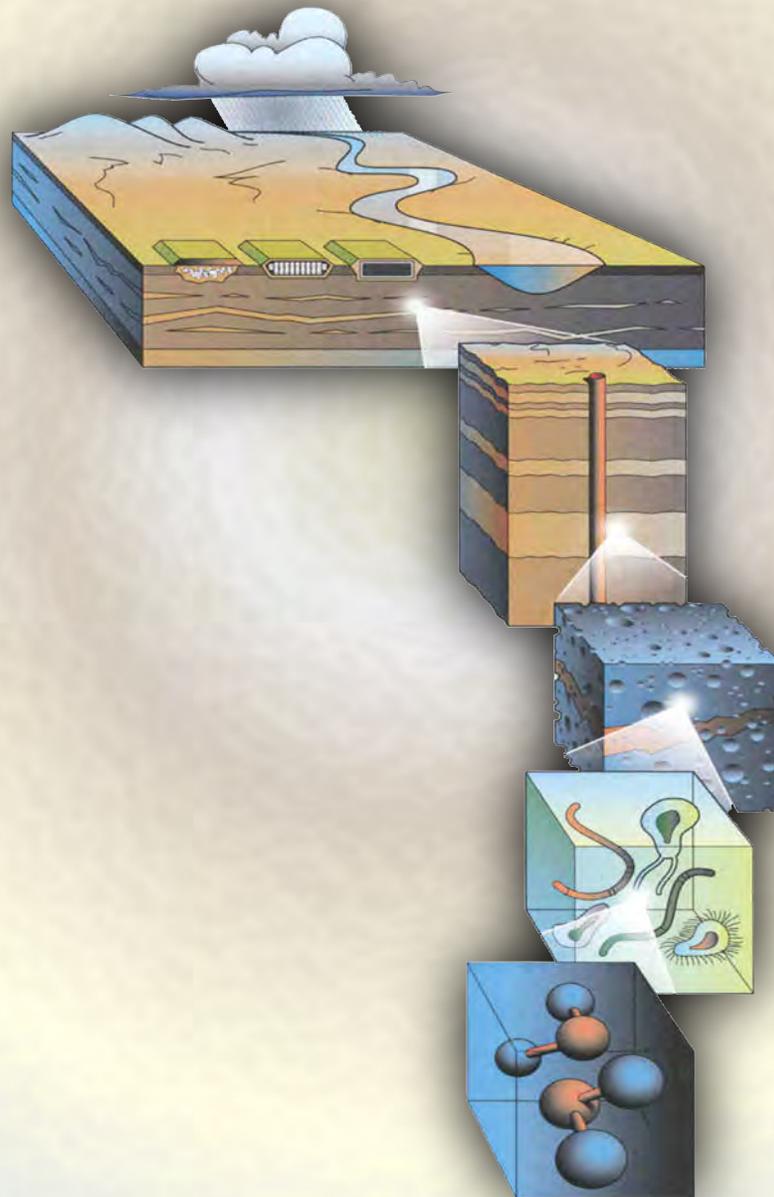




Subsurface Biogeochemical Research (SBR) Contractor-Grantee Workshop

April 26 - 28, 2011



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Subsurface Biogeochemical Research (SBR)
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Abstracts

University-Led Research Abstracts
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Subsurface Biogeochemical Research

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

University-Led Research

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

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

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

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

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

University-Led Research

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

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

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

University-Led Research

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

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

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

The sorption of Np in its most environmentally prevalent oxidation state of +V onto SRBL geome-dia was examined to determine the mobility and transport of Np in the subsurface. The studies demonstrated that Np(V) is not sorbed onto the sandy material under any environmentally relevant conditions. In contrast, Np(V) begins to sorb onto the clay material under slightly basic conditions. According to hydrolysis constants, the neutral $\text{NpO}_2(\text{OH})$ species is likely the form that is sorbed. At very high pH values, the Np(V) can be directly precipitated, but these conditions are not environmentally relevant.

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

GPR Imaging of Channeled Flow in Fractured Bedrock

University-Led Research

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

GPR experiments were conducted at the Altona Flat Rock fractured sandstone field site using surface GPR reflection to image a water-saturated subhorizontal bedrock fracture 7.6 m below surface. Three-dimensional GPR data were acquired at 50 MHz and 100 MHz frequencies covering approximately a 65 m² area at a 0.25 m x 0.5 m grid. Fracture water salinity was controlled by re-circulation of saline traced formation water in a dipole flow setup encompassing the region monitored by GPR. Comparison of GPR reflection amplitudes between background clean water (9.3 mS/m), and traced water of 200 mS/m and 600 mS/m fluid electrical conductivity showed that regions of low reflection amplitude remain mostly unchanged when water salinity changes. In contrast, regions of high reflection amplitude show a large increase in response to fluid salinity increase. This result is in agreement with the expectation that fracture regions with greater aperture, and therefore greater clean water reflection amplitude, conduct most of the saline tracer. GPR amplitude reveals 1 m to 1.5 m wide flow channels trending across the survey area and the flow dipole field. The spatial scale of these channels corresponds roughly to hydrodynamic dispersivity measured from interwell saline tracer breakthrough. GPR imaging offers the capability to define the geometry of flow channeling and reduce the uncertainty of transport predictive modeling in bedrock groundwater systems.

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

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

University-Led Research

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In this project, novel microscale experimental approaches are being applied to understand how microscale biotic and abiotic reactions and their combined interactions control uranium mobility in the subsurface at the Hanford 300 Area IFRC. Microscale geochemical gradients (dissolved O₂ (DO) and H₂ concentrations, redox potential, and pH) were measured in Hanford 300A subsurface sediment biofilms and correlated with the average U speciation. These measurements revealed significant differences in geochemical parameters across a sediment biofilm/water interface in the presence and absence of U(VI) under oxic and anoxic conditions. While the pH was relatively constant within the biofilm, the redox potential, DO, and H₂ concentrations were heterogeneous at the microscale (<500–1000 μm). Under bulk aerobic conditions, the addition of U(VI) resulted in the development of microenvironments with high DO levels (DO hotspots) that were not observed in the absence of U(VI).

The presence of U(VI) under anoxic conditions resulted in a decrease in H₂ concentrations. U LIII-edge X-ray absorption spectroscopy (XANES and EXAFS) showed that 80–85% of the U was present as U(IV). Our results demonstrate the importance of microscale geochemical gradients for understanding U mobility in the subsurface. Extracellular polymeric substances (EPS) in the subsurface sediment biofilms are hypothesized to play important roles in U biotransformations and mobility. To investigate the roles of EPS in U immobilization, we used *Shewanella* HRCR-1, an isolate from the Hanford Reach of the Columbia River, as a model, and characterized the composition of EPS using FTIR and proteomics to provide insight into the potential interactions of EPS with uranium. Redox proteins including the homologues of two *S. oneidensis* MR-1 outer membrane c-type cytochromes, MtrC, and OmcA, which have been implicated in extracellular electron-transfer reactions, were identified in the EPS fractions. The presence of these proteins was also confirmed by immunoblot analysis. We further quantified the contribution of EPS to U(VI) immobilization via sorption and reduction by EPS fractions. We found that, in reduced form, the isolated cell-free EPS fractions could reduce U(VI). Polysaccharides in the EPS likely contributed to U(VI) sorption and dominated the reactivity of loosely associated EPS, while redox active components (e.g., outer membrane c-type cytochromes), especially in bound EPS, likely facilitated U(VI) reduction. As a novel noninvasive tool in microscale studies, NMR was used to determine material-transport rates within live biofilms.

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

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

University-Led Research

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

This project was begun in September 2010, and to date we have prepared oxide-free, clay-sized fractions (<2.0 μm and <0.5 μm) from Hanford sediments collected from Well C6209 59-60 ft b.g.s. and from the Mercer Clay formation in Cambria County, Pennsylvania. Both these sediments contain high Fe concentrations in their clay fractions and are located adjacent to steep redox gradients. Elemental and iron contents were determined by complete dissolution in HF/H₂SO₄, and Fe(II) content was determined by an HF/H₂SO₄-phenanthroline assay. For the Hanford clay fractions, we measured an iron content of 11.2% (9.98 mmol Fe/g) and an Fe(II) content of 1.4%. The unaltered clay fractions were then completely reduced using the citrate-bicarbonate-dithionite (CBD) method. The unaltered and fully reduced clay fractions were then characterized by a suite of complementary techniques, including synchrotron radiation x-ray diffraction (SR-XRD), Fe K-edge x-ray absorption spectroscopy (including EXAFS and XANES), and transmission electron microscopy with electron energy loss spectroscopy (TEM-EELS). In the near future, these samples will also be analyzed with Mössbauer spectroscopy. Combined with aqueous and solid-phase elemental analyses and Fe(II/III) speciation measurements, we plan to synthesize all these data together to better define the structural coordination of Fe in natural phyllosilicates. Currently, the speciation of Fe in these materials is difficult to assign because of the multiple possible forms of Fe, such as “structural Fe(III)” (likely in the octahedral sheet of the TOT layer), “structural Fe(II)” (originally present in the unaltered material and newly formed by CBD reduction), “surface-complexed Fe(II)” (to edge sites), and “interlayer-exchanged Fe(II)” (to basal plane).

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

Inorganic Controls on Neptunium Mobility in the Subsurface

University-Led Research

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

This project is currently in Year 1. We have optimized synthesis methods for calcite (CaCO_3), gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), borax ($\text{Na}_2\text{B}_4\text{O}_5(\text{OH})_4 \cdot 8\text{H}_2\text{O}$), and arcanite (K_2SO_4) that provide crystals of suitable size for detailed characterization. These minerals present several possible incorporation sites for the neptunyl ion, including interstitial substitution and substitution for monovalent or divalent cations. Following optimization of synthesis conditions, each mineral has been re-grown in aqueous systems containing hundreds to thousands of ppm Np(V). Analyses of these minerals are under way using laser-ablation and solution-mode inductively-coupled-plasma mass-spectroscopy (ICP-MS).

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

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

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

University-Led Research

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

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

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

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

University-Led Research

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Our research seeks to resolve the coupling between silicate weathering and radionuclide sorption-desorption during disequilibria that ensue when high-level radioactive waste (HLRW) is introduced to circumneutral soil, sediment, or rock. Focus is on the near-field region of caustic liquid waste release to the Hanford vadose zone, where hyperalkaline solutions (pH 11-13⁺) accelerate incongruent aluminosilicate weathering, leading to likely neoformation of zeolites, feldspathoids and carbonates (latter depending on PCO_2) that can sequester radionuclides into bound form during nucleation and crystal growth. Whereas cesium-137 and strontium-90 and nitrate are incorporated into aging-induced recalcitrant forms within product solids, iodine-129 (as iodide) is very weakly retained under such conditions. Even for more strongly sorbed/precipitated Cs and Sr, however, the thermodynamic stability of the product is challenged when the caustic source is removed and replaced with more dilute background pore water (BPW), characteristic of groundwater recharge.

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

Neo-precipitates range from 3-10% of the reacted sediment mass, depending on treatment regime (6 or 12 mo, with or without CO₂, high or low contaminant concentration). The principal controls on contaminant effluent release – and that are now included in the model – are (i) generalized Sr and Cs cation exchange on both native sediment and neo-precipitate surfaces, (ii) frayed edge site (FES) Cs exchange, and (iii) neo-precipitate dissolution/ transformation. These controls are reflected in various components of the data set, including (1) column experiments conducted with aluminosilicate precipitates in isolation revealed nonstoichiometric release of Cs and Sr relative to Al and Si; (2) small loss of sodalite or cancrinite was observed over hundreds of pore volumes BPW, while effluent solutions were characterized by a low and nonstoichiometric molar ratio of neo-phase Si to NO₃; (3) growth of Ca-Willhendersonite occurred at the expense of Ca-Chabazite, and was observed to contain both Sr and Cs after extensive leaching. The associated slight shortening of aluminosilicate bond distances due to the structural transformation, along with the near perfect Si-Al ordering, renders extra framework cations less exchangeable.

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

University-Led Research

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

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

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

Geoelectrical Measurement of Multiscale Mass Transfer Parameters

University-Led Research

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

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

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

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

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

University-Led Research

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

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

- (1) Exchangeable ions that reach exchange equilibrium within a few days at room temperature. This population accounts for about 2% of the K and about 5% each of the Rb and Cs in the soil samples.
- (2) Ions that undergo restricted exchange, at rates that are very low at room temperature. Acid-extraction rates increase nearly tenfold when temperature is increased to 50°C and again by a somewhat smaller factor when temperature is increased to 75°C. This population accounts for about 25% of the K, roughly 60% of the Rb, and nearly 90% of the Cs in the soil samples. At room temperature and low acidity, however, only 12% to 20% of the Rb in five samples equilibrated with added isotopically enriched Rb over 8 months, which indicates that most of the Rb in this population exchanges so slowly that it is effectively fixed in the soil.
- (3) Ions extractable by acid only very slowly at 75°C. We consider these ions to be bound in silicate structures—most likely in remnants of primary muscovite within HIV particles—that dissolve only very slowly at 75°C. Most of the K, but not most of the Cs, and Rb, is in the silicate structures.

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

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

University-Led Research

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

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

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

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

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

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

University-Led Research

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

Samples of groundwater were taken from the boreholes FW215 (Nov-2009) and FW106 (Jul-, Sep-, Oct-2010) by the ORNL personnel, shipped on ice to our laboratory and kept under anoxic conditions at 2-4 °C before use for isolation, typically within 2-3 d of sampling. Isolation was done under aerobic and anaerobic conditions by using (1) plating, (2) enrichment anaerobic culture, (3) serial dilutions in microtiter plates and (4) ichip technique. As nutrient media, we used R2/R2A under aerobic conditions and various combinations of electron donors/acceptors for anaerobic cultivation to encourage development of the metal-reducing organisms. After isolation and purification, the identity of isolates was established by sequencing their ribosomal genes (16S, 18S, 25S, ITS). The obtained isolates were tested for their resistance to major toxic compounds detected in FW106 at concentrations ranging from zero to up to 10-100 times above the ambient. Growth was followed by OD₆₀₀ in plate readers. In parallel microcosm experiments, groundwater samples were amended with various C-sources and incubated under oxic or anoxic conditions at 20°C; growth was recorded from the rate of CO₂ production with membrane-inlet mass spectrometry and residual dynamics of added and endogenous organic compounds (GC-MS).

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

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

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

University-Led Research

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

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

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

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

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

University-Led Research

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

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

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

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

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

University-Led Research

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

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

We further studied the interaction of Hanford colloids with fulvic and humic acids, and we found that the colloids form more stable suspensions in the presence of fulvic and humic acids. We monitored colloid transport in the field lysimeters at the Hanford 300N Lysimeter Site. The facility consists of six lysimeters, each 7.6 m deep. Fiberglass wicks were installed at 1, 2, 4, and 7 ft below surface to collect vadose zone water. We applied Eu-hydroxycarbonate and carboxylate-modified polystyrene colloids to the lysimeters. The lysimeters were irrigated with different flow rates. (Two lysimeters were exposed to natural rainfall, while the others were being irrigated regularly with multiples of the average recharge--124 mm/year, 248 mm/year, and 248 plus a 100 mm Chinook event.) Wick outflow has been analyzed for pH, electrical conductivity, colloid concentrations, electrophoretic mobility, and particle size. Soil cores were taken in August 2010 to determine depth profiles of colloid concentrations. Colloids have been detected in the outflow, but most of the colloids still remain in the top 30 cm of the soil. We instrumented one additional lysimeter in the Hanford 200 Area (Field Lysimeter Test Facility) with fiberglass wick samplers in late February 2010. No colloids were applied to this lysimeter, which we used to monitor *in situ* colloid mobilization.

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

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

University-Led Research

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

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

University-Led Research

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

Research has commenced toward the completion of targeted goals.

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

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

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

University-Led Research

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

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

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

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

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

University-Led Research

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

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

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

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

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

University-Led Research

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

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

Biogenic and chemogenic iron sulfide solid and reduced U solid were successfully produced and scaled-up for the kinetic studies. From XRD, the generated iron sulfide and U(IV) solid phases were confirmed to be mackinawite (FeS) and uraninite (UO₂(s)), respectively. Interestingly, Mössbauer characterization of FeS solids gave a different pattern for biogenic vs. chemogenic FeS. The presence of structural Fe(III) or am-FeS are suspected in the biogenic samples. XAS, TEM, and Mössbauer spectroscopy data analyses are ongoing to confirm S, Fe, and U structural properties and to explain possible differences in reactivity of biogenic and chemogenic samples.

Biogenic solids were produced by *Desulfovibrio vulgaris*. Over a range of pH (6.5 to 8.6) and ratios of lactate:SO₄ (0.5:1 to 1.9:1) and SO₄:Fe (as dissolved Fe(II)), 1:1 to 1:0.11), *D. vulgaris* primarily produced mackinawite (verified by XRD). Higher lactate:SO₄, lower SO₄:Fe, or weakly acidic pH enhanced mackinawite crystallization. Hydrogen and pyruvate as alternate electron donors showed enhanced FeS production compared to lactate. A study of U reduction by *D. vulgaris* was also initiated. *D. vulgaris* enzymatically reduced U(VI) to UO₂(s) when U(VI) was the only electron acceptor, with 90% conversion within 10 hours. U(VI) reduction was faster in the presence of sulfate, implicating abiotic reduction of U(VI) by biogenic H₂S. XAS data of biogenic UO₂(s) gave first shell O atoms at 2.33 Å and second shell U neighbors at 3.84 Å, consistent with previously reported XAS values.

Batch oxidation-kinetic studies were performed using chemogenic FeS(s) and UO₂(s) to identify the mechanism and rates of oxidation in the presence of oxygen under typical groundwater conditions. FeS (5 g/L) inhibited the rate of oxidation of UO₂ (1 g/L) compared to a control experiment without FeS, with elemental S implicated. When all elemental S was depleted, the Fe(III) oxide solids produced from FeS oxidation accelerated UO₂ oxidation to U(VI). Experiments with biogenic FeS and UO₂ are planned to confirm whether similar reaction products and oxidation trends will be observed.

Two UO₂(s) column experiments were prepared for oxidation studies using: (1) chemogenic FeS (2.75 g FeS(s)/kg) and UO₂(s) (0.5 g UO₂(s)/kg), or (2) UO₂(s) alone (0.5 g UO₂(s)/kg sand): packed in mixture with RABS sediments from the Rifle CO site. U effluent data suggest FeS improves the retention of U during oxygen-saturated groundwater flow compared to the column with no FeS. Solid phases will eventually be characterized to assess solid-phase changes responsible for the U, Fe, and S effluent behavior. Future studies are planned for introducing biogenic FeS and UO₂ into the columns.

A Spectroelectrochemical Sensor for Pertechnetate

University-Led Research

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

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

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

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

University-Led Research

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

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

Occurrence and Stabilization of Colloidal and Nanoparticulate Mercury Sulfides

University-Led Research

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

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

We performed additional experiments with zinc-sulfide-DOM nanoparticles, which behave similarly to mercury sulfides. In these experiments, zinc sulfide was precipitated in the presence of dissolved cysteine, an amino acid that was used as an analogue for thiol functional groups in DOM that bind metals such as Zn²⁺ and Hg²⁺. We used small angle X-ray scattering (SAXS) and X-ray absorption spectroscopy (XAS) to probe the cluster size, aggregate structure, and coordination environment of Zn-S-cysteine nanoclusters that formed during the early stages of precipitation. Our results demonstrated that cysteine slowed aggregation rates of ZnS clusters by coordinating to Zn atoms on the edges of clusters. Furthermore, in solutions with molar ratios of 2:1 cysteine:ZnS, we observed larger cluster subunits compared to solutions with 1:1 cysteine:ZnS, indicating that the cysteine did not block growth sites on the surface of monomer subunits.

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

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

University-Led Research

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

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

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

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

University-Led Research

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

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

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

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

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

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

University-Led Research

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

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

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

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

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

University-Led Research

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

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

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

University-Led Research

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Cr(VI) is a highly toxic contaminant that has been introduced into many shallow subsurface environments. Although Cr(VI) is typically quite mobile, it can be retarded via adsorption and reduction reactions. The primary aim of this exploratory research is to develop methods for applying surface complexation models (SCMs) to complex mineral mixtures, including natural sediments. To achieve this goal, the following tasks are planned: (1) measurement of Cr(VI) sorption on kaolinite, montmorillonite, hydrous manganese oxide (HMO) and g-alumina as a function of ionic strength, pH, pCO₂ and sorbate/sorbent ratio, (2) development of SCMs describing Cr(VI) adsorption on kaolinite, montmorillonite, HMO and g-alumina, (3) measurements of Cr(VI) sorption on model mixtures of kaolinite, montmorillonite, HMO, g-alumina, goethite and hydrous ferric oxide (HFO), (4) testing of component additivity (CA) SCM predictions of Cr(VI) sorption on the model mixtures, (5) measurements of Cr(VI) sorption on bulk natural sediments with differing quantities of organic carbon and HMO/HFO before and after step-wise extractions, and (6) development of CA methods to describe Cr(VI) sorption on natural sediments.

Cr(VI) adsorption has been measured on g-alumina (5 g/L) as a function of pCO₂ (0, atmospheric, 2.5%), pH (4-10), ionic strength (.001, .01 or 0.1 M NaNO₃) and Cr(VI) concentration (10⁻⁴ to 10⁻⁶ M). Adsorption decreases with increasing pH, varying little with ionic strength or pCO₂, except that adsorption is inhibited at low pH under high ionic strength and high pCO₂. The data have been used to develop constant capacitance, double layer, and triple layer models (CCM, DLM, TLM) of Cr(VI) adsorption on g-alumina. Of these, the ionic-strength-dependent parameters of the CCM are best able to reproduce the observed adsorption behavior over broad ranges in solution condition. Kinetics experiments to test Cr(VI) uptake and release have been initiated with montmorillonite, kaolinite and HMO. Initial data suggests that Cr(VI) uptake at pH 3 is slower on HMO (days, rather than hours) than on g-alumina, with sorbed Cr(VI) released quickly and completely at pH 10. In contrast, very slow sorption/desorption kinetics are observed on kaolinite; at pH 3, sorption continues to increase slowly for at least two weeks. Uptake of Cr(VI) is very rapid at pH 3 (minutes to hours) on untreated montmorillonite, but is irreversible at pH 10 over several days, most likely due to reduction of the Cr(VI) by either organic matter or Fe(III) in the clay lattice.

Planned experiments will use kaolinite and montmorillonite after extraction of Fe and/or organic matter (e.g., using peroxide, oxalate, dithionite) to test whether Cr(VI) sorption on these clays is reversible in the absence of reduction. Work planned for the remainder of Year 1 will include measurement of Cr(VI) adsorption on montmorillonite, kaolinite, and HMO as a function of pH, ionic strength and pCO₂, with development and comparison of SCMs for these sorbents. Work in Year 2 will focus on measurement of Cr(VI) sorption on model mixtures of minerals and on natural sediments. These data will be used to test SCM component additivity predictions of Cr(VI) sorption.

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

University-Led Research

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

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

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

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

University-Led Research

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

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

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

(3) *Multiscale Modeling and Up-Scaling.* We seek to account for pore-scale variation in physical and mineralogical properties, flow velocities, and (for unsaturated conditions) wetting fluid/grain surface areas, in reactive transport models. To accurately upscale reaction rates, we are using a combination of lattice Boltzmann modeling, reactive-transport-network flow modeling, and continuum-scale finite difference models. A specific application is to understand how carbonate rocks in contact with CO₂-rich brines change due to the precipitation or dissolution of fast-reacting minerals such as calcite and dolomite. In preliminary results from our geochemical model alone (no transport), we were able to identify the conditions that lead to significant changes to the pore volume, due to the precipitation of calcite and dolomite. The transport part of the model is being developed. We are fortunate to have established a collaboration with Prof. Hilfer of the University of Stuttgart, who has provided us with a carbonate rock image in the form of a voxelized cube.

Microbial Uranium Reduction and Monitoring Tools

University-Led Research

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

Diverse groups of microorganisms affect the oxidation state of metals and, therefore, control the mobility of toxic radionuclides in subsurface environments. Relevant to uranium (U) redox speciation are bacteria that reduce predominantly water-soluble and mobile hexavalent U, U(VI), to U(IV), which has lower solubility and typically forms the uraninite mineral UO_2 . Recent studies with *Desulfitobacterium* spp. suggested that the ability to reduce U(VI) to U(IV) is a shared feature among members of this group. Interestingly, extended X-ray absorption fine structure (EXAFS) analysis demonstrated that U associated with the solid phase in the *Desulfitobacterium* cultures was a mononuclear U(IV) species instead of the typical nanoparticulate uraninite with U-U coordination. More detailed studies indicated that solution conditions affected the solid phase U, and in the presence of phosphate, non-uraninite U(IV) was formed both in cultures of Gram-negative (i.e., *Anaeromyxobacter*) and Gram-positive (*Desulfitobacterium*) bacteria, and also in abiotic systems that used 9,10-anthrahydroquinone-2,6-disulfonate (AQH₂DS) as the reductant. *Desulfitobacterium* cultures and abiotic systems with AQH₂DS produced mononuclear U(IV) also in the absence of phosphate whereas *Anaeromyxobacter dehalogenans* strain 2CP-C reduced U(VI) to U(IV)-uraninite. These findings suggest that distinct biomolecular U(VI) reduction mechanism(s) operate in Gram-positive *Desulfitobacterium* spp. and Gram-negative *Anaeromyxobacter* spp.

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

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

Advances in the Molecular Analysis of Communities Associated with Uranium Bioremediation

University-Led Research

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

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

University-Led Research

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

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

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

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

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

University-Led Research

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

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

University-Led Research

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

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

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

Radiochemically Supported Microbial Communities

University-Led Research

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

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

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

University-Led Research

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

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

University-Led Research

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

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

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

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

University-Led Research

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

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

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

University-Led Research

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

Research is focused on identifying the primary redox proteins that catalyze metal oxidation, environmental factors that influence protein expression, and molecular-scale geochemical factors that control the rates of biotic and abiotic interactions among U(IV) minerals, Fe(III) phases, and nitrogen oxide species formed during nitrate reduction. In these studies, we are using the widespread *Thiobacillus denitrificans*, the first autotrophic bacterium reported to catalyze anaerobic U(IV) oxidation, which is also capable of Fe(II) oxidation. A key part of the research program is the identification and characterization of uranium and iron species and phases produced during coupled oxidation-reduction processes under either biotic (enzymatic) or abiotic conditions in batch and column experiments with model solids and aquifer sediments. Reactive transport modeling will be used to synthesize molecular-scale reactions and bench-scale kinetic studies to develop a mechanistic basis for rate expressions. For oxidation experiments, biogenic UO₂(s) was synthesized under anaerobic conditions (using *Shewanella oneidensis* strain MR-1) following previously published methods. Precipitated solids were washed (1 M NaOH) and characterized by synchrotron X-ray absorption spectroscopy (collected at the Stanford Synchrotron Radiation Lightsource) at the U L-III edge. Spectroscopic results indicated poorly crystalline products of U(IV) with the local atomic structure of UO₂(s) that were stable in the X-ray beam with samples held at 77 K. After additional characterization, biogenic UO₂(s) will be slurry-loaded with ground quartz into small-volume columns to investigate rates of abiotic and biotic oxidative dissolution in anaerobic, flow-through experiments.

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

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

University-Led Research

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

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

To translate experimental results into numerical analysis of U fate and transport, we developed a reaction network based on Sani et al. (2004) to simulate U(VI) bioreduction with concomitant UO_2 reoxidation in the presence of hematite or ferrihydrite. The reduction phase considers SRB reduction (using lactate) with the reductive dissolution of Fe(III) solids, which is set to be microbially mediated as well as abiotically driven by sulfide. Model results show the oxidation of HS^- by Fe(III) directly competes with UO_2 reoxidation as Fe(III) oxidizes HS^- preferentially over UO_2 . The majority of Fe reduction is predicted to be abiotic, with ferrihydrite becoming fully consumed by reaction with sulfide. Predicted total dissolved carbonate concentrations from the degradation of lactate are elevated ($\log(p_{\text{CO}_2}) \sim -1$) and, in the hematite system, yield close to two orders-of-magnitude higher U(VI) concentrations than under initial carbonate concentrations of 3 mM. Modeling of U(VI) bioreduction with concomitant reoxidation of UO_2 in the presence of ferrihydrite was also extended to a two-dimensional field-scale groundwater flow and biogeochemically reactive transport model for the South Oyster site in eastern Virginia. This model was developed to simulate the field-scale immobilization and subsequent reoxidation of U by a biologically mediated reaction network.

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

University-Led Research

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

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

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

University-Led Research

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

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

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

Quantifying Microbe-Mineral Transformation Using Dielectric Spectroscopy

University-Led Research

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This project involves laboratory experiments to investigate three hypotheses:

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

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

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

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

University-Led Research

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

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

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

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

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

University-Led Research

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

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

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

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

University-Led Research

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

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

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

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

University-Led Research

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

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

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

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

University-Led Research

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

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

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

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

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University-Led Research

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

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

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

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

[1] Zhang, S., et al. 2010. *Env. Sci. Technol.*, 44, 9042–9048.

[2] Schwehr, et al. 2009. *Env. Sci. Technol.*, 43, 7258–7264.

[3] Zhang, et al. 2011. *Env. Sci. Technol.*, submitted.

[4] Li, H.-P., et al. 2011. *Appl. Environ. Microbio.*, accepted.

[5] Kaplan, D.I., et al. 2011. *Env. Sci., Technol.*, accepted.

[6] Otosaka, S., et al. 2011. *Sci. Tot. Environ.*, submitted.

[7] Xu, C. et al. 2011. *Geochim. Cosmochim. Acta*, submitted.

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

University-Led Research

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

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

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

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

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

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

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

University-Led Research

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

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

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

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

University-Led Research

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

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

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

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

University-Led Research

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

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

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

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

Uranium Biomineralization by Natural Microbial Phosphatase Activities in the Subsurface

University-Led Research

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

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

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

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

Identifying Biomarkers and Mechanisms of Toxic Metal Stress with Global Proteomics

University-Led Research

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

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

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

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

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

Defining the Molecular-Cellular-Field Continuum of Mercury Detoxification

University-Led Research

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

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

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

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

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

Manganese Redox Mediation of UO₂ Stability and U Fate in the Subsurface: Molecular- and Meter-Scale Dynamics

University-Led Research

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Long-term stability of U(IV) is imperative to the success of U bioremediation in the environment. Injection of electron donors such as acetate and establishment of reducing conditions is being tested to reductively precipitate U(VI) as U(IV) species. During this process, reductive dissolution of Fe and Mn oxides will occur, followed in time, by the establishment of sulfidogenic conditions. After the injection of electron donor ceases, groundwater conditions will slowly return to more oxidizing conditions. As this occurs, strong oxidants such as MnO₂ can form through microbial activity under suboxic conditions, jeopardizing the stability of U(IV). The focus of our project since it started in June 2010 has been (1) to investigate whether MnO₂ formation can occur under low O₂ conditions in both laboratory and *in situ* field experiments, (2) to examine the fate of U once it is oxidized by MnO₂ and the effects of pH and carbonate concentration, and (3) to determine whether direct contact between MnO₂ and U(IV) is necessary for U re-oxidation and where the reaction is localized.

Laboratory experiments with a model Mn-oxidizing bacterium demonstrated that Mn oxidation may proceed at relatively high rates, even when the O₂ concentration is very low (0.1%). Kinetic analysis revealed that the bacteria had a high affinity for O₂. To test whether Mn oxidation can proceed under field-relevant conditions, we developed a method to encapsulate active Mn(II)-oxidizing bacteria in a diffusible gel (gel puck) that can be incubated *in situ* in the wells at the Rifle IFRC site. We have isolated a variety of strains from Rifle sediments and are testing their efficacy in the gel pucks. We are planning to deploy these gels at the Rifle site later this spring. During this past field season at Rifle, we deployed sediments in mesh bags and suspended at various depths to probe the field DO concentrations that support Mn oxidation. We also deployed *in situ* column experiments, which received an inflow of 25 μM Mn²⁺ to probe the accumulation of Mn oxides. Finally, we have performed *in situ* experiments to verify that U(IV) can be reoxidized by MnO₂ at the Rifle aquifer through the use of diffusive gels. Mesh bags, columns and gel pucks with U(IV)/MnO₂ were retrieved in November 2010 and are being characterized using ICP-OES, XAS, and SEM/TEM. Beamtime at SSRL was allocated to this project, and XAS measurements will be made in March and April 2011.

To understand the reaction between MnO₂ and U(IV), biogenic uraninite or monomeric U(IV) were embedded in gels along with a Mn oxidizer and incubated in a basic medium with 5% oxygen and 100 μM Mn(II). Although Mn oxidation was evident in the gel pieces, no U(VI) was released from the gel into the medium, although U(VI) was present in the gel. These gel pieces will be analyzed by electron microscopy, XAS, and microXAS to explore the spatial relationships among U(VI), Mn oxides, and the Mn(II)-oxidizing bacteria.

Finally, experiments are being conducted to further explore the reactions occurring at the MnO₂ mineral interface, specifically the adsorption of U(VI) to MnO₂ and the oxidation of U(IV) on MnO₂ surfaces. Results to date show that U(VI) adsorbs strongly to MnO₂ above pH 4, and that carbonate has a strong negative effect above pH 8 due to the formation of U(VI)-carbonate complexes. Samples from these experiments will be analyzed by XAS to determine the molecular-scale structure of the adsorbed U(VI) and the effects of pH and carbonate.

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

University-Led Research

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

In situ bioremediation of uranium with a slow-release electron donor, emulsified vegetable oil (EVO), was tested in Area 2 of the U.S. DOE IFRC site, Oak Ridge, TN. The EVO containing (w/w) 60% vegetable oil, 6% biodegradable surfactant, 0.3% yeast extract, and 0.05% $(\text{NH}_4)_3\text{PO}_4$, was injected one time into the subsurface. Groundwater samples were collected from one up-gradient (W8) and seven down-gradient wells (W1–7) to monitor temporal dynamics of geochemistry and functional genes of microbial communities over nine-months using GeoChip. Detrended correspondence analysis (DCA) of all detected genes suggested that significant changes in microbial communities occurred down-gradient but not in the up-gradient control. Corresponding to the geochemical changes, genes involved in biodegradation of the EVO (acetogenesis, CH_4 production and oxidation) and reduction of the electron acceptors (denitrification, cytochrome, sulfate reduction, dissimilatory and assimilatory nitrate reduction) were significantly enriched. The detected genes revealed enrichment of denitrification genes *narG*, *nosZ*, and *dsr* from *Desulfotomaculum* and *Desulfovibrio*, and cytochromes of *Geobacter*, *Anaeromyxobacter*, and *Desulfovibrio* after EVO injection, followed by a decline as EVO was consumed. These changes closely correlated with the concentration changes of acetate and the electron acceptors. This study demonstrated that a one-time injection of EVO provided e-donors for the effective bioreduction of U(VI) and *Geobacter*, *Anaeromyxobacter*, *Desulfovibrio*, and *Desulfotomaculum* were detected in abundance.

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

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

University-Led Research

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Natural redox conditions regulate technetium (^{99}Tc) species and mobility. Under reducing environments, highly mobile pertechnetate ($\text{Tc}^{\text{VII}}\text{O}_4^-$) is readily reduced to sparingly soluble Tc^{IV} oxides, (e.g. $\text{TcO}_2 \cdot n\text{H}_2\text{O}$). Numerous studies have investigated the reduction of TcO_4^- by naturally occurring abiotic and biotic processes, on the premise that these could be used for long-term immobilization of ^{99}Tc . The majority of those processes involve biogenic generation of Fe(II) and iron minerals, which subsequently reduce Tc. While effective for short-term immobilization of ^{99}Tc , one major difficulty with this strategy is that the (re)oxidation of Tc^{IV} oxides is relatively facile, and remobilization is likely to occur under oxidative environmental conditions.

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

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

To clarify the role of individual chemical species in sequestering ^{99}Tc in a biotic system, we performed series of abiotic reduction experiments. Sulfide was added in doses varying from 0 to 10 mM to simulate different stages of the biotic development of sulfidogenesis. At low sulfide doses (0, 0.1, 0.5, and 1 mM), mimicking the initial period of biotic sulfate reduction, all aqueous sulfide was scavenged during pre-equilibration by nZVI. Following $^{99}\text{TcO}_4^-$ addition, ^{99}Tc removal rates fit first-order kinetics and correlated strongly with the sulfide dose. However, the two higher sulfide doses (5 and 10 mM), representing the intermediate and end stages of biotic sulfate reduction, resulted in a significant level of aqueous sulfide present before $^{99}\text{TcO}_4^-$ addition. This excess aqueous sulfide appeared to substantially drive the reaction away from first-order kinetics, resulting in low but persistent levels of aqueous ^{99}Tc , even after 100 hours of reaction. Reoxidation experiments indicated that sulfidic conditions resulted in slower ^{99}Tc reappearance rates and significantly lower aqueous ^{99}Tc concentrations after more than 100 hours, compared to nonsulfidic conditions. The amount of ^{99}Tc released to the aqueous phase decreased as the sulfide dose increased.

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

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

University-Led Research

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

[1] Boggs, M. A.; Dong, W.; Gu, B.; Wall, N. A. *Radiochimica Acta* 2010, 98, 583-587.

[2] Boggs, M. A.; Travis, M.; Samuel, L.; Islam, M. R.; Dong, W.; Gu, B.; Wall, N. A. *Environmental Science & Technology* 2011, Accepted.

[3] Wall N.A. and Gu B. Invited Presentation. Pacifichem, 12/15-20, 2010, Honolulu, HI, USA

[4] Boggs M.A., Minton T.D., Lomasney S., Wall N.A. Oral presentation at ACS/NORM regional meeting, June 20-23, 2010, Pullman, WA, USA.

[5] Islam, M. R.; Boggs, M. A.; Wall, N. A. *Radiochimica Acta* 2011, In preparation,

[6] Karunathilake, N.; Boggs, M. A.; Wall, N. A. *Radiochimica Acta* 2011, In preparation.

[7] Gu, B.; Dong, W.; Liang, L.; Wall, N. A. *Environmental Science & Technology* 2010, Submitted.

[8] Gu B., Dong, W., Wall N.A., Lou Y. Oral presentation at Goldschmidt, June 13-18, 2010, Knoxville, TN, USA

Viral infection of Subsurface Microorganisms and Metal/radionuclide Transport

University-Led Research

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

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

Imaging Biofilms in Opaque Porous Media

University-Led Research

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

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

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

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

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

University-Led Research

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

U(VI) sorption affinity was evaluated with non-nanoporous alpha-alumina (SSA of 8 m²/g) and crushed natural goethite (SSA of 5 m²/g), as well as nanoporous natural goethite (SSA of 60 m²/g; nanoporosity of 30 μL/g for < 5 nm pore) and alumina materials with large nanopores (SSA of 147 m²/g; nanoporosity of 90 μL/g for < 5 nm pore) and small nanopores (SSA of 230 m²/g; nanoporosity of 145 μL/g for < 5 nm pore). Sorption of 100 μM U(VI) onto 0.2 g solid material in 10 mL of 5 mM HCO₃⁻ solution under near-neutral pH was rapidly completed for nanoporous goethite and Al oxides, whereas only ~30–60% sorption occurred on non-nanoporous oxides. Bicarbonate and pH exerted a minor effect on U(VI) sorption on nanoporous goethite and Al oxides, but played a significant role in hindering U(VI) sorption on non-nanoporous goethite and Al oxides. The majority of U(VI) remained on the surface of nanoporous goethite and Al oxides after extraction with 50 mM NaHCO₃, whereas nearly all U(VI) was released from non-nanoporous materials. Sorption and desorption results collectively suggest that nanoporous materials have significantly higher U(VI) sorption affinity than non-nanoporous materials.

Microbial reduction of soluble U(VI) to sparingly soluble uraninite (UO₂) is considered an efficient remediation technique for subsurface uranium contamination. An important unanswered question for *in situ* bioremediation is whether U(VI) sorbed on a reactive mineral surface is subject to effective and rapid reduction, as is the case for aqueous U(VI). To investigate the chemical linkage between U(VI) sorption affinity and the redox reactivity on a mineral surface, U(VI) bound to the surfaces of non-nanoporous alpha-alumina and nanoporous alumina (2~5 μmole U/g) was reduced by 1 mM hydroquinone (AH₂DS) over 1 day to 3 weeks in an anaerobic chamber. The reduction of U(VI) sorbed on the alumina surfaces was estimated by comparing the U(VI) desorption in anoxic bicarbonate solution (10~1000 mM) between 1 mM AH₂DS- reduced and unreduced samples. Duplicate samples were analyzed by U L_{III}-edge x-ray absorption spectroscopy for uranium speciation. The results indicate complete reduction to nanoparticulate uraninite of U(VI) sorbed on non-nanoporous alumina in 2 days, whereas little to no reduction was observed with U(VI) sorbed on nanoporous alumina with large pores (15% U(IV)) and small nanopores (5% U(IV)) after 1 weeks. This result is attributed to the higher sorption affinity of U(VI) to nanoporous surfaces than to non-nanoporous surfaces.