can help overcome problems that hamper chromate remediation by bacteria. Pursuit of these approaches requires a better understanding of the biochemistry, biological role, and regulation of bacterial chromate-reducing enzymes, and identification of suitable candidates for such studies. We have cloned and expressed genes encoding six such candidates, and have demonstrated that they fall into two distinct classes of enzyme. The Class I enzymes are the more effective chromate reducers, and are also effective in catalyzing quinone reduction. Conversely, the Class II enzymes are less effective in reducing chromate but highly effective in reducing quinones and, furthermore, nitrocompounds, which are also important environmental pollutants. As our primary concern is chromate bioremediation, we have to date focused primarily on two Class I enzymes, ChrR from *Pseudomonas putida* and YieF from *Escherichia coli*. Regulatory studies indicate that both enzymes appear to be under the control of starvation-type promoters, which is promising for bioremediation applications, because it facilitates improvements that would ensure high-level expression under low-nutrient field conditions. A *chrR* mutant strain exhibits diminished chromate reduction, and also reduced viability in the presence of chromate, highlighting the importance of these enzymes. The mutant also displays increased sensitivity to compounds such as paraquat and H_2O_2 that are strong oxidizing agents. These enzymes reduce quinones divalently. This is important, as fully reduced quinones have the property to quench reactive oxygen species (ROS), whose generation is a major reason for chromate toxicity in bacteria. Divalent quinone reduction also prevents ROS generation from metabolically produced or environmental quinones by other cellular enzymes that reduce them univalently. YieF also quenches respiratory chain-associated reactive-oxygen-species generation in respiring-membrane vesicles. Moreover, as opposed to most biological enzymes that reduce chromate univalently to Cr(V) [hence setting up a redox cycle that results in the generation of large quantities of reactive oxygen species (ROS)], YieF does so divalently, as evidenced by rapid mixing experiments permitting measurement of redox changes at ms scale (which failed to reveal generation of seminquinone flavoprotein in the enzyme during chromate reduction), and by direct fluorimetric determination of ROS produced during chromate reduction. These experiments show conclusively that YieF generates only the absolutely unavoidable amount of ROS during chromate reduction, given the fact that this reaction involves a four-electron reduced enzyme catalyzing a reaction requiring only three electrons. These enzymes therefore constitute a broad antioxidant defense mechanism whose biological role encompasses protection against chromate and quinone toxicity and also offers a possible avenue for enhancing bacterial resistance to deleterious field conditions. In conclusion, the two enzymes whose molecular biology, biochemistry, and physiology we have examined in detail are highly attractive candidates for protein and genetic engineering interventions to enhance bacterial chromate-reducing potential and antioxidant defense.

Construction of a Transcriptional Fusion Library for Expression Analysis in *Shewanella oneidensis* MR-1

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Whole-genome microarrays hold promise for modeling the behavior of gene expression in response to varying the environmental conditions. Advances in this technology make it possible to simultaneously measure expression from all genes present in a genome in a single experiment. A complementary approach for characterizing differential gene expression can be provided by analysis of reporter activity mediated by transcriptional fusions. Because reporter activity can be measured in living cells in real time, the use of transcriptional fusions is more amenable to high-throughput continuous-expression analysis under many growth conditions than are microarrays. We describe the construction of a targeted reporter library (62 constructs) in *Shewanella oneidensis* MR-1, whereby promoter-containing DNA sequences upstream to genes associated with electron transport, adhesion, and cell signaling were cloned in the pProbe-NT broad-host range vector upstream to the plasmid-encoded green fluorescent protein (GFP). The copy number of this vector in MR-1 was estimated to be one by southern hybridization analysis and was shown to be stable over many generations during growth in media lacking antibiotic selective pressure. Initial testing, using a screening assay developed to measure the effect of growth in suspension versus on solid surfaces, suggests that expression of the promoter upstream to *mtrDEF* is significantly higher in MR-1 cells growing on surfaces than in suspension (broth cultures). Similar, but less dramatic, differences were observed for other promoters. High-throughput approaches for transfer of plasmid constructs into MR-1 regulator mutants and subsequent expression analyses are also under development.

Studies of Multi-Heme Cytochromes from *Geobacter sulfurreducens*

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Microbial reduction of U(VI) to U(IV) is a promising strategy for the in situ remediation of uranium, and thus is a primary focus of the NABIR program. The *Geobacteraceae* family predominates in the reduction of uranium in subsurface environments. Current studies are focusing on the model organism, *Geobacter sulfurreducens*, which is capable of using Fe(III) as its terminal electron acceptor. Intensive functional genomics and physiological studies are in progress in Prof. Derek Lovley's laboratory, and the complete genome sequence of this organism is available from TIGR. The *G. sulfurreducens* genome contains a large number (>100) of cytochromes *c*; they function in the metal-reduction pathways. We are studying two types of cytochromes that are required for the reduction of Fe(III). These are the three-heme cytochromes (c_7 family), and the membrane-bound 89 kDa cytochrome FerB, which contains 12 hemes. The c_7 three-heme cytochromes are related to the well-studied c_3 four-heme cytochromes found in sulfur-reducing bacteria. The c_7 cytochromes appear to be characteristic of a family of bacteria that can reduce Fe(III). We are also developing heterologous expression systems for the production of multi-heme cytochromes *c*.

The c_7 cytochrome (PpcA) was originally purified from the periplasm of *G. sulfurreducens*; it was shown to reduce Fe(III), U(VI), and Cr(VI). We found, using BLAST searches, that the bacterial genome codes for four other homologs of the c_7 protein and, in addition, for three other proteins that are polymers of the c_7 domains. Two of the polymers have four repeats, and one has nine repeats; they contain 12 and 27 hemes, respectively. At present it is not clear under what conditions the c_7 homologs are expressed and how they are used by *G. sulfurreducens*.

We have previously expressed cytochrome PpcA in *E. coli*; and purified it, crystallized it, and determined its structure by x-ray diffraction using 1.45 Å data. The core of this molecule is formed by the three hemes, surrounded by the 71 amino acid residues of the protein. We have located the chromate binding site. The packing of the molecules in the crystal lattice can serve as a model for the structures of the poly-c_7 cytochromes. Comparing the sequences of the c_7 domains in the polymers with the c_7 sequences suggests that the polymers are a new type of cytochrome, with different heme coordination for one of the hemes in each domain. Structural studies of these closely related molecules will help elucidate the features that are responsible for their functions.

Global Analysis of *Shewanella oneidensis* Strain MR-1 Proteome Using Accurate Mass Tags

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Bacterial strains such as *Shewanella oneidensis* strain MR-1 are key organisms in the bioremediation of metals, due to their ability to enzymatically reduce and precipitate a diverse range of heavy metals and radionuclides. Important in these processes is the need to develop improved enzymatic pathways in these organisms. As a first step, the proteome of the organism—defined as the entire protein complement of the cell expressed under a given set of conditions—must be completely characterized. A single genome can exhibit many different proteomes, depending on stage in cell cycle, cell differentiation, response to environmental conditions (nutrients, temperature, stress, etc.), or the manifestation of disease states. Therefore, the study of proteomes under well-defined conditions can provide a better understanding of complex biological processes, which requires faster and more sensitive capabilities for the characterization of cellular constituents.

We have utilized a new technology, based on the combination of global tryptic digestion, high-resolution liquid chromatography, and tandem mass spectrometry and high field FTICR mass spectrometry, to define a proteome of an organism. One approach for protein identification is based on global approaches for protein digestion and accurate mass analysis; this approach resulted in the generation of an "accurate mass tags" (AMTs) database for *S. oneidensis*. Combined with analysis of intact proteins, we can identify post-translational modifications of these proteins. Additionally, we have extended the use of this AMT database based on stable-isotope labeling to quantitatively determine the changes in protein abundance from cells grown aerobically and anaerobically.

Protein Engineering in the Hg(II) Resistance (*mer*) Operon

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NABIR Research Objectives. Proteins of the widely found bacterial mercury resistance (*mer*) operons convert reactive inorganic [Hg(II)] and organic [RHg(I)] mercurials to relatively inert monoatomic mercury vapor, Hg(0). We study the metalloregulator MerR, and the two enzymes that mediate this process: MerA, the mercuric reductase; and MerB, the organomercurial lyase.

Results and Plans. We have generated a single polypeptide (107aa), which contains the coiled-coil metal binding domain (MBD) of the MerR homodimer. In whole cells, and also in purified in vitro cells, MerR and MBD bind several thiophilic metals and metalloids (1). XAFS data show that MBD forms a HgS_3 structure like MerR, and both form a CdS_4 structure; the source of the 4th S may be a solvent thiol. ICP-MS stoichiometry is 1 for both proteins. We have fused the MBD protein to Lpp-OmpA; fluorescent antibody, protease sensitivity, and antibody pull-downs indicate that MBD is surface displayed at ~20,000 copies per cell, providing >600% increase in Hg(II) binding over uninduced cells. Near-term plans include kinetic and thermodynamic studies of MerR and MBD interactions with Hg, Cd, Co, Pb, and U using equilibrium and nonequilibrium dialysis, isothermal titration calorimetry, and various spectroscopies with colleagues in the UGA Center for Metalloenzyme Studies. We can also now regularly get MerR to form small crystals and will use various strategies to get them large enough for a run at APS.

***MerB*.** The 4 cysteines of the 212-amino-acid organomercurial lyase MerB have distinct roles in protonolysis; and the protein itself, not the solvent, is the immediate source of the proton (2). Formation of a Hg(II)-trapped state of MerB is the basis for its requirement of a 2-fold molar excess of thiol (2). We have cryostabilized crystals of MerB, collected 3.3 Å data at UGA, and will be collecting data at APS in February with UGA crystallography collaborator Cory Momany.

***MerA*.** With Sue Miller (UCSF), we are defining the role of the mobile N-terminal domain of MerA (NmerA) by expressing full-length MerA and the catalytic Core with or without an independently expressed NmerA domain in wild-type (WT) *E. coli* and in strains lacking the ability to make glutathione (GSH), the major cellular thiol buffer. These constructs are stable and express well in GSH^- strains. We are now comparing Core and Core+NmerA with WT MerA in Hg resistance and in ^{203}Hg volatilization in WT *E. coli* and in GSH^- strains. We hypothesize that Core alone cannot compete for Hg(II) coordinated to other thiols, but that it requires the mobile NmerA domain to pluck Hg(II) from GSH or cytosolic proteins and to present it to the active site. So we expect that in GSH^- cells, Core will be slow in Hg volatilization compared to WT MerA; and that NmerA, even as a separate protein, will repair Core's defect. Understanding this "bucket brigade" mechanism in MerA will illuminate efforts to broaden MerA's substrate range to radionuclides of interest to DOE and the role(s) of these common N-terminal domains in general.

1. Song, L. (ASM Student Travel Awardee), Caguiat, J., and Summers, A.O. (2002). Poster Q180, 102nd General Meeting of the ASM, Salt Lake City, Utah, May 2002.
2. Pitts, K.E. and Summers, A.O. (2002). The roles of thiols in the bacterial organomercurial lyase (MerB). *Biochemistry*, **41**:10287–10296.

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

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The biotransformation of metals and radionuclides by subsurface bacteria offers considerable potential for environmental remediation of contaminated sites. The effectiveness of bioremedial technologies will increase, provided the biological mechanisms of metal reduction are better understood and manipulated for optimum benefit. Metal-reduction rates are accelerated by dissimilatory metal-reducing bacteria in the presence of humic compounds. This is a result of the redox cycling nature of humics as a function of their quinone moieties. *Shewanella algae* BrY and *S. oneidensis* MR-1 produce melanin, a pigment common to many microorganisms. Melanin is classified as a humic compound that has quinone moieties and redox cycling behavior. Under anaerobic conditions, melanin serves as a terminal electron acceptor and soluble electron shuttle to iron minerals. Melanin is also detected on the bacterial surface, which results in increased iron mineral reduction rates, by as much as tenfold.

Melanin production by *S. colwelliana* is a product of the *melA* gene, which encodes for the enzyme p-hydroxyphenyl pyruvate dioxygenase (pHPPD) and is part of the phenylalanine/tyrosine pathway. For melanin to be produced through this pathway, tyrosine is broken down to p-hydroxyphenyl pyruvate, then to homogentisic acid, which is then excreted from the cell. Outside the cell, homogentisic acid is autooxidized and self-polymerized to form quinone-rich melanin polymers. A nucleotide sequence similar to the *melA* gene of *S. colwelliana* was detected on the genome of *S. oneidensis* MR-1 via a BLAST search, revealing a 92.5% amino acid similarity to *melA*. Other genes responsible for melanin production, such as tyrosinase, were not detected on the *S. oneidensis* MR-1 genome.

Melanin production by *S. oneidensis* MR-1 may be an important mechanism for electron transfer to insoluble metals. To evaluate the role melanin plays in metal reduction, melanin production will be inhibited under various growth conditions with the triketone sulcotrione [2-(2-chloro-4-methane sulfonylbenzoyl)-1,3-cyclohexanedione], a pHPPD inhibitor. In addition, deletion mutants, deficient in melanin production, will be developed by deletion mutagenesis of the *melA* gene. The resulting cultures will be evaluated for soluble and insoluble metal-reduction capabilities. These results will determine the significance of melanin production as a mechanism for metal reduction. Since melanin production from *S. oneidensis* MR-1 requires extracellular homogentisic acid, the role of the Type II secretion system in melanin production will be determined using insertion mutagenesis techniques.

Genes for Uranium Bioremediation in the Anaerobic Sulfate-Reducing Bacteria

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The ubiquitous sulfate-reducing bacteria of the genus *Desulfovibrio* have the capacity to change the redox state of a number of toxic heavy metals, including the conversion of soluble uranium(VI) to the less soluble uranium(IV). Because this process has potential for application in bioremediation efforts of contaminated environments, the identity of the reductases and the parameters that delimit their functioning are of great interest. Previous experiments explored the role of cytochrome c_3 (cyt c_3) in uranium reduction by *Desulfovibrio desulfuricans* strain G20. A derivative mutated in the gene for the dominant cyt c_3 was able to reduce U(VI) at a rate of about one-half that of the wild type using lactate or pyruvate as the electron donor. When hydrogen was the electron donor for U(VI) reduction, this mutant was more severely impaired, exhibiting a rate about 10% that of the wild type. The interpretation of these data was that the electrons from hydrogen are transferred primarily to U(VI) through a cyt c_3–dependent pathway, while some of the electrons from the organic acids lactate and pyruvate are able to bypass the cyt c_3 pathway. A possible candidate for an alternative electron carrier comes from the work of Wade and DiChristina (*FEMS Microbiol. Lett. 184*(2):143-148, 2000), who showed that a component common to nitrite reduction of *Shewanella putrefaciens* strain 200 (possibly nitrite reductase itself) was necessary for U(VI) reduction by that bacterium. To explore the functioning of nitrite reductase in U(VI) reduction by *Desulfovibrio* strains, a kinetic analysis of simultaneous U(VI) and nitrite reduction by the mutant strain lacking the dominant cyt c_3 was performed. Initial experiments indicate that U(VI) and nitrite reduction proceed independently in both the mutant and the wild type. Interestingly, the cyt c_3 mutant reduces nitrite at a rate about twice that of the wild type when pyruvate is the electron donor. Attempts are under way to construct a mutation in the gene for nitrite reductase for further testing of its contribution to U(VI) reduction.

With access to the genome sequence of *Desulfovibrio vulgaris* Hildenborough, a computational approach for predicting conserved regulatory elements in the intergenic regions has been adopted. Intergenic regions are grouped both by putative metabolic networks and by putative conserved regulons of *E. coli*. Statistically significant regulatory elements are being determined that will be analyzed for function in the laboratory. One such unique element has been identified that is currently being tested. The data collected will serve as a framework for future experimental studies of the regulatory networks of *D. vulgaris*.

Single-Molecule Dynamics of a Flavin Reductase Involved in Metal Reduction and EDTA Degradation

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The general *Eschericia coli* flavin reductase (Fre) has been reported to reduce metals, including Fe(III)-siderophores, Cob(III)alamin, and chromate. Fre can also supply reduced flavins for reduced flavin-utilizing monooxygenases, including EDTA and NTA monooxygenases. Thus, Fre is studied at the single-molecule level to probe its conformational dynamics on multiple time scales. We demonstrate the use of electron transfer (ET) as a spectroscopic ruler to measure the minute structural changes in single-protein molecules. Binding assays and structure simulation have demonstrated that Fre binds flavin adenine dinucleotide (FAD) tightly with a K_d value of 29 nM. For Fre with bound FAD, the fluorescence of FAD is quenched by a nearby tyrosine residue via photo-induced ET. The fluorescence lifetime of the FAD in a single-enzyme molecule is probed on a photon-by-photon basis, which allows the determination of the potential of mean force between the flavin fluorophore and a nearby tyrosine, and the observation of conformational fluctuation at multiple time scales spanning at least five decades. This phenomenon can be explained in terms of anomalous diffusion on a rugged energy landscape, and indicates the existence of long-lived conformers at room temperature. The characterization of Fre by both single-molecule techniques and conventional approaches has provided insights into flavin biochemistry that are important in metal bioremediation.

PROGRAM ELEMENT 4
Biogeochemistry

Impact of Ferrihydrite Biomineralization on Contaminant and Microbial Dynamics

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Soils and sediments are complex assemblages of organic and inorganic material that are seldom at sustained equilibrium. Coupled biological, chemical, and physical factors dictate the evolutionary pathways of the system. The transition from aerobic to anaerobic conditions, for example, has profound impacts on the reactive mineralogical constituents and the operating biochemical pathways. We have recently explored the alteration in reactive phases and contaminant sequestration mechanisms upon reductive dissolution of ferrihydrite by dissimilatory iron-reducing bacteria under hydrodynamic conditions. A complex mineral assemblage results that is dominated by the production of goethite and magnetite with small quantities of green rust. The principal bacterial role to supply an Fe(II) source, with the resulting ferrous-iron concentration being the dominant factor controlling the biomineralization pathway. At low Fe(II) concentration (less than 0.4 mM at pH 7), goethite is the dominant product, with magnetite being the major product at higher ferrous iron concentrations. Abiotic experiments confirm the role of Fe(II) in the mineralization process.

On the basis of the resulting particle morphology and lattice fringe images, goethite is produced through a dissolution/reprecipitation process. Accordingly, formation rates of goethite, as elucidated with x-ray absorption spectroscopy, provide conservative estimates for the rate of ferrihydrite dissolution. The rate of Fe(II)-catalyzed ferrihydrite dissolution is surprisingly rapid—7×10^{-9} mol m^{-2} s^{-1} in the presence of 2 mM Fe(II) at pH 7. In fact, these rates are sufficient to support dissimilatory iron reduction even at micromolar levels, challenging the assumption that the low solubility of iron oxide minerals limits ferric-iron accessibility. These observations further suggest that Fe(II)-induced transformations towards more crystalline phases are a major consumption pathway of reactive ferric hydroxides, dramatically altering nutrient and contaminant sequestration (suppressing, for example, arsenic and phosphorus retention >50-fold).

The fate of redox active contaminants such as chromium and uranium will be impacted dramatically as a consequence of reductive biomineralization. Adsorption properties will be modified appreciably with the shift in mineralogy, and the development of reactive ferrous-iron-bearing phases (solution, surface, and solid) will have important ramifications on reductive stabilization. While the extent of contaminant adsorption on ferric (hydro)oxides will generally decrease upon biomineralization (a consequence of the crystallization and diminished surface area), the potential for reductive stabilization will be enhanced. In fact, considering the phases resulting from our biomineralization experiments, chromate reduction will be controlled nearly equally by green rust, Fe(II)- saturated goethite, and aqueous Fe(II). Similarly, the pertechnetate anion will be rapidly reduced by these ferrous-bearing phases. Uranyl reduction, on the other hand, remains dominated by biological (enzymatic) pathways, albeit chemical avenues—principally pathways involving green rust and Fe(II)-bearing goethite—are significant.

Biogeochemical Processes Controlling Microbial Reductive Precipitation of Radionuclides

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This project will identify and quantify coupled biogeochemical reactions that control reductive precipitation of U(VI) and Tc(VII). Both batch and continuous flow experimental methods in combination with aqueous geochemical and spectroscopic analyses are being used. Most equilibrium speciation models predict the dominant U(VI) aqueous species in groundwater will be uranyl-carbonate complexes. A recent description of a calcium-uranyl-carbonate (Ca-U(VI)-CO_3) complex indicates this species may dominate the aqueous speciation of U(VI) in many environments, including FRC and UMTRA sites. Bacterial reduction of U(VI) in bicarbonate-buffered solution in presence and absence of Ca was monitored. XAFS measurements confirmed the presence of a Ca-U(VI)-CO_3 complex in the initial solutions containing Ca. Ca, at concentrations of 0.45–5 mM, caused a significant decrease in rate and extent of bacterial U(VI) reduction. Reduction of U(VI) by facultative (*Shewanella putrefaciens* strain CN32) as well as obligate (*Desulfovibrio desulfuricans*, *Geobacter sulfurreducens*) anaerobes was affected by the presence of Ca. U(VI) reduction ceased when system Eh reached –0.034 ± 0.001 V, based on the $Ca_2UO_2(CO_3)_3 \rightarrow UO_{2,cr}$ couple. Results are consistent with the hypothesis that U is a less effective electron acceptor when present as the $Ca_2UO_2(CO_3)_3$ complex. Microbial Tc(VII)O_4^- reduction under identical conditions was unaffected by the presence of Ca, suggesting inhibition of U reduction was not a result of generalized inhibition of microbial activity. Preliminary investigations probing influence of periplasmic precipitation of UO_2 on viability of *S. putrefaciens* CN32 suggest the viable CN32 population decreases during U reduction by 100- to 1000-fold, as measured by cultivation on TSB+fumarate agar, but that viability appears not to be directly linked to UO_2 precipitation. Higher populations of viable cells were maintained with lactate as an electron donor compared to H_2. U precipitates were observed in association with cell periplasm and outer membrane as reduction proceeded, suggesting it proceeded concurrently on both sites. Manganese (III, IV) oxides are among the most powerful naturally occurring oxidants and may promote oxidative dissolution of uraninite ($UO_{2,cr}$) and TcO_2, even under anaerobic conditions. Thermodynamic calculations predict, and batch laboratory experiments confirm, manganese oxides oxidize UO_2 and TcO_2 under a broad range of solution conditions. Yet the rate this solid–solid redox reaction occurs is poorly known. Initial characterization focused on oxidation of a dissolved organic (oxalate) by Mn oxides. Oxalate oxidation by Nsutite (γ-$Mn^{IV}O_2$), Birnessite (δ-$Mn^{IV}O_2$), Manganite (γ-$Mn^{III}OOH$), and Hausmannite ($Mn^{II}Mn^{III}Mn^{III}O_4$) is well described by a 2nd-order kinetic model, which accounts for aqueous speciation and Mn sorption (treated as equilibrium reactions), changing surface area as solids are reductively dissolved (kinetic reactions), and the electron transfer reaction (kinetic). For these minerals, generation of Mn^{2+} as reaction progresses has little impact on reaction rate and extent. By contrast, oxalate oxidation by Bixbyite ($Mn^{III}Mn^{III}O_3$) exhibits a decrease in rate of reaction as Mn^{2+} is generated, and cannot be described by a single 2nd-order kinetic expression. Rate inhibition is removed when alternate sites for Mn^{2+} sorption are included, and rate of oxalate loss is described by a single 2nd-order expression. Thermodynamic calculations predict the propensity for oxalate oxidation follows the order Hausmannite > Bixbyite > Manganite > Birnessite > Nsutite. Kinetic data follow the order Manganite > Hausmannite > Birnessite > Bixbyite > Nsutite. Rate constants for oxalate oxidation by various Mn oxides span several orders of magnitude. Reaction network and rate parameters identified from batch systems described results from continuous flow experiments. Results provide baseline characterization of the reactivity of Mn oxides for future experiments investigating the reactivity of UO_2 with the same Mn oxides.

The Biogeochemistry of Pu and U: Distribution of Radionuclides Affected by Microorganisms and their Siderophores, Reductants, and Exopolymers

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Radionuclide-contaminated environments are often oxic, including the Rocky Flats Environmental Technology Site (RFETS), and the contaminated groundwater at the NABIR Field Research Center (FRC). Radionuclide distribution within such environments is affected by indigenous biogeochemical processes, including the metabolic activities of aerobic microorganisms, key members being the ubiquitous *Pseudomonas* and *Bacillus* genera. Because of the chemical similarities between the actinides uranium (U) and plutonium (Pu), and iron (Fe), the metabolic processes of these microorganisms that affect the biogeochemistry of Fe could also significantly affect Pu and U distribution. We are determining the extent to which metabolic processes involved in Fe acquisition and in exopolymer production affect the distribution of Pu and U between the aqueous and solid phases. Our focus is on determining the equilibrium distribution of Pu and U between these phases, in the presence and absence of microorganisms, and in relation to Fe bioavailability. Secondly, using transposon mutagenesis, we are determining to what extent microbial processes (including siderophore production and metabolism, and exopolymer and reductant production) directly or indirectly influence aqueous/solid phase distribution of Pu and U.

In addition to considering chelators and potential metal-reducing exudates, we are investigating the limits of direct metal reduction. The metal reductase activity of a microorganism is believed to be governed by its c-type cytochrome content. In order to catalytically reduce solid metal oxides such as iron(III) oxides and metal–ligand complexes, the redox potential of these compounds needs to be in the range accessible to cytochrome C. We have examined the redox properties of commonly used iron(III) complexes (such as NTA, EDTA, citrate, siderophores, etc.), which cover a wide range of redox potentials (+200 to 400 mV), and have started examining the ability of *Shewanella* and *Geobacter* sp. to use these iron complexes as terminal electron acceptors.

The results of the research program will contribute to NABIR's stated needs to understand both "the principal biogeochemical reactions that govern the concentration, chemical speciation, and distribution of metals and radionuclides between the aqueous and solid phases" and "what alterations to the environment would increase the long-term stability of radionuclides in the subsurface."

Microbial Stabilization of Plutonium in the Subsurface Environment

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Plutonium contamination is widespread in the surface soils and subsurface sediments throughout the DOE complex. Pu is generally considered to be relatively immobile; however, transport of Pu, albeit at markedly low concentrations, has been observed at many DOE sites. The focus of this award is to elucidate the processes that lead to enhanced stabilization (i.e., immobilization) of dissolved and colloidal Pu species, through the action of natural microbial communities. The work this year consisted of three broad research avenues: (1) the production of exopolysaccharides (EPS) by *Clostridium* sp. and free-living marine bacteria, (2) the evaluation of stability constants for Pu binding to acid polysaccharides, and (3) evaluations of the biotransformation of Pu complexes by *Pseudomonas fluorescens.*

Production of EPS. Compounds produced by *Clostridium* sp., grown in an anaerobic medium, were characterized by GC-EI-MS. Results show that acid polysaccharides (mainly galacturonic acid) dominated in the colloidal fraction (>1 kDa). Results from gel electrophoretic-focusing experiments using ethanol-extracted acid polysaccharides labeled with Pu(IV) are being compared to results from field studies. Free-living bacteria in the marine environment were tested to determine if they respond to quorum-sensing autoinducer molecules (AHLs). The presence of quorum-sensing systems in free-living aquatic bacteria may indicate that these systems are widespread in natural habitats. Quorum sensing may be involved in the production of Pu-binding EPS ligands. Results demonstrate that free-living bacteria at low concentrations do indeed respond to ambient concentrations of these molecules.

Pu complexation with organic ligands. An experimental methodology was developed for evaluating stability constants for Pu(IV) complexation, with organic ligands at environmentally relevant concentrations (e.g., $10^{-15} - 10^{-10}$ M). The ligand-exchange method was evaluated with citric acid and applied to alginic acid, an acid polysaccharide. Analysis of the experimental data indicates that the Pu–alginic acid complex is 1:2 (Pu/alginic) acid with a log $\beta_{1,2}$ value of 13.47 ± 0.022 at I = 0.1 M. Experiments are underway to evaluate Pu(IV) complexation with galacturonic acid, the primary component of the EPS produced by *Clostridium* sp.

Biotransformation of Pu compounds. Biotransformation of equimolar Pu nitrate, Pu hydroxide, and Pu citrate by *Pseudomonas fluorescens* under aerobic conditions is being investigated. The effect of excess citric acid on the rate and extent of biotransformation of equimolar Pu citrate is also being examined. Pu–organic complexes and the degradation products of citric acid are identified by electrospray ionization-mass spectrometry (ESI-MS). Speciation of Pu before and after microbial activity is determined by solvent extraction technique and by XANES and EXAFS at the NSLS. Studies on the effect of aerobic and anaerobic microbial processes on Pu mobilization and immobilization as well as Pu speciation in Pu-contaminated soil collected from the Rocky Flats Environmental Technology Site are in progress. Pu-contaminated soil and Pu-contaminated soil spiked with Pu nitrate and amended with carbon, nitrogen, and phosphorus are incubated under aerobic or anaerobic conditions.

Hydrogen as an Indicator to Assess Biological Activity during Trace-Metal Bioremediation

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The design and operation of a trace-metal or radionuclide bioremediation scheme requires that specific redox conditions be achieved at given zones of an aquifer for a predetermined duration. Tools are therefore needed to identify and quantify the terminal electron acceptor processes (TEAPs) that are being achieved during bioremediation in an aquifer, and to ensure that this is done at a high spatial resolution. Dissolved hydrogen concentrations have been shown to correlate with specific TEAPs during bioremediation in an aquifer. Theoretical analysis has shown that these steady-state hydrogen levels are solely dependent upon the physiological parameters of the hydrogen-consuming microorganisms, with hydrogen concentrations increasing as each successive TEAP yields less energy for bacterial growth. The assumptions for this statement may not hold during a bioremediation scheme in which an organic substrate is injected into the subsurface and where organisms may consume hydrogen and carbon simultaneously.

A series of batch experiments were conducted to study the utilization of acetate and hydrogen by *Geobacter sulfurreducens* under iron-reducing conditions. Microcosms were set up to investigate the utilization of hydrogen and acetate when either of them is the sole electron donor and when both are present and utilized simultaneously as an electron donor. These experiments were conducted for varying initial conditions of the hydrogen and acetate concentration, and the disappearance of these compounds plus the evolution of Fe^{2+}, as well as biomass, were monitored over time. The results of these studies indicate that the biokinetic coefficients describing the rate of hydrogen utilization are not affected by the simultaneous utilization of acetate, and that the biokinetic coefficients describing the rate of acetate utilization are not affected by the simultaneous utilization of hydrogen.

A model was developed using the batch dual-donor experiments, which indicate that the steady-state hydrogen concentration in the presence of an organic as electron donor is not only dependent on the biokinetic coefficients of the TEAP, but also the concentration of the organic substrate. Column experiments performed to measure steady-state H_2 concentrations under dual electron-donor conditions were compared to the model formulation. Steady-state H_2 concentrations in a flow-through column using NABIR Field Research Site (Oak Ridge, TN) soil were higher than observed for iron-reducing conditions in the field even though evidence suggests that iron reduction was the only active TEAP in the column. These dual-donor steady-state H_2 concentrations correlated with the dual-donor model formulation. Additional column experiments are currently being performed under iron-reducing conditions to further characterize the effect of dual electron-donor utilization on steady-state dissolved hydrogen concentrations. This includes an additional column experiment similar to that described above (Field Research Site soil, indigenous organisms) as well as a more controlled column experiment using glass beads, synthetic iron (ferric citrate), and the iron-reducing, H_2 and acetate-oxidizing bacteria (*Geobacter sulfurreducens*) used in the batch experiments.

The Role of Biogeochemical Dynamics in the Formation of U(VI) Solids under Oxic Conditions

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To assist the U.S. Department of Energy (DOE) with long-term stewardship issues associated with bioremediation of uranium (U), the overall goal of this work is to define the mechanisms by which microorganisms facilitate the formation of insoluble hexavalent U solid phases. Under anoxic conditions, microbial reduction of U(VI) to U(IV) can potentially decrease groundwater U contamination by lowering solubility and by slowing migration through the soil. However, such biological alteration must be considered temporary unless long-term anoxia can be maintained. When oxic conditions return, U(IV) will likely oxidize to U(VI), which is generally more soluble and potentially more mobile. For example, in U ore deposits in which uraninite (consisting of reduced, tetravalent U as UO_{2+x}) is the parent material, exposure to oxidizing conditions results in alteration to U(VI) minerals, with the U(VI)-phosphates frequently defining the boundaries of the ore body. Of the U(VI)-containing minerals, these U(VI)-phosphates are of interest because they are the least soluble of the U(VI) solids found in nature. Additionally, U(VI) solids such as metaschoepite have been observed as the products of the oxidation and corrosion of spent nuclear fuel, in which U is present in the reduced form.

Microorganisms present in soils may play a role in the formation of U(VI)-phosphate solid phases. However, the role of microorganisms in transformation and precipitation of actinide-containing minerals is not well understood. Bacteria are believed to influence actinide geochemistry through various mechanisms that are a part of the biogeochemical cycle of U. Our research links important geochemical and microbiological aspects of this problem, providing a fundamental basis for predicting the complex and dynamic interplay of biological treatment strategies.

We are investigating the role of model microorganisms such as *Bacillus sphaericus* and *Shewanella putrefaciens* on U(VI) sorption, solid phase formation, and transformation. These species are commonly found in soils as well as microbial consortia isolated from the NABIR Field Research Center at Oak Ridge National Laboratory. Data will be presented on the relative abilities of these bacteria to sorb U over a wide pH range, with special attention to the sorption behavior at low concentrations of U ($\leq 10^{-6}$ M), such as would be commonly found in the environment. We observe maximum sorption between pH 5 and 6. Cell-surface functional groups responsible for U interaction are identified using TRLFS (Time-Resolved Laser-induced Fluorescence Spectroscopy) and EXAFS (X-ray Absorption Fine Structure Spectroscopy). This information can then be used to design remediation systems that stimulate biological activity to favor the formation of U(VI)-phosphate phases. We are also investigating the impact of these bacteria on the transformation of the U(VI) oxide hydrates (e.g., metaschoepite, $[(UO_2)_8O_2(OH)_{12}]\cdot 12H_2O$) to U(VI) phosphates such as metaautunite, $Ca[(UO_2)(PO_4)]_2\cdot 6H_2O$. The abiotic transformation pathway of metaschoepite to metaautunite has been previously elucidated by Sowder (1998) and indicates that dissolution and reprecipitation of the U crystal structure is required, thus presenting a potential kinetic barrier for nucleation and reprecipitation. Our current biotic studies suggest that the bacterial surfaces may serve as nucleation sites, possibly via the surface sorption sites identified by TRLFS.

Biogeochemistry of Uranium Under Reducing and Re-oxidizing Conditions: An Integrated Laboratory and Field Study

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Reduction and subsequent precipitation of uranium (U) and chromium (Cr) by sulfate-reducing bacteria (SRB) and dissimilatory iron-reducing bacteria (DIRB) is well known. The use of SRB also has the potential to immobilize other toxic metals because of the large number of metals (Hg, Pb, Cd, Cu, Ni, Zn) that form stable sulfide compounds.

This project builds on our prior research to integrate laboratory and field studies focused on the NABIR FRC at Oak Ridge, TN. At the FRC, in-situ coupon experiments were conducted in a well penetrating the saturated zone of Area 3, an acidic, uranium-contaminated zone at the FRC. Hematite mineral surfaces were exposed to groundwater and indigenous flora. The bacterial community that developed over a 2-month period on hematite surfaces contained 13 Operational Taxonomic Units (OTUs) that fell primarily within the β- and γ-Proteobacteria, with over half of the recovered clones represented by a strain that exhibited 95% similarity to an *Alcaligenes* sp. and approximately 50% of the remaining clones represented by a strain that exhibited 96% similarity to a *Frateuria* sp. These strains were not represented in the more phylogenetically-diverse microbial community that colonized hematite mineral surfaces recovered from an uncontaminated site at the FRC. We developed a Ag staining method for imaging of reducing zones on mineral surfaces, including Fe oxides. To our knowledge, this is the only microscopic technique that can image Fe(II) zones on the surface of an Fe-bearing mineral with monolayer sensitivity.

In addition to field studies, laboratory experiments have been a combination of batch studies and under flowing conditions to better represent aquifer conditions for U(VI) reduction. *Desulfovibrio desulfuricans* was grown in a flat plate reactor using lactate. Uranium, in form of soluble U(VI), was continuously fed to the reactor at a concentration of 30 mg/L for eight (8) months, during which time U(VI) was completely removed from solution. Bulk water chemistry and microbial activity measurements indicate that uranium was immobilized by two simultaneous processes: (1) chemically, by reduction and precipitation with microbially generated hydrogen sulfide, and (2) and by direct reduction. The Fe(III)- and U(VI)-reducing bacterium, *Shewanella oneidensis* was grown in six fracture flow reactors (FFRs) of different geometries. The spatial and temporal distribution of substrate (tracked using a tracer dye (brilliant blue FCF)), was greatly influenced by attached growth. In all FFRs, bacterial density was observed higher at the entrance, likely due to the presence of higher electron donor concentrations. Results suggest that spatial heterogeneity of attached bacteria depends on the geometry of the fracture zone. These data will provide a baseline for developing nutrient delivery strategies for improving spatial distribution of microbial activity and U(VI) reduction capability in fracture flow systems.

Our earlier assessment of Al toxicity to SRBs was extended to include the effects of Al speciation. Thermodynamic speciation calculations and Al-27 nuclear magnetic resonance spectra provided evidence for the presence of colloidal clusters of the Al-13 tridecamer ion in the suspensions yielding the greatest decreases in SRB population.

Transformation and Stabilization of Chromium in Subsurface Environments

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Chromium is one of the major heavy-metal contaminants in the environment. Oxidized hexavalent chromium species, such as chromate CrO_4^{2-} and dichromate $Cr_2O_7^{2-}$, are highly toxic, and potential carcinogens and mutagens. By contrast, the reduced Cr(III) that readily forms insoluble oxides and hydroxides [$Cr(OH)_3$] is regarded as less toxic or nontoxic to organisms. Thus, the reduction of environmental Cr(VI) to Cr(III) constitutes a valuable mechanism of detoxification that can be accomplished by both abiotic and biotic processes. Abiotic reduction occurs through reaction with labile species, such as hydrogen sulfide and thiols, which probably play a crucial role in transforming Cr(VI) under reducing conditions. Reduction of Cr(VI) by bacteria also occurs under reducing conditions, characteristic of the cytoplasmic milieu, after an active uptake of Cr(VI) inside the cell. Most such bacterial reductions entail enzyme reductases that generally have active sulfhydryl sites. Thus, reduced-sulfhydryl sulfur also may be involved in the cytoplasmic reduction of chromate by bacteria. We conducted mechanistic studies of the reduction of Cr(VI) with several sulfur nucleophiles including hydrogen sulfide, and several thiols, 3-mercaptopropionic acid, cysteine, and glutathione, at a pH range of 4–9 at ambient temperature. XANES spectroscopy performed at the NSLS revealed changes in the speciation of both chromium and sulfur. With thiols, the main products generated at low pH were disulfides. However, on increasing the pH into the basic range, the oxidation proceeded to the level of sulfonates with intermediate stages, including sulfinates. Hydrogen sulfide was oxidized to thiosulfate and sulfate.

The biochemical pathway was examined using a highly resistant *Bacillus* sp. Because sulfhydryl sites are known to be active sites of enzyme reductases, we examined bacterial growth and reduction of Cr(VI) by *Bacillus* in the presence of varying concentrations of two major sulfur species, sulfate and thiosulfate. Our results established that bacteria reduce hexavalent chromium to its trivalent form. While a large fraction of the reduced chromium is associated with the bacterial mass, it is not clear whether it is present internally in the bacterial cytoplasm or externally on their outer surface. While changes in sulfate did not affect the reduction rate, raising the thiosulfate concentration in the medium from 0.05 mM to 1.0 mM markedly increased the reduction rate. Thiosulfate enhances the reduction of Cr(VI) probably by accelerating the biosynthesis of chromate reductase enzymes, although other mechanisms may be involved. Our results show that the enzymes or any other substances mediating the reduction reside mostly in the cytoplasm.

Influence of Reactive Transport on the Reduction of U(VI) in the Presence of Fe(III) and Nitrate: Implications for U(VI) Immobilization by Bioremediation/Biobarriers

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The coupling of biogeochemical and transport processes is important to the bioremediation of metals and radionuclides in the field, but experimental research systems with transport and biologically mediated redox reactions are severely lacking. We propose to examine the reduction of U(VI) in the presence of nitrate and Fe(III)-containing minerals under conditions representative of biostimulation. This research will establish (1) mechanisms by which the fluxes of electron acceptors, electron donors, and other species can be controlled to maximize the transfer of reductive equivalents to the aqueous and solid phases; and (2) associated process models that describe the transport and reaction of U(VI) and iron species under conditions relevant to bioremediation.

This research will utilize DOE subsurface sediments collected from the Hanford site and the Oak Ridge FRC, and synthetic porous media designed to have specific bioavailable iron-mineral phases and contents. The facultative dissimilatory metal-reducing bacteria *Shewanella putrefaciens* (strain CN32) and *Shewanella oneidensis* (strain MR-1) will be adopted as test organisms. Experimental research will be conducted using sediment-packed column systems, and will be focused on three main areas: (1) the importance of the abiotic reduction of U(VI) by biogenic Fe(II); (2) the influence of the transport process on Fe(III) reduction and U(VI) immobilization, with an emphasis on methods for controlling the fluxes of aqueous species to maximize uranium reduction; and (3) the reductive capacity of biologically reduced sediments (with respect to reoxidation by convective fluxes of O_2 and NO_3^-) and the long-term stability of immobilized uranium mineral phases after bioremediation processes are complete. The proposed research is unique in the NABIR portfolio, and it will provide scientifically based information that will be useful in the design and assessment of bioremediation strategies for U(VI) as well as other metals and radionuclides.

We will report on early results from our research. The material presented will focus on results from (1) flow-through stirred systems for the examination of sorption and abiotic reduction of U(VI) by Fe(II) under conditions of fluid transport; (2) U(VI) transport experiments in column systems containing Hanford and Oak Ridge FRC sediments under various concentrations of carbonates; and (3) collaboration with the EMSL users' facility for use of NMR and SEM/TEM to examine the structure of biofilms of the iron-reducing bacterium S. oneidensis MR-1 at subpore scales within porous media.

Reduction of TcO_4^{-2} by Biogenic Fe(II) in Sediments from DOE's Oak Ridge and Hanford Sites

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Technetium-99 is an important subsurface contaminant at DOE Sites, including Hanford, Oak Ridge, and Savannah River. At Hanford, it is the most important risk-driving contaminant, and its subsurface inventory and mobility is key to all long-term site assessments and risk analyses. ^{99}Tc is persistent with a long half-life (2.3×10^5 years) and often exists in the subsurface as the mobile, pertechnetate [$Tc(VII)O_4^{2-}$] anion. ^{99}Tc can be immobilized from water by reduction of $Tc(VII)\ O_4^{2-}$ to insoluble Tc(IV) oxides, and this reductive process may be manipulated for remedial purposes. This research is investigating the role of mineral-bound, biogenic Fe(II) in the heterogeneous reduction of Tc(VII) in the absence and presence of NO_3^-, a key DOE co-contaminant.

Last year, we reported that $Tc(VII)O_4^{2-}$ is rapidly reduced to Tc(IV) by biogenic Fe(II) produced by metal-reducing bacteria (*Shewanella putrefaciens*) in both Oak Ridge and Hanford sediments. Surprisingly, $Tc(VII)O_4^{2-}$ was quantitatively reduced even in the presence of high concentrations of NO_3^-, which are present in most DOE groundwater plumes. The reaction of NO_3^- with biogenic Fe(II) and Tc(IV) is kinetically inhibited. This work has lead to the development of kinetic models that describe the reduction rate of $Tc(VII)O_4^{2-}$ as a function of biogenic Fe(II) concentration. Research over the past year has developed a mechanistic basis for these models by identifying and characterizing the forms of biogenic Fe(II) that are most reactive with $Tc(VII)O_4^{2-}$. This task has been accomplished by using FRC sediments that have been biologically and abiotically reduced, treated with various chemical agents to remove hypothesized reactive Fe(II) phases, and respiked with various concentrations of specific Fe isotopes (^{56}Fe and ^{57}Fe) to allow the monitoring of specific Fe(II) pools. These materials have been studied both before and after reaction with $Tc(VII)O_4^{2-}$, and the results have provided intriguing insights on the reactivity of various Fe(II) forms (adsorbed surface complexes on Fe(III) oxides, exchangeable Fe(II) on phyllosilicates, structural Fe(II) in phyllosilicates, and other unique biotic phases) with $Tc(VII)O_4^{2-}$. Specific reactive Fe(II) phases have been identified and monitored after reaction with $Tc(VII)O_4^{2-}$ using wet chemical techniques, analytical transmission electron microscopy, variable temperature ^{57}Fe Mossbauer spectroscopy, and x-ray absorption spectroscopy. An exciting result has been the finding that metal-reducing bacteria generate an apparently unique surface phase in the FRC sediment that is quite reactive with Tc(VII). It exhibits a distinct Mossbauer signal, but has not yet been definitively identified. Additional studies this year will target the identification of this phase and characterize mineralogic and chemical factors controlling the oxidation rate of $Tc(IV)O_2 \bullet nH_2O$ from Oak Ridge and Hanford sediments.

NABIR
Environmental Management Projects

In-line Uranium Immunosensor

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The goal of this project is to develop an in-line uranium immunosensor that can be used to determine the efficacy of specific biostimulation approaches. The DOE–NABIR program is presently funding a project to develop and test a handheld immunosensor that will provide a rapid, portable assay for hexavalent uranium. For in situ stabilization processes (i.e., the "push-pull" experiments or nutrient "recycling" trials), however, an in-line sensor that could monitor the removal of uranium (or other radionuclides) over longer periods of time would have distinct advantages. It would operate autonomously and provide near real-time data about uranium immobilization in the absence of personnel at the site. In fact, the idea for this sensor was generated after discussions with investigators performing in situ stabilization experiments at the NABIR Field Research Center at Oak Ridge National Laboratory. This site is proposed for the testing and initial deployment of the sensor. The project is a joint effort between the Blake laboratory at Tulane and Sapidyne Instruments, Inc, and has three technical goals:

1. Construction of an in-line immunosensor for hexavalent uranium after engineering discussions with the final users;
2. Incorporation of the reagents already developed for the handheld immunosensor into this device and testing of its performance capabilities with hexavalent uranium spiked into buffer and groundwater samples;
3. Determination of the capabilities of the in-line sensor during upcoming field tests at the FRC.

A prototype device (a modification of a KinExA 3000 instrument already available in the PI's laboratory) has been installed. A commercially available antibody-antigen-capture reagent system with binding properties similar to the uranium system was identified. This system (antibiotin-biotin-biotin-BSA) was used to test the ability of the prototype sensor to (1) provide a linear dose response with antibody concentration; (2) respond appropriately to increasing quantities of antigen; and (3) autonomously dilute reagents from stock solutions. After minor design changes, the first prototype passed all three performance tests. Work is underway to finalize the design of the optics modules, which will determine the space and power requirements of the final unit.

Field Investigations of Lactate-Stimulated Bioreduction of Cr(VI) to Cr(III) at Hanford 100H

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DOE faces challenging problems in cleaning up 18 major facilities, including those with sites contaminated with hexavalent chromium [Cr(VI)] associated with the production of weapons-grade nuclear materials, plating and boiler operations, and equipment repair and fabrication. Cr(VI), which is a strong carcinogen, is highly mobile, whereas trivalent Cr(III) is less hazardous and practically immobile under reduced conditions. Cr(VI), which is widely distributed at one of the most contaminated DOE sites, Hanford, is migrating to the Columbia River. One of the most promising new remediation technologies for chromium-contaminated groundwater is in situ bioreduction. The proposed field project is a direct extension of a NABIR project that received 3 years of support and demonstrated the biogeochemical transport effects of carbon on stimulation of bioreduction of chromium in soil cores, and supports the SCFA Technical Targets, "Metals and radionuclide source zone stabilization and treatment," and "Biogeochemical processes that determine contaminant fate."

The overall objective of the proposed project is to carry out field investigations to demonstrate the feasibility of a cost-effective, in situ remediation technology, using lactate-stimulated bioreduction of dissolved Cr(VI) to form an insoluble mineral precipitation of Cr(III) at the Hanford 100H area. Specific goals will include: (1) design of a field test to develop criteria for full-scale deployment of in situ Cr(VI) bioreduction via lactate stimulation for use at DOE sites; (2) providing of field testing and monitoring (including geophysical methods) of the effects of lactate biostimulation on microbial community activity, redox gradients, transport limitations, and other reducing agents, and comparison of the field results with those of our previous NABIR laboratory work; (3) assessment of the kinetic rates and conditions that may cause reoxidation of Cr(III) to Cr(VI) after biostimulation is terminated; (4) assessment of the use of bioremediation in conjunction with other alternative remediation technologies, such as a pump-and-treat approach, for the Hanford 100H area. The results of this project will be used to develop a conceptual model of chromium bioreduction in groundwater on a field scale and to provide recommendations for field deployment of lactate-stimulated bioremediation.

In Situ Immobilization of ^{99}Tc at the Hanford Site by Stimulation of Subsurface Microbiota

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Microorganisms, either directly or indirectly, can alter the oxidation states of uranium and technetium resulting in their precipitation as sparingly soluble solid phases. This process, in concept, can render these contaminants immobile for long time periods. Previous and ongoing NABIR research has established the basic biogeochemical principles underlying the direct and/or Fe(II)-facilitated reduction of ^{99}Tc by microbes. The objective of this project is to evaluate the applicability of these principles to the problem of groundwater contamination by ^{99}Tc at the Hanford Site. Technetium-99 is a radionuclide that contributes significantly to estimates of future human health risk at Hanford because of its longevity and mobility in the subsurface environment. It exists at high concentrations (up to 30,000 pCi/L) in the central areas of the site where the groundwater table is deep, and is predicted to move to the Columbia River within the next decade. It also has been observed at lower concentrations (600 pCi/L) in shallow groundwater near the river in the 100 H area. This project focuses on laboratory studies to establish the existence and metabolic requirements of microorganisms native to Hanford sediments that are capable of directly or indirectly mediating $Tc(VII)O_4^{2-}$ reduction. If lab-scale experiments are successful, biostimulation tests using a single-well push-pull approach will be used to evaluate the viability of in situ reduction of ^{99}Tc by native microorganisms (in collaboration with Jack Istok, Oregon State University), and a field-scale biostimulation experiment will be designed. This project will determine if bioremediation of ^{99}Tc is feasible and ultimately may lead to low-cost reduction of risk from ^{99}Tc at the Hanford Site.

Currently, we are performing initial experiments on samples of the Ringold Formation recently collected from a saturated subsurface zone contaminated with U and Tc. Sediment samples have been used in microcosm experiments and microbial enrichments to probe for the presence of microorganisms capable of reducing TcO_4^{2-} or Mn and Fe. Microcosms have been amended with various electron donors including acetate, lactate, formate, or glucose with and without nitrogen and phosphorous amendment. Metal-reducing activity will be monitored in these microcosms over time and those exhibiting activity will be used to establish microbial enrichments for the specific type of metal-reducing activity identified. Samples of the Hanford Formation from the subsurface of 100 H Area will be collected later this year for use in biostimulation experiments.

BASIC:

Bioremediation and Its Societal Implications and Concerns

Testing a Stakeholder Participation Framework for Fielding Bioremediation Technologies

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This research is investigating stakeholder attitudes about the use of bioremediation technologies, with the objective of reducing conflict among stakeholders. The research protocol includes four closely related components. First, we are testing a framework for stakeholder participation that prescribes appropriate stakeholder involvement strategies based on stakeholders' trust of the other parties involved in technology-deployment decision making. Second, we are assessing conflict among stakeholders regarding the acceptability of in situ bioremediation (i.e., immobilization) as a means to reduce risks posed by radionuclides and metals in the environment. Third, we are assessing the role that awareness of risk exposure plays in the willingness of stakeholders to engage in problem solving and making risk tradeoffs. Fourth, we are assessing the potential of using the results of these first three components to forge consensus among stakeholders regarding the use and oversight of bioremediation technologies and stakeholder involvement in the decision process. This poster presentation describes the results of empirical tests of hypotheses related to the first three objectives. Data used in these tests are the result of more than 75 interviews conducted during the summer of 2002 in the regions around Oak Ridge, TN, Los Alamos, NM, and Hanford, WA. Current activities include collecting additional data through a large telephone survey in these same geographical regions. After processing all data, we will be communicating our results to the interested and affected parties by holding workshops for DOE employees and contractors, and for community members in each of the three study areas.

Use of Stases when Negotiating and Defining Issues in Public Discussions

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Increasingly, scientists are being asked to meet nonscientists to discuss science and technology research and initiatives. The success of these interventions varies enormously, indicating the need for better communication tools and processes. One largely overlooked communication instrument has been the use of *stases*, the category sets used (largely unconsciously) by speakers for negotiating and defining issues in public discussions. In our previous work in BASIC, we observed patterns of such category sets in the unscripted interactions between NABIR scientists and nonscientists, and characterized them in ways suitable for preparing for future interactions. Our current work verifies and extends the method, both for preparing and analyzing interactions with a variety of stakeholder groups.

Briefly stated, transcripts of stakeholder/scientist naturalistic (i.e., unscripted but purposive) interactions have shown discussants using four general category sets, close to those anticipated by traditional stases: (a) *factual information*, including findings, approach, and outcomes of research; (b) definitions and *clarifying information*; (c) speculations, questions, and predictions about practical *courses of action and applications* for the work; and (d) observations and questions about the *quality and value* of the work. Recently, we confirmed the existence of these "public science stases" by independent human coding, and examined transcripts with content-analysis software (Diction 5.0 and WordStat) to form dictionaries of word usage and co-location of topics. Also, content analysis of recent newspaper articles about bioremediation and NABIR science, as samples of recent public concerns, is currently extending the available dictionary of topics and terms, so that we can better characterize the complexity of social concerns by accurately diversifying our category sets, and correlating category sets with other social indicators.

Our analysis suggests that the category sets can serve as indicators of significant commonalities and differences in frames of reference. Deviations in stasis patterns, for instance, reveal how individuals (a) develop innovative communication patterns when confronted with patterns outside of their communication repertoire, (b) expand the context for interactions, and (c) surface productive and unproductive communication norms. Over time, use of the category sets can suggest ways in which communication goals and behaviors change.

Besides its value for NABIR scientists, we are examining the technique in the light of public discussions of science and current methods of discourse analysis: Do some categories generally co-occur? Are some categories more prominent in certain atmospheres of controversy or opinion (e.g., particularly when the public keenly feels a lack of control over events)? This work will also be useful in identifying systemic changes in communication environments (e.g., identifying communication indicators that occur as precursors to outrage or crises) and for constructing communication strategies (e.g., how to explain risk in ways that are meaningful to specific audiences).

Our related work shows that the four category sets are primary but not exhaustive. What other category sets would be helpful for preparing for or answering public concerns? Using these and other related category sets, are techniques of "mapping" interactions meaningful additions to current discourse analysis? Is the "dictionary" of categorical words in NABIR science similar to those in discussions of other scientific topics or in discussions of controversial public-policy topics? How valuable is this approach for scientists, members of the public, or communication practitioners?

Science-Informed Regulatory Policy

David J. Bjornstad (PI) and Amy K. Wolfe

Oak Ridge National Laboratory, Oak Ridge, Tennessee

Science-informed regulatory policy asks the question, How should biotechnology R&D develop information to support the regulatory process as a normal course of study? This project stems from the assumption that current biotechnology R&D is merely the first phase of a series of developments, extending into the future, that will challenge the ability of the regulatory process to bring a sound scientific basis to issues that increasingly will challenge existing ethical and philosophical norms. By taking steps to ensure that a firm foundation of information is compiled through the normal workings of the R&D process, it is argued that government-sponsored R&D can facilitate and support a regulatory system that, in turn, can help ensure that the benefits of R&D are realized at the earliest possible point in time.

This project will produce three papers:

- The first paper reviews the evolution of the current U.S. regulatory framework for biotechnology products.
- The second describes the challenges the regulatory system must face to accommodate the emerging science, and the role that risk analysis can play in defining the needs of science and society.
- The third provides an overview of the types of science that could be supplied.

This project is scheduled for completion in mid-2003.

Public Perception of Bioremediation Strategies and Long-Term Stewardship at Department of Energy Sites

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This project was designed to identify the range of concerns held by the public about bioremediation strategies for in situ clean up at Department of Energy (DOE) sites. Our interdisciplinary team worked with members of the public and other experts to identify those characteristics of DOE that contribute to public perceptions of risks associated with bioremediation. Two primary methods were used to collect data. First, a "consensus conference" was conducted with a panel of lay members who worked with panel-identified experts to articulate their concerns about bioremediation in general, and at the specific study site (Idaho National Engineering and Environmental Laboratory). Using information from the consensus conference, a structured survey was created and administered via computer-aided telephone interview (CATI).

We found that citizens were able to learn quickly about bioremediation through the consensus conference. In their consensus report, they conclude that bioremediation could be an effective cleanup approach at INEEL and other sites. Their remaining concerns relate to potential health risks for employees on site, communication with the public about cleanup progress, and developing an actively engaged public to ensure continuity in commitment to remediation goals. The lay panel also identified needs for further research on bioremediation technologies.

From the survey, we found that while only about 32% of respondents from the general population in the four counties around INEEL knew about bioremediation, 85% trusted the DOE "a lot" or "some" to experiment with new cleanup technologies. When asked the "most important" characteristic of DOE that engendered trust in the agency, 26% of the respondents reported that technical competency was the most important, 25% identified attention to human health issues, and 23% said the most important thing was for DOE to be honest about its activities.

The Determinants of Social Acceptability of Bioremediation Technologies

Amy K. Wolfe (PI) and David J. Bjornstad

Oak Ridge National Laboratory, Oak Ridge, Tennessee

Because technologies that pass the test of technological acceptability frequently fail the test of social acceptability, our research has focused on gaining insight into the determinants of social acceptability of bioremediation. In our previous NABIR BASIC research, we developed a framework for considering social acceptability, analyzed data from a series of DOE site-specific advisory board meetings, and reviewed opportunities for public involvement in decision making about remediation options at DOE sites. As a separate exercise, we explored the social acceptability of phytoremediation. The work reported here represents the last phase of our current efforts. Specifically, we report the findings from a series of simulation workshops in which participants were charged with determining the conditions under which proposed bioremediation field research or deployment would be acceptable.

We conducted a total of six simulation workshops, involving three sets of scenarios. Workshops consisted of four parts: (1) background information, (2) scenario 1, (3) scenario 2, and (4) debriefing. Background information described DOE legacy wastes; the challenges associated with remediating subsurface chromium, mercury, plutonium, uranium, and technetium; and categories of remediation options, including bioremediation. Scenario 1 focused on the acceptability of four proposed bioremediation *field research* projects, all ultimately targeted at immobilizing subsurface chromium, mercury, plutonium, uranium, and technetium. Scenario 2, which took place several years after Scenario 1, focused on the acceptability of a proposed *use* of one bioremediation technique, genetically engineered microorganisms, for remediation. The agency proposing research or cleanup, "Fedagency," was fictitious, as were the sites and contamination scenarios.

Participants in any single workshop were divided into two or three subgroups. We altered selected variables among subgroups and across workshops. For example, while all participants were told they were a part of an advisory group, sometimes that group was national instead of local. In some subgroups, participants were not given roles to play. However, in most subgroups, participants were assigned roles such as president of the League of Women Voters or Chamber of Commerce, retired scientist, property owner, union leader, or environmental activist. Among the other variables manipulated were site location (e.g., proximity to residential areas), site size and complexity, and pressures to remediate.

Although groups varied considerably in their interactions and responses, we are able to draw some conclusions, which are described in this presentation. For instance, the kinds of issues raised regarding field research tended to be different from those raised for deployment. Technological and risk attributes tended to influence, but not determine, participants' acceptability judgments.

Field Research Activities

NABIR Field Research Center, Oak Ridge, Tennessee

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NABIR research objectives and results. To encourage hypothesis-based field research and process-level understanding, the NABIR program has established a Field Research Center (FRC) for NABIR investigators. The FRC provides a site for investigators to conduct field-scale research and to obtain DOE-relevant subsurface samples for laboratory-based studies of bioremediation. Currently, the NABIR program has a single Field Research Center (FRC) located on the U.S. Department of Energy's Oak Ridge Reservation (ORR) in Oak Ridge, Tennessee. Staff from Oak Ridge National Laboratory's Environmental Sciences Division has operated the FRC since April 2000. Both contaminated and background (uncontaminated control) areas are located on the ORR's Y-12 National Security Complex in Bear Creek Valley. The initial focus of research at the FRC has been on in situ biostimulation experiments to promote the immobilization of uranium and technetium.

The FRC is used by NABIR investigators for various purposes, including:

- A source of subsurface samples
    - Over 500 groundwater and sediment samples (cores and composites) have been collected and shipped from the background and contaminated sites for use by 8 national laboratories and 15 universities
    - Characterization and source of humic material
- Evaluation of new characterization and monitoring methods
    - Deployment of coupons (or bug traps) for rapid assessment of in situ microbial activity (University of Tennessee, ORNL, INEEL, and others)
    - Microcosm studies, microbial enrichments, and analyses of DNA, RNA, and PLFAs
    - Development of microarray technology for assessment of community dynamics
    - Improvement of mathematical models for prediction of community structure and dynamics
    - Field-portable immunoassay instruments and reagents to measure chelators and mobile forms of uranium (Tulane University)
    - Characterization of the subsurface with surface and crosswell geophysics (ORNL and LBNL)
    - In situ uranium assay with downhole NaI detector (ORNL)
- Multidisciplinary in situ accelerated bioremediation research projects
    - In situ uranium reduction experiments using push-pull techniques (Oregon State University and Oklahoma University located in Areas 1 and 2)
    - Field-scale bioreduction of uranium (Stanford and ORNL located in Area 3)
    - In situ immobilization of uranium in structured porous media via biomineralization at the fracture/matrix interface (PNNL, ORNL, and University of Alabama located in Area 2)

Additional information and data can be obtained at the FRC website: (http://www.esd.ornl.gov/nabirfrc/).

Biostimulation of Uranium Reduction In Situ and Coupled Ex Situ Groundwater Treatment at the NABIR Field Research Center

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Previously, we characterized site hydrogeology, geochemistry, and microbiology in Area 3 of the NABIR Field Research Center in Oak Ridge, Tennessee. This site is adjacent to the S-3 pond cap, and contains high levels of uranium (~30 mg/L in the water, with 300–400 mg/kg on the soil), nitrate (8–10 g/L), aluminum (~0.5 g/L), and low pH (~3.6), plus a range of other metals and volatile organics. In preparation for planned biostimulation experiments, we have installed two injection and extraction well pairs. One well pair defines an outer flow cell. The second well pair defines an inner flow cell nested within the outer cell. We intend to stimulate U(VI) reduction within the inner cell by intermittent addition of an electron donor. Prior to this, we will neutralize acid within the outer and inner cells, and remove the aluminum and nitrate. This will be accomplished by pumping water from the outer extraction cell to an aboveground treatment train where it will be treated before reinjection. The treatment train includes vacuum strippers to remove volatile organics, a two-step precipitation process to remove metals, and a fluidized bed bioreactor to remove nitrate.

We have also evaluated the connectivity of the injection/extraction well pairs in a tracer study using bromide. Breakthrough profiles indicate that water is efficiently transported between the injection and extraction wells, with short breakthrough times. Simulations of the tracer study are being used to better define the leakage between the inner and outer cells, and to simulate the proposed field-scale experiments. The objective of the proposed experiments is to determine the rate and extent of uranium reduction in the field environment under controlled conditions. To prepare for these studies, we are performing batch and column microcosm studies, and we have evaluated uranium removal rates with different electron donors. Ethanol was the most efficient electron donor tested for stimulation of uranium removal. Experiments are ongoing to identify the microorganisms involved, and to elucidate interactions between the kinetics of U removal and desorption of U(VI) from the sediment.

Factors Controlling In Situ Uranium and Technetium Bioreduction and Reoxidation at the NABIR Field Research Center

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Field studies by our group have demonstrated that it is possible to stimulate in situ microbial activity in the shallow unconfined aquifer formed in residuum overlying the Nolichucky shale in FRC Area 1. Over fifty push-pull tests were performed in six wells to determine the effect of electron donor additions on rates of nitrate, Tc and U reduction. Injected test solutions consisted of site groundwater containing ~ 150–200 mM nitrate, 5–10 μM U(VI), and 31,000 pCi/L ^{99}Tc amended with a bromide tracer, sodium bicarbonate for pH adjustment, and 20–500 mM of either acetate, ethanol, or glucose. Injected test solutions in control wells consisted of site groundwater containing tracer and sodium bicarbonate but no added electron donors. In all cases, successive additions of electron donor resulted in increased rates of donor utilization, with maximum rates of up to 25 mM/hr. Rates of nitrate removal also increased to a maximum rate of ~4 mM/hr. Injected Tc was rapidly reduced (concurrently with nitrate removal) with maximum rates of ~ 800 pCi/L. Injected U was initially rapidly reduced, but then U concentrations increased concurrently with nitrite production, which we hypothesize is due to the reoxidation of reduced U by denitrification intermediates (NO_2^-, N_2O, and NO). To test this hypothesis, additional tests were performed using test solutions prepared from Area 2 well GW835, which had much lower nitrate concentrations (~5 mM). In these tests, nitrate and nitrite concentrations remained below detection and U reduction continued throughout the experiment, with no apparent reoxidation. No-donor addition experiments were conducted in wells previously treated with an electron donor with a decrease in rates of nitrate, Tc, and U reduction. These results indicate that it is possible to stimulate Tc and U reduction in the presence of extremely high initial nitrate concentrations in the moderate-pH (~5–6) portion of Area 1. Microcosm studies have indicated extremely low levels of microbial activity in sediments collected from the low-pH (<4) portion of Area 1. A series of field tests is in progress to attempt to stimulate microbial activity by a series of electron donor (ethanol) additions performed over a series of months. In addition, we have begun a series of field tests in Area 2 wells to examine the effect of electron donor additions in an environment characterized by circumneutral pH and low (<10 mM) nitrate concentrations. Results from these tests will be available at the PI meeting.

Biogeochemistry and Microbially Mediated U(VI) Reduction at Uranium Mill Tailings Sites, Colorado Plateau, USA

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Uranium Mill Tailings Remedial Action (UMTRA) sites continue to provide a low-cost, low-risk venue for field sampling and experimentation to assess both natural bio-attenuation and engineered bio-remediation of uranium. Data from three sites—Shiprock, NM; Gunnison, CO; and Old Rifle, CO—suggest that microbially mediated reduction of U(VI) to U(IV) occurs locally in uranium contaminant plumes. Laboratory-scale experiments on contaminated sediments demonstrate that amendment of sediment samples with electron donor results in loss of U(VI) from the aqueous phase due to microbial reduction of U(VI) to U(IV). Single-well push-pull tests at two sites partially corroborate accelerated, microbially mediated U(VI) reduction at a larger scale. To more effectively test acceleration of U(VI) reduction at the field scale, we collaborated with DOE's UMTRA Groundwater Project to conduct a biostimulation experiment at the Old Rifle UMTRA site in which an electron donor was metered into the subsurface under natural gradient conditions. Specifically, ca 2 mM acetate was amended to the subsurface over a period of three months in a 15 m × 18 m × 2.5 m volume comprised of 3 upgradient monitoring wells, 20 injection wells, and 15 downgradient monitoring wells. Uranium contamination occurs in the groundwater flow system consisting of alluvial sands and gravels of the Colorado River overlying the relatively impermeable Wasatch Formation. Data collected during the experiment include geohydrologic, geochemical, and microbiologic parameters. Details are reported by collaborating NABIR investigators, but overall results of the experiment are as follows. Uranium concentrations decreased as much as 70% downgradient from the injection zone, consistent with uranium- and iron-reducing conditions dominating during the first half of the experiment. During the second half of the experiment, sulfate-reducing conditions dominated, resulting in a decreased rate of U(VI) reduction. Overall, results show the importance of maintaining metal-reducing conditions to achieve the desired experimental end-point of maintaining groundwater uranium concentrations below applicable standards. One of the data sets collected during the experiment is a passive multilevel sampler transect through the center of the experimental volume. Profiles from the two downgradient wells closest to the injection gallery demonstrate the ability of this approach to uniformly decrease U(VI) concentrations under in situ field conditions with typical physical and geochemical heterogeneity. An exception is the uppermost few cm of the saturated zone, in which U(VI) actually increased during the experiment, presumably due to either evaporative concentration of U(VI) or changes in geochemical parameters controlling U(VI) adsorption. Both processes could be the result of a drop in the elevation of the water table during the experiment.

In Situ Immobilization of Uranium in Structured Porous Media via Biomineralization at the Fracture/Matrix Interface

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We are beginning a series of interdependent tasks that will culminate in an in situ field-scale biostimulation experiment at Area 2 of the NABIR Field Research Center (FRC). The field experiment will evaluate the feasibility of stimulating microbial U(VI) reduction activity in targeted pore fractions of structured porous media. The research plan is designed to evaluate the hypothesis that U(VI) in low-permeability porous regions (micropores) of saprolite at the FRC can be immobilized and isolated from mobile groundwater by stimulating localized in situ microbial U(VI) reduction in hydrologically accessible fractured zones (meso- and macropores). Such activity will cause precipitation of insoluble UO_2 within the mesopore domain, thereby reducing or eliminating a long-term source of groundwater contamination that is otherwise extremely difficult to remediate. Planned research elements include field hydrologic and geophysical characterization, sediment wet chemical analysis and evaluation of microbial metal reduction potential, bench-scale reactive transport experiments using intact sediment blocks, and the field-scale biostimulation experiment. The research will result in improved understanding of complex interactions between biogeochemical transformation and hydrologic flow and transport processes in structured porous media, and will lead to development of a general strategy for controlled bioremediation of metals and radionuclides in such subsurface environments. These results will also enhance our ability to upscale laboratory bioremediation experiments to the field scale.

Tasks being undertaken in the first year include: (1) collection of undisturbed saprolite material and initiation of laboratory column experiments, (2) detailed site survey (geophysical and hydrologic) for selection of specific experimental zone, (3) collection of sediment from the experimental site and use in batch slurry analyses of microbial uranium reduction, and (4) development of a three-dimensional field-scale modeling capability for design and interpretation of the planned experiments.

Student Presentations

Characterization of *Deinococcus radiodurans* for Actinide Precipitation

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We study *Deinococcus radiodurans*, a nonpathogenic prokaryote able to withstand high doses of ionizing radiation, for bioremediation and biostabilization of heavy metals and actinides to limit the migration of these contaminants. Our goal is to develop a system for *D. radiodurans* to bioprecipitate actinides similar to that of a genetically engineered strain of *Pseudomonas aeruginosa* capable of polyphosphate accumulation, inducible degradation and secretion, and UO_2^{2+} precipitation.

The radioresistance of *D. radiodurans* to ionizing gamma radiation has been well studied, yet is not well understood.[1] Several DNA repair mechanisms have been discovered, yet survivability studies are typically done under nutrient-rich conditions at a single growth stage. Although a minimal irradiation medium has been described,[2] a clear study of the effect of different variables on survivability in a nutrient-starved environment has not been performed. We have shown that *D. radiodurans* grown in typical TGY complex media displays increasing radioresistance with increasing age of the culture at the time of harvest; however, the dose below which there is little loss of reproductive viability does not change significantly with the growth phase. Higher irradiation rates in nutrient-starved medium display higher survivability. These results indicate that there is a threshold below which the radiation dose is not sufficient to overcome the cells' passive defense mechanisms, such as radical scavenging by carotenoids in the cell wall. Additional radiation resistance is then due to active resistance induced by cell damage, which can be related to aging. Using the 88-Inch Cyclotron at Lawrence Berkeley National Laboratory, we also studied the effects of light-ion irradiation in aqueous suspension, which shows increasing lethality corresponding to increasing linear energy transfer (LET) values of the radiation. Previous studies only examined heavy ion effects on cells supported on solid medium.[3]

To better understand the chemistry of nonengineered *D. radiodurans*, we studied the interaction of strain R1 with UO_2^{2+} in dilute salt solution. R1 sorbs uranyl more than two orders of magnitude less than the engineered *P. aeruginosa*. We are increasing the ability of *D. radiodurans* to sequester uranium via metabolic engineering. Chemical studies of the cell-uranyl binding strength and pH optima support spectroscopic data indicating that a carboxyl surface group, consistent with known characteristics of *D. radiodurans* S-layer,[4] interacts with and binds the uranyl. Studies—including Infrared Spectroscopy, Laser Fluorescence Spectroscopy, and Extended X-ray Absorption Fine Structure Spectroscopy (EXAFS)—are underway to further elucidate the mechanism of uranyl complexation to the cell surface, and to further characterize a strain provided that has been engineered to accumulate polyphosphate.

[1]Battista, J.R., A.M. Earl, and M.J. Park. 1999. Why is *Deinococcus radiodurans* so resistant to ionizing radiation? *Trends Microbiol. 7*, 362–365.
[2]Venkateswaran, A., S.C. McFarlan, D. Ghosal, K.W. Minton, A. Vasilenko, K. Makarova, L.P. Wachett, and M.J. Daly. 2000. Physiologic Determinants of Radiation Resistance in *Deinococcus radiodurans*. *Appl. Environ. Microbiol. 66*, 2620–2626.
[3]Dewey, D.L. 1969. The Survival of *Micrococcus radiodurans* Irradiated at High LET and the Effect of Acridine Orange. *Int. J. Radiat. Biol. 16,* 583–592.
[4]T.E. Lister, and P.J. Pinhero. 2001. *In vivo* atomic force microscopy of surface proteins on *Deinococcus radiodurans*. *Langmuir. 17*, :2624–2628.

Mathematical Modeling and Analysis of Test Data at FRC

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In preparation for a field-scale demonstration of uranium reduction, we have:

1. Developed mathematical models of flow, transport, and biogeochemical transformations, and
2. Analyzed pumping and tracer-test data to determine system parameters.

Mathematical models include analytical solutions, MODFLOW, a new finite-volume flow model, particle tracking, and PHREEQC. We will present results from the application of some of these models to data analysis. The pumping tests have provided information about the system's conductivity and specific storage and, to a lesser extent, anisotropy degree and direction. The tracer test has been useful in determining breakthrough curves, and in demonstrating the presence of fast-flow pathways and the possibility that this is a dual-porosity system.

Community Composition of Iron(III)-Reducing Bacteria from Subsurface Sediments of the Field Research Center

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To test the bioremediation potential of the acidic uranium-contaminated sediment in Area 1 at the FRC, we focused on Fe(III)-reducing bacteria (FeRB), as they represent the indigenous microorganisms capable of immobilizing uranium in situ. Thus, we extracted DNA from more than 20 Fe(III)-reducing MPN tubes cultured at both neutral and low pH. Over 850 clones were screened in order to detect the organisms most suited for in situ bioremediation at this site. Through cloning and sequencing experiments, we found considerable differences in phylotype between Fe(III)-reducing enrichments from background and contaminated FRC sediment. In the background Fe(III)-reducing MPN tubes (pH7), over two-thirds of the 16S rRNA gene sequences obtained were closely related to members of the *Geobacteraceae* family. In contrast, sediment from the contaminated pH 7 Fe(III)-reducing MPN tubes revealed that almost half of the 16S rRNA gene sequences were 96% similar to the halogen-respiring species *Anaeromyxobacter dehalogenenans*, but no *Geobacteraceae* sequences were detected. In the Fe(III)-reducing MPN tubes cultured at low pH (4 to5), only 16S rRNA gene sequences closely related to gram-positive organisms were detected. From the contaminated enrichments cultured at low pH, the most predominant 16S rRNA gene sequences were closely related to the gram-positive organisms *Brevibacillus* and *Paenibacillus*. T-RFLP analysis of enrichment cultures strongly supported the cloning and sequencing results. Our current work is focused on the determination of bioremediation potential by following selected groups of FeRB before and after sediment biostimulation through the addition of selected carbon sources (in conjunction with push-pull experiments being carried out by Istok et al.). Using DNA extracted directly from the sediment, both MPN-PCR and real-time PCR are being conducted in order to quantify the differences in abundance of *Geobacter*-, *Anaeromyxobacter*-, *Paenibacillus*-, and *Brevibacillus*-type 16S rRNA gene sequences. Our results suggest that new model Fe(III)-reducing organisms should be pursued to aid in the ongoing development of bioremediation strategies for uranium contamination in acidic subsurface sediments.

Factors Influencing Nitrate-Dependent U(IV) Oxidation

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It has recently been shown that the addition of nitrate to U(IV)-containing sediments leads to the oxidation and solubilization of uranium. Therefore, when designing a bioreductive remediation strategy for nitrate- and uranium-contaminated sites, it may be necessary to consider geochemical and microbiological factors that may result in post-treatment U(IV) oxidation and remobilization. We assessed factors that could influence nitrate-dependent oxidation of U(IV) in anoxic, alluvial sediments and groundwater in an attempt to determine controls on this process. Preincubations were initially conducted to reduce Fe(III) (complete within 10 d), sulfate, and 100 μM U(VI) (complete within 30 d and 99 d, respectively). Spatially resolved x-ray fluorescence and XANES on sediments from these incubations showed that uranium was in fact reduced (not sorbed) and associated with Fe(II) (likely FeS). Upon addition of 10 mM nitrate to the incubations, U(IV) was completely oxidized after 9 d, and U oxidation occurred concomitantly with Fe(II) and reduced sulfur oxidation.

Since oxidation of biogenic U(IV) may be limited by nitrate concentration, we assessed the effects of 0.1, 1, and 5 mM nitrate on U(IV) oxidation, and found only 10, 18, and 25 μM U(VI) produced, respectively, of 320 μM U(IV) initially present. Upon exhaustion of nitrate, microbially catalyzed oxidation of Fe(II), U(IV), and reduced sulfur stopped. In incubations receiving 50 mM nitrate, Fe(II), U(IV), and reduced sulfur oxidation continued for at least 16 d. These results indicate that in the presence of nitrate, microbially catalyzed U(IV), Fe(II), reduced sulfur, and organic carbon oxidation occur concomitantly.

As intermediates in the nitrate-reduction process are known to oxidize U(IV), we assessed the effect of washing sediments to remove the organic electron donor on nitrate-dependent U(IV) oxidation. Washed sediments with U(IV) and 5 mM nitrate were incubated in a dissolved organic carbon-free mineral solution. U(IV) oxidation occurred quite rapidly, providing further evidence that U(IV) oxidation is mediated by lithotrophic microbial activities. Since previous work has shown that the presence of sulfide will inhibit the oxidation of U(IV) by oxygen, we assessed the effect of various inorganic reductants on nitrate-dependent U(IV) oxidation. The addition of 10 mM sulfide, Fe(II), or FeS to sediments containing U(IV) and 5 mM nitrate resulted in the inhibition of U(IV) oxidation, and in the case of sulfide, inhibited nitrate-reducing activity. In all cases, this inhibition was less pronounced at lower reductant concentrations. The extent of U(IV) oxidation is apparently dependent on nitrate concentration and on the presence of sulfide, Fe(II), and FeS. Therefore, the presence and amount of these reductants may be used to predict the stability of U(IV) in a site where U(IV) bioreduction has been used to immobilize uranium.

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