



University-Led Research Abstracts  
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# University-Led Research

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

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We conducted three experiments to investigate the spectral induced polarization (SIP) source mechanisms in microbially active environments.

In the first experiment, we investigated the SIP response of different biofilm components. Our results show that: increasing cell concentration increases the imaginary conductivity ( $\sigma''$ ); increasing the concentration of alginate decreases  $\sigma''$  however, addition of bacteria cells causes an increase in  $\sigma''$ ; increasing the phenazine (metabolite) concentration causes an increase in  $\sigma''$ . This effect is enhanced in the presence of microbial cells; increasing the DNA concentration decreases  $\sigma''$ . Addition of cells did not cause an increase in  $\sigma''$ . Preliminary modeling suggests that in the absence of conductive biominerals the  $\sigma''$  is a quantitative indicator of bacterial cell density.

In the second experiment, we examined the SIP response of common iron minerals associated with metabolic activity of microorganisms. Both pyrite and magnetite show high  $\sigma''$  and real ( $\sigma'$ ) conductivities compared to hematite, goethite, and siderite. The  $\sigma''$  spectra of both pyrite and magnetite exhibit a well-defined characteristic relaxation peak below 10 kHz, not observed with the other iron minerals. The  $\sigma''$  of a mixture of iron minerals is dominated and linearly proportional to the mass fraction of the highly conductive (pyrite and magnetite) iron minerals. The  $\sigma''$  magnitude increased with decreasing grain size diameter of magnetite and pyrite with a progressive shift of the characteristic relaxation peak towards higher frequencies. Our results suggest that SIP techniques can be used to track the formation of semi-conductive mineral phases such as pyrite and magnetite.

In the third experiment, we investigated biomineralization of iron minerals by *Geobacter sulfurreducens* and its  $\Delta$ pilA mutant. Wild type (WT) and  $\Delta$ pilA mutant cultures were supplemented with acetate, Fe citrate and elevated concentrations of phosphates. The WT and  $\Delta$ pilA strains promoted the accumulation of Fe(II) and decrease of phosphate in the aqueous phase. While the WT favored the formation of structurally ordered flattened prismatic crystals, composed of O, P and Fe, resembling vivianite, the deposits recovered from  $\Delta$ pilA culture had a similar elemental composition but its morphology was a rosette. The Fe/P ratio in the deposits recovered from the WT culture was higher than in deposits recovered from  $\Delta$ pilA culture. The synthesis of vivianite by *Geobacter* is significant because vivianite has been shown to abiotically reduce U(VI). We anticipate characterizing the processes that control the biogeochemical cycling of iron and biogeophysical responses from porous media populated with iron reducing bacteria communities.

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

Joel Blum (jdblum@umich.edu) - Univ of Michigan, J Blum (PI), B Gu, ORNL; J Demers, Univ Mich; F He, ORNL; W Zheng, ORNL (Co-PIs).

This project utilizes stable Hg isotopes in the East Fork Poplar Creek (EFPC) watershed, TN to place new constraints on methylmercury (MeHg) production, transport and degradation. We seek to better understand mechanisms leading to decoupling of inorganic Hg and MeHg concentrations in the EFPC ecosystem. We are conducting ecosystem level studies of natural samples and experimental studies of Hg isotope fractionation. Natural samples have been collected from: wetland sediments and pore waters; stream sediments, surface waters and pore waters; stream bottom periphyton; and young-of-year stoneroller minnows and redbreast sunfish. We have: 1) completed three seasonal sampling campaigns and analyses of THg, MeHg, and isotopic composition of sediments, suspended particulates, surface waters, pore waters, and periphyton from EFPC, Hinds Creek and adjacent wetlands; 2) analyzed the isotopic composition of archived fish and invertebrate tissues obtained from the ORNL EA Group; 3) collected samples of dissolved gaseous mercury (DGM) from EFPC stream water, and 4) conducted photochemical and dark oxidation experiments with EFPC stream water to investigate Hg isotope fractionation.

Recently published results from this project show Y-12 impacted sediments have a distinct Hg isotopic composition that can be traced from EFPC to Poplar Creek and the Clinch River, and that the isotopic composition of Hg in sediments within the EFPC vary little along the flow path (Donovan, Blum, Demers, Gu, Brooks, Peryam, 2014, Environ. Sci. Technol. 48, 3666-3674). In contrast, additional analyses show that the Hg isotopic composition of total suspended solids and the dissolved phase within EFPC change with distance downstream. There are shifts in the average isotopic composition of Hg in suspended sediment (THgp) from Y-12 ( $\delta^{202}\text{Hg} = -0.46\text{‰}$ ,  $\Delta^{199}\text{Hg} = -0.03\text{‰}$ ) to downstream reaches ( $\delta^{202}\text{Hg} = 0.08\text{‰}$ ,  $\Delta^{199}\text{Hg} = -0.08\text{‰}$ ). Changes in isotopic composition of dissolved Hg (THgd) is less consistent across seasons and along the flow path. The isotopic composition of DGM ranged in  $\delta^{202}\text{Hg}$  values from 0.97‰ to 1.10‰ and in  $\delta^{199}\text{Hg}$  values from -1.52‰ to -0.29‰, differing significantly from that of stream water, suspended particulate matter, and sediments. Analyses of riparian wetland and hyporheic pore waters suggests that near-stream sources of Hg may be isotopically distinguishable from THgp and THgd within the stream channel. Analyses of biota suggest different isotopic compositions of MeHg and inorganic Hg in the stream ecosystem. Combining field measurements with experimental results we assess potential sources and processes driving changes in Hg concentrations and isotopic compositions along the EFPC flow path.

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

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Iron-bearing phyllosilicate minerals help establish the hydrogeological and geochemical conditions of redox transition zones because of their small size, limited hydraulic conductivity and redox buffering capacity. The bioreduction of soluble U(VI) to sparingly soluble U(IV) can promote the reduction of clay-Fe(III) through valence cycling. The reductive precipitation of U(VI) to uraninite was previously reported to occur only after a substantial percentage of clay-Fe(III) had been reduced. Using improved analytical techniques we show that concomitant bioreduction of both U(VI) and clay-Fe(III) by *Shewanella putrefaciens* CN32 can occur. Soluble electron shuttles were previously shown to enhance both the rate and extent of clay-Fe(III) bioreduction. Using extended incubation periods we show that electron shuttles enhance only the rate of reduction (overcoming a kinetic limitation) and not the final extent of reduction (a thermodynamic limitation). The first 20% of clay-Fe(III) in nontronite NAu-2 was relatively “easy” (i.e., rapid) to bioreduce while the next 15% of clay-Fe(III) was “harder” (i.e., kinetically-limited) to bioreduce and the remaining 65% of clay-Fe(III) was effectively biologically un-reducible. In abiotic experiments with NAu-2 and biogenic uraninite, 16.4% of clay-Fe(III) was reduced in the presence of excess uraninite. In abiotic experiments with NAu-2 and AH2DS, 18.5 to 19.1% of clay-Fe(III) was reduced in the presence of excess and variable concentrations of AH2DS. A thermodynamic model based on published values of the non-standard state reduction potentials at pH 7.0 ( $E'_H$ ) showed that the abiotic reactions between NAu-2 and uraninite had attained apparent equilibrium. This model also showed that the abiotic reactions between NAu-2 and AH2DS had attained apparent equilibrium. The final extent of clay-Fe(III) reduction was well correlated to the standard state reduction potential at pH 7.0 ( $E^{\circ}_H$ ) of all of the reductants used in these experiments (AH2DS, CN32, dithionite, uraninite).

We also examined the reduction of nitrobenzene by CN32 in the presence of montmorillonite SWy-2 (low Fe(III) content) and nontronite NAu-2 (high Fe(III) content). Nitrobenzene and other nitroaromatic compounds (NACs) have been selected for study because their redox properties are well known and they have previously been used as redox probe molecules to examine the reactivity of clay-Fe(II). We found that the abiotic reduction of nitrobenzene became increasingly important as clay-Fe(II) accumulated in the system.

## **Inorganic Geochemical Controls on Neptunium Transport: Co-Precipitation in Minerals**

Peter Burns (pburns@nd.edu) - University of Notre Dame(PI).

Neptunium-237, with a half-life of 2.14 million years, is a contaminant of concern for the U.S. Department of Energy. Multiple oxidation states are accessible, but under subsurface conditions of environmental importance the Np(V) oxidation state is strongly favored. This cation forms a linear dioxo cation, the neptunyl ion, which is soluble in aqueous solutions. Whereas it is tempting to assume that the Np(V) uranyl ion will behave similarly to the U(VI) uranyl ion in the subsurface, there are a variety of reasons to expect otherwise. Specifically, the Np(V)-O bonds within the neptunyl ion are weaker than those in the U(VI) uranyl ion, making the neptunyl ion O atoms more reactive. Also, the charge of the neptunyl ion is lower than that of the uranyl ion, which will impact charge balance mechanisms for co-precipitation reactions.

We hypothesize that the Np(V) neptunyl ion may be co-precipitated into a variety of low temperature mineral structures that form in the subsurface. The analogous situation has been demonstrated in several studies focusing on uranyl ions, but the only studies for neptunyl have examined calcite.

In order to derive a predictive understanding of Np(V) incorporation into a variety of minerals, we are experimentally examining incorporation of the neptunyl ion in several mineral phases that present different cation sites that are incorporation targets. The chosen mineral phases are part of the carbonate, sulfate, nitrate and borate families and contain either monovalent or divalent cations. Materials have been characterized by a variety of methods including inductively coupled plasma mass spectrometry (solution and laser ablation mode), UV-vis spectroscopy, X-ray photoelectron spectroscopy and time resolved laser fluorescence spectroscopy.

Results to date demonstrate both that incorporation of U(VI) and Np(V) in such structures differ significantly between the two actinyl ions, and also between structure types. We are thus bringing crystal chemical models to bear on the study of the local environments of incorporation.

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

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Phosphate addition is an in situ remediation approach that may enhance the sequestration of uranium without requiring sustained reducing conditions. However, the geochemical factors that determine the dominant immobilization mechanisms upon phosphate addition are insufficiently understood to design efficient remediation strategies or accurately predict U(VI) transport. The overall objective of our project is to determine the dominant mechanisms of U(VI)-phosphate reactions in subsurface environments. Our research approach seeks to determine the U(VI)-phosphate precipitates that form from homogeneous solutions, characterize the effects of phosphate on U(VI) adsorption and precipitation on smectite and iron oxide minerals, and investigate how phosphate affects U(VI) speciation and fate in field sediments.

Our initial studies investigated the products of U(VI)-phosphate reactions in the absence and presence of sorbing mineral phases. In homogeneous systems, autunites form even when other U(VI) phosphates are more thermodynamically stable. In the presence of Ca, U(VI) may also sorb to or coprecipitate with calcium phosphates. In heterogeneous systems, phosphate has no macroscopic effect on U(VI) adsorption to a smectite clay despite LIFS and EXAFS spectra showing a clear change in surface speciation. In contrast, phosphate enhanced U(VI) adsorption to goethite at pH 4 but suppressed adsorption at pH 6 and 8. For both smectite and goethite, autunites precipitated at high phosphate and U(VI) concentrations but only after a critical supersaturation was exceeded.

Our current activities focus on U(VI)-phosphate reactions in Rifle and Hanford site sediments. Synthetic groundwaters designed to match the major element compositions at the sites were employed for both sediment types. Batch studies show little to no U(VI) sorbs to Rifle sediments except at high (1 mM) phosphate concentrations. The addition of phosphate to the influent of columns of Rifle sediments results in substantial sustained decreases in effluent U(VI) concentrations. Sequential extractions of sediments from the columns confirmed the accumulation of uranium with time and observed a gradual shift of solid-associated uranium to less mobile species. EXAFS and LIFS analysis of these sediments suggested that U(VI) was immobilized by both precipitation of an autunite mineral and adsorption. Batch studies of Hanford sediments showed distinct behavior from the Rifle sediment system. Substantial U(VI) adsorption occurred and was enhanced by phosphate addition, with an autunite-type precipitate forming at initial U(VI) and phosphate concentrations above 25  $\mu\text{M}$  and 100  $\mu\text{M}$ , respectively. Ongoing column experiments are investigating U(VI) retention in Hanford sediments in the presence of phosphate.

## Uranium fate in acidic waste-weathered sediments: Scaling of molecular processes to predict reactive transport

Jon Chorover (chorover@cals.arizona.edu) - University of Arizona (PI), Peggy O'Day; Carl Steefel; Karl Mueller; Wooyong Um; John Zachara; Estela Reinoso-Maset; Nico Perdrial; Angelica Vazquez-Ortega; Masa Kanematsu (Co-PIs).

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

Hypotheses: (1) Reactive transport modeling of future U releases from the vadose zone of acid-waste weathered sediments can be constrained by combining information on contaminant bonding environment with quantification of contaminant phase partitioning and meso-scale kinetic data on contaminant release from the waste-weathered porous media; (2) Although field contamination and laboratory experiments differ in their diagenetic time scales, sediment and synthesized U minerals dissolution, neophase nucleation, and crystal growth reactions that occur during the initial system disequilibrium leave a strong imprint that persists with memory effects over subsequent longer-term equilibration time scales.

Research Approach: An iterative measure-model approach is applied to elucidate mechanistic underpinnings of reactive contaminant transport in weathering geomedia.

Experimental design: Crib waste simulants were reacted with Hanford sediments in batch and flow-through systems. Coupling of contaminant uptake to mineral weathering was monitored both during waste-sediment interaction, and after, when waste-weathered sediments are subjected to infusion with circumneutral background pore water (BPW).

Results: We completed bench-scale batch weathering to one year and flow-through column experiments where Hanford Sediments were reacted with U-bearing synthetic crib waste (SCW) solutions as a function of pH, U and phosphate concentrations. PO<sub>4</sub> exerted strong control over U speciation at all pH with rapid precipitation of meta-ankoleite [K(UO<sub>2</sub>PO<sub>4</sub>)•3H<sub>2</sub>O] leading to near complete immobilization of U. Boltwoodite [K(UO<sub>2</sub>)(HSiO<sub>4</sub>)•1.5H<sub>2</sub>O] increased even in PO<sub>4</sub>-containing systems. Without PO<sub>4</sub>, U speciation was controlled by the rate of silicate weathering. In this case, U immobilization was limited to 25-50% as precipitated becquerelite [Ca(UO<sub>2</sub>)<sub>6</sub>O<sub>4</sub>(OH)<sub>6</sub>•7H<sub>2</sub>O] or compreignacite [K<sub>2</sub>(UO<sub>2</sub>)<sub>6</sub>O<sub>4</sub>(OH)<sub>6</sub>•7 H<sub>2</sub>O] and boltwoodite. Carbonate dissolution buffered influent solutions to pH ~?, which promoted silicate weathering and boltwoodite precipitation. Dissolution of synthetic compreignacite and Na-compreignacite yielded dissolution rates of 8.89•10<sup>-13</sup> and 2.48•10<sup>-12</sup> mol m<sup>-2</sup> s<sup>-1</sup> (normalized to BET surface area), respectively. Desorption experiments of weathered sediments? revealed an incremental desorption of U for phosphate-free systems, indicating dissolution of compreignacite, becquerelite and schoepite, but solutions were saturated with respect to Mg-zippeite. In contrast, slow dissolution of meta-ankoleite and autunite in PO<sub>4</sub> systems showed low U release.

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

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Research conducted at the University of Southern California, Montana State University, and at Rensselaer Polytechnic Institute in Troy, New York, continues to embrace controlled cultivation technologies for evaluating the response of signature microorganisms to changes in environmental conditions. We are particularly interested in the activity and strategies invoked by attached microbial populations within highly-integrated "biofilm" communities.

*Desulfovibrio vulgaris* RCH-1 is a sulfate-reducing bacterium that was isolated from chromium-contaminated groundwater at the 100H Hanford Site. This organism formed biofilms under conditions of sulfate limitation at environmentally relevant temperature of 20°C. A variety of extracellular structures were observed by a variety of microscopic techniques. Apart from some metal deposits near the bacterial cell surface, metal precipitation occurred extracellularly predominantly on thin, elongated structures that in cross section appeared as stacks of membranous structures, or as complex geometrical enclosed shapes whose inside were always devoid of bacteria. Non-osmicated biofilms that were stained with a solution of uranyl acetate revealed an unstained thin core structure, which upon osmication becomes black, indicating that the thin structure is lipid-based. We also observed membrane vesicles nearby or docking to the extracellular structures, and these observations supported a membrane lipid-based origin. Serial section lipophilic dye FM1-43 in cryostat-sections revealed that the membrane structures persist for tens of micrometers. EDS imaging revealed presence of Fe, O and P, but not sulfide, and these results suggested the metal deposits are not solely the result of inorganic chemistry interactions of metals ions with hydrogen sulfide. The biofilm and metal deposition was visualized in 3D with SBF/SEM, and showed a heterogeneous distribution of metal precipitates away from cells. Assessing metal interactions at in situ temperatures in biofilms rather than optimal growth temperatures in bulk-phase and under electron donor- and acceptor-limitation with field isolates provides relevant insight into metal reduction for respective field sites.

Methods for visualizing the growth of extracellular structures in real time and under physiologically relevant and metabolically active conditions were developed by members of our extended team at USC and initially applied those methods to the facultative metal reducing bacterium *Shewanella oneidensis*. Recent results provide new insights into the strategies and components invoked for efficient extracellular charge transfer within electron acceptor-limited biofilms, many of which are consistent with observations made with *D. vulgaris* RCH-1. These approaches are currently being applied to the field isolate *Geobacter. daltonii* FRC32T using

## Methods to Quantify Mercury Bioavailability in Sulfidic Sediments

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The production of methylmercury (MeHg) in the environment depends, in part, on the speciation of inorganic mercury and the portion that is available for uptake into methylating microorganisms. This research investigated two methods to quantify the bioavailable fraction of mercury in sediments and other settings relevant for MeHg production.

The first approach is a selective leaching protocol that utilized an anoxic leachate comprised of glutathione, a strong chelating ligand for Hg that may mimic sulfhydryl-containing ligands on the surface of methylating microorganisms. For a pre-defined leaching procedure (1 mM glutathione, 1 gram of sediment per 10 mL, and 0.5 h holding time), the results indicated a correlation between glutathione-leachable mercury concentrations and the native methylmercury concentrations in 10 different marine sediments. Further experiments were performed with anaerobic sediment microcosms that were amended with different types of Hg with a known variation in methylation potential. The glutathione-leachable mercury concentrations in these microcosms experiments also correlated to MeHg production. The results demonstrated that a thiol-based leaching protocol may be a more appropriate approach than other methods (e.g. tin-reduction, sequential extractions) for the determination of Hg bioavailability in sediments.

Because of the promising results of the glutathione-based leaching test, we explored the use of diffusive gradient in thin-film (DGT) samplers, a thiol-based passive sampler that can be used as an in situ method for Hg bioavailability. This method has been used previously to determine dissolved metal concentrations in water. However, when DGT samplers are deployed in sediment settings, it is unclear how the presence of small particles affects the performance of the samplers. To test this question, DGT samplers were exposed to solutions containing different formulations of HgS nanoparticles. The amount of Hg accumulated onto DGT was quantified and compared with the dissolved Hg(II) in the solution (quantified by 0.02- $\mu$ m filtration). The speciation of Hg on the resin layer was also identified by X-ray absorption spectroscopy. The results indicated that although the nanoparticles could not pass through the diffusion layer, they deposited on the surface of this layer and possibly clogged the pores of the sampler. As a result, the migration of dissolved Hg through the diffusion layer was retarded in the presence of the nanoparticles. Therefore, the Hg concentration predicted by DGT is smaller than the actual concentration in the bulk solution. The findings of our study have important implications for interpretation of Hg data obtained by DGT samplers in anoxic settings.

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

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Discharge of contaminated groundwater to surface waters is of concern at many DOE facilities. For example, at the Savannah River Site (SRS), uranium contaminated groundwater is discharging into natural wetlands. The numerous sharp biogeochemical transitions occurring in wetlands have profound effects on the ultimate fate of redox-sensitive trace metals and radionuclides, including uranium.

The goal of this research is to provide new insights on how plant-induced alterations to the sediment biogeochemical processes affect key uranium reducing microorganisms, uranium reduction, and uranium spatial distribution. We have formulated the following hypotheses. (1) U(VI) discharged from ground- to surface-waters can be immobilized effectively as U(IV) in the sediments at the groundwater-surface water interface. The electron donor required to stimulate the microorganisms capable of reducing U(VI) is provided by wetland plants via their root exudates and root turnover. (2) Oxygen released into the sediments by plants reoxidizes Fe(II), forming iron oxy(hydroxi)des, which provide the bioavailable Fe(III) for long-term bacterial iron-reducing activity, which is key for a sustained biological uranium reduction.

To test the above hypotheses, we operated small-scale wetland mesocosms to simulate the discharge of uranium-contaminated groundwater to surface waters. The mesocosms were operated for 80 days, followed by analysis for solid-associated chemical species, microbiological characterization, micro-X-ray fluorescence ( $\mu$ -XRF) mapping of Fe and U on the root surface, and U speciation via X-ray Absorption Near Edge Structure (XANES).

Bacterial numbers including *Geobacter* sp., Fe(III), as well as total uranium, were highest on roots, followed by sediments near roots, and lowest in zones without much root influence. Results from the  $\mu$ -XRF mapping on root surfaces indicated a strong spatial correlation between Fe and U, while the analysis via XANES showed that a significant fraction of uranium was reduced to U(IV), indicating that iron cycling in the rhizosphere also results in uranium reduction and immobilization. Under high iron loading, results indicate that co-precipitation of U(VI) with iron might have been an important U removal process.

A series of depth-discrete dialysis samplers were set up in an uranium-contaminated wetland on the SRS to monitor seasonal U dynamics. Vertical profiles of dissolved U are consistent with our laboratory findings, indicating that during the growing season when there is more active iron reduction, the dissolved U in the sediment pore water is significantly lower.

## **Integrated geophysical measurements for bioremediation monitoring: combining NMR, magnetic methods and SIP.**

Kristina Keating (kmkeat@andromeda.rutgers.edu) - Rutgers University (PI), Rutgers University; Dimitris Ntarlagiannis, Rutgers University; Kenneth Williams, LBNL (Co-PIs).

Our research aims to develop borehole measurement techniques capable of monitoring subsurface processes, such as changes in pore geometry and iron/sulfur geochemistry, associated with remediation of heavy metals and radionuclides. Previous work has demonstrated that geophysical method spectral induced polarization (SIP) can be used to assess subsurface contaminant remediation; however, SIP signals can be generated from multiple sources limiting their interpretational value. Integrating multiple geophysical methods, such as nuclear magnetic resonance (NMR) and magnetic susceptibility (MS), with SIP, could reduce the ambiguity of interpretation that might result from a single method. Our research efforts will entail combining measurements from these methods, each sensitive to different mineral forms and/or mineral-fluid interfaces, providing better constraints on changes in subsurface biogeochemical processes and pore geometries significantly improving our understanding of processes impacting contaminant remediation.

The Rifle Integrated Field Research Challenge (IFRC) site was used as a test location for our measurements. The Rifle IFRC site is located at a former uranium ore-processing facility in Rifle, Colorado. Leachate from spent mill tailings has resulted in residual uranium contamination of both groundwater and sediments within the local aquifer. Studies at the site include an ongoing acetate amendment strategy, native microbial populations are stimulated by introduction of carbon intended to alter redox conditions and immobilize uranium. To test the geophysical methods in the field, NMR and MS logging measurements were collected before, during, and after acetate amendment. Next, laboratory NMR, MS, and SIP measurements were collected on columns of Rifle sediments during acetate amendment. The laboratory experiments were designed to simulate field experiments; changes in geophysical signals were expected to correlate with changes in redox conditions and iron speciation.

Field MS logging measurements revealed vertically stratified magnetic mineralization, likely the result of detrital magnetic fraction within the bulk alluvium. Little to no change was observed in the MS data suggesting negligible production of magnetic phases (e.g. magnetite, pyrrhotite) as a result of sulfidogenesis. Field NMR logging measurements maintained high levels of noise contamination requiring significant signal processing, and analysis suggests that any changes may be difficult to differentiate from simultaneous changes in water content. Laboratory MS and NMR measurements remained relatively stable throughout the course of the acetate amendment experiment, consistent with field measurements. However, SIP measurements changed during acetate amendment finding consistent with previous research indicating the formation of iron-sulfide mineral phases.

## **Identification of a c-type cytochrome involved in Mn(IV) reduction in *Anaeromyxobacter dehalogenans* strain 2CP-C**

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*Anaeromyxobacter dehalogenans* strains are metabolically versatile Deltaproteobacteria that conserve energy from the reduction of a variety of electron acceptors, including insoluble manganese dioxide (MnO<sub>2</sub>) and ferric oxides/oxyhydroxides (FeOOH). The goal of this research effort was to identify c-type cytochromes involved in electron transfer to the insoluble electron acceptor MnO<sub>2</sub>.

The characterization of deletion mutants has revealed a number of c-type cytochromes involved in electron transfer to solid metal oxides in *Shewanella* spp. and *Geobacter* spp; however, knockout mutants often do not display distinct phenotypes indicating the limitations of this approach for making functional assignments to specific c-type cytochromes. A genetic system for *Anaeromyxobacter* is not available and functional assignment of c-type cytochromes is lacking. To identify *A. dehalogenans* c-type cytochromes involved in electron transfer to solid metal oxides, protein expression profiles of strain 2CP-C cells grown with acetate as electron donor and MnO<sub>2</sub>, ferric citrate, ferric oxyhydroxide, nitrate or fumarate as electron acceptors were compared. Whole cell lysates were subjected to trypsin proteolysis and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Distinct c-type cytochrome expression patterns were observed in cells grown with the different electron acceptors. *A. dehalogenans* strain 2CP-C cells grown with insoluble Mn(IV) expressed 25 c-type cytochromes out of the 69 c-type cytochromes encoded on the strain 2CP-C genome. An uncharacterized c-type cytochrome (Adeh\_1278) was uniquely expressed in strain 2CP-C cells grown with MnO<sub>2</sub>. PCR and reverse transcriptase-qPCR demonstrated that the Adeh\_1278 gene was transcribed in cells grown with MnO<sub>2</sub> but not with any of the other tested terminal electron acceptors. Apparently, the transcription of the Adeh\_1278 gene and Adeh\_1278 expression correlated with Mn(IV) reduction activity in *A. dehalogenans* strain 2CP-C. Adeh\_1278 has three heme binding motifs and is predicted to occur in the periplasm. The identification of Adeh\_1278 as a protein uniquely expressed during growth with MnO<sub>2</sub> suggests its utility as a biomarker for MnO<sub>2</sub> reduction and demonstrates the value of the LC-MS approach for making functional assignments to c-type cytochromes. The distinctive expression of c-type cytochromes in response to growth with different terminal electron acceptors offers opportunities for functional (i.e., activity) in situ monitoring using environmental metaproteomics or transcript-targeted approaches.

## Going Wireless: Fe(III) Oxide Reduction without Pili by *Geobacter sulfurreducens* Strain JS-1

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Previous studies have demonstrated that the electrically conductive pili of *Geobacter sulfurreducens* strain DL-1 are essential for optimal extracellular electron transfer to Fe(III). *G. sulfurreducens* strain KN400 reduces poorly crystalline Fe(III) oxide more rapidly than strain DL-1. In order to further study the mechanisms for Fe(III) oxide reduction in KN400, the gene for PilA, the structural pilin protein, was deleted. Deletion of the PilA gene inhibited Fe(III) oxide reduction. However, slow rates of Fe(III) reduction were detected after extended (> 30 days) incubation in the presence of Fe(III) oxide. After seven consecutive transfers the adapted PilA-deficient strain, designated strain JS-1, reduced Fe(III) oxide as fast as the wild type. Microarray, proteomic, and gene deletion studies indicated that this adaptation was associated with greater production of the c-type cytochrome PgcA, which was released into the culture medium. Multiple lines of evidence suggested that PgcA acted as an electron shuttle, promoting electron transfer from the outer cell surface to Fe(III) oxides in strain JS-1. In contrast, PgcA was not required for effective Fe(III) oxide reduction in the wild-type strain. Strain JS-1 competed well with the wild-type strain when both were grown together on Fe(III) oxide. However, when 50% of the culture medium was replaced with fresh medium every three days, the wild-type strain out-competed strain JS-1. This result was attributed to the need for JS-1 to continuously replace the PgcA being removed from the medium, putting JS-1 at a competitive disadvantage, similar to the apparent selection against electron shuttle producing Fe(III) reducers in most soils and sediments. The long period necessary for the PilA-deficient strain of KN400 to adapt for effective Fe(III) oxide reduction, and the fact that deleting the gene for PgcA in the wild-type had no impact on Fe(III) oxide reduction, suggest that pili-mediated Fe(III) oxide reduction is more representative of the mechanism by which *G. sulfurreducens* reduces Fe(III) oxide in soils and sediments. The ability of KN400 to adapt to the loss of pili demonstrates that caution may be warranted in extrapolating to natural environments the mechanisms for Fe(III) oxide reduction elucidated in studies with cultures maintained for long periods under laboratory conditions that do not mimic those found in soils and sediments. The substantial plasticity encoded in the genomes of microorganisms capable of extracellular electron transfer, coupled with unnatural laboratory selection pressures, have the potential to lead to physiological responses that might not be found in natural environments.

## **SMART (Subsurface Microbial Activity in Real Time) Technology for Real-Time Monitoring of Subsurface Microbial Metabolism**

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Real-time estimates of in situ anaerobic microbial activity are needed in order to elucidate the impacts of environmental perturbations, such as seasonal changes in temperature and nutrient availability, as well as to assess the effectiveness of bioremediation strategies. Current methods typically involve invasive sampling of the environment, which can alter microbial activities, and often require the addition of tracers and/or expensive analytical equipment, adding complexity and expense. Therefore, we developed SMART (Subsurface Microbial Activity in Real Time) technology as a novel approach to monitoring microbial metabolism in anaerobic sediments and submerged soils. The SMART approach is based on microbial fuel cell concepts that have previously been adapted to detect changes in the availability of relatively high concentrations of organic compounds in wastewater, or during in situ bioremediation of uranium-contaminated groundwater. However, in most soils and sediments, rates of microbial activity are not linked to the concentrations of organic substrates, but rather to the turnover rates of the substrate pools, which have steady state concentrations in the  $\mu\text{M}$  range. In order to determine whether levels of current produced at a graphite anode would correspond to the rates of microbial metabolism in anaerobic sediments, small graphite anodes were inserted at various depths in sediment cores and connected to graphite brush cathodes in the overlying water. Currents produced were compared with the rates of [2- $^{14}\text{C}$ ]-acetate turnover because acetate is a central intermediate in the anaerobic degradation of organic matter, regardless of the predominant terminal electron-accepting process. Over a wide range of in situ metabolic rates, there was a direct correlation between current production and the rate that [2- $^{14}\text{C}$ ]-acetate was metabolized to  $^{14}\text{CO}_2$  and  $^{14}\text{CH}_4$  in sediments in which Fe(III) reduction, sulfate reduction, or methane production was the predominant terminal electron-accepting process. At comparable acetate turnover rates, currents were higher in the sediments in which sulfate-reduction or Fe(III) reduction predominated than in methanogenic sediments. This was attributed to reduced products (Fe(II), sulfide) produced at distance from the anode contributing to current production in addition to the current generated from the microbial oxidation of organic substrates at the anode surface in all three sediment types. These results suggest that the SMART technology will serve as a simple, inexpensive, and effective approach for real-time monitoring of rates of anaerobic microbial metabolism in a diversity of anaerobic soils and sediments with applicability to the study of subsurface bioremediation and the impact of environmental perturbations on microbial activity.

## **Radiocarbon Signature of Microbial DNA from a Reducing Zone of a Floodplain aquifer, Rifle Colorado**

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Biogeochemical redox cycles in groundwater systems frequently mobilize metals and metalloids that directly impact human health. The microbes catalyzing these biogeochemical reactions require organic carbon as an electron donor. The source(s) of these electron donors in groundwater systems has been at best poorly constrained. We recently developed a method to filter large volumes of groundwater (>2000 gallons), extract and purify the DNA, and to radiocarbon date the DNA to determine organic carbon sources (Mailloux et al., 2013). Results from one high arsenic field site in Bangladesh characterized by rapid infiltration indicated that microbes preferentially used organic carbon that was slowly advected through the aquifer. Microbes did not utilize young anthropogenic organic carbon nor did they utilize sediment derived organic carbon. At the Department of Energy's Field Research Challenge (IFRC) site near Rifle, Colorado portions of the recently recharged shallow aquifer are reducing with elevated levels of arsenic and iron. In addition, this aquifer harbors novel microbial assemblages. This aquifer offers a unique opportunity to study organic carbon sources given the different potential and resolvable organic carbon sources. The organic carbon sources include modern organic carbon from recent recharge, slowly advected organic carbon represented by dissolved organic matter (DOM), and older sediment organic carbon. Estimated groundwater flow rates are 80 m per year indicating that the site is flushed approximately annually. Two samples of DOM from the site were dated at 2980 and 3340 y. Bulk radiocarbon sediment ages ranged from 4750 to 11,600 y. Well LQ-107 with 2.2 mg/l Fe and 62 ug/l arsenic was sampled to determine organic carbon sources. Water was filtered using a 10<sup>3</sup> filter housing and 0.2 μm serial nylon filters. The planktonic microbial population was sampled by filtering 6213 gallons. DNA was extracted and purified to remove organic carbon derived from the cell lysates, humic materials, and buffers. DNA was radiocarbon dated at the Center for AMS at Lawrence Livermore National Laboratory. The Radiocarbon DNA age was 3325±40 y. These results indicate that the microbial population at the site is utilizing the DOM for cell division. Future plans include sampling more wells and completing development of an RNA radiocarbon method.

Mailloux, B.J., Trembath-Reichert, E., Cheung, J., Watson, M., Stute, M., Freyer, G.A., Ferguson, A.S., Ahmed, K.M., Alam, M.J., Buchholz, B.A., Thomas, J., Layton, A.C.,

## **Real-time, on-site and quantitative detection of bioavailable radionuclides and heavy metals in contaminated water at DOE sites**

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Quantitative, real-time detection of radionuclides and heavy metal ions on-site remains a significant challenge while monitoring environmental resources such as groundwater and surface water. At the Integrated Field Research Center in Rifle, subsurface water contaminated with soluble and labile U(VI) is being converted to insoluble U(IV) by microbial action to fix the uranium and therefore an on-site sensor that can specifically quantify U(VI) in groundwater will provide a tool for monitoring uranium speciation and remediation progress.

Lu and coworkers have reported a DNAzyme based fluorescent uranium sensor with a detection limit of 11 ppt and high selectivity. It is specific for U(VI) as DNAzyme's are specific to a particular oxidation state of a metal. ANDalyze Inc. commercialized several DNAzyme based heavy metal sensors including sensors for uranium, lead, copper, zinc, cadmium and mercury using a combination of dry form sensor cartridge and a hand-held fluorimeter. The tests can be done in 2 minutes. The focus of our poster is the commercialized uranium sensor with a detection range of 2 - 60 ppb and a typical uranium recovery of 75-125% for field testing.

Previously, we collaborated with Ken Williams and Phil Long at Rifle and with Tonia Melhorn and Kenneth Lowe at ORNL to test our uranium sensor with samples from Rifle and ORNL sites. Twelve ORNL samples with ppm level uranium were analyzed after dilution and the results compared well to ICP (80-120% recovery). The Rifle samples had lower recovery of ~ 50%. We then optimized the protocol to test samples at Rifle which require a simple acidification to ~pH 5 prior to analysis by adding few drops of dilute acid. With the optimized protocol, we traveled to the Rifle site to perform field analysis of samples. Eight samples were analyzed using two each from plots A, B, C and D and the results compared to ICP. The samples had a uranium recovery of ~ 70-110% (compared to ICP results) which works well for quick, on-site field analysis. Since most samples showed a lower result than ICP, we are testing various uranium complexes that maybe found in the Rifle subsurface to test their effect on the sensor's ability to quantify U(VI) and therefore improving sensor performance.

To add capability of remote monitoring, we are currently in the process of developing a continuous monitor which will allow for unattended operations up to 30-days, while testing samples at a specified frequency.

## **Strikingly Different Form of Organic Carbon in Soils of Temperate and Permafrost Soils: Implications for Carbon and Metal Cycling**

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Soils are major reservoirs of organic carbon (OC), and play a central role in the mobility of carbon between the land and the atmosphere. Among different soils, a significant fraction of global soil OC is stored in permafrost soils, and thawing and rapid losses of OC in the form of CO<sub>2</sub>, associated with warming of polar regions, raises a serious concern for the stability of carbon in polar soils. Studies have shown that variations in soil mineralogy and biogeochemistry, and climatic conditions can modify the soil OC retention, which in turn alter the elemental cycles. The focus of our research is on how variations in vegetation and soil biogeochemical conditions control the dynamics of OC and associated major and trace elements in soils of different climatic conditions.

In this study, we examined OC forms associated with soils of temperate and polar climates using X-ray Absorption and NMR spectroscopic techniques, and high-resolution FT-ICR ESI-mass-spectrometry. In addition, to assess OC dynamics, we also evaluated the forms of S, P, and Cl bound to OC, and their time-dependent concentration variations. We compared the chemistries of these elements in Arctic soils with soils collected from temperate coniferous and deciduous forests.

Our studies indicate that the organic carbon is rich in lipids, and poor in aromatic and carboxylic carbon in the high Arctic soils when compared to soils of other climates. The O/C ratios of the extractable organic carbon also indicate that it is O-poor. This is in direct contrast to the organic matter found in soils of tropical and temperate climates, which is rich in lignins, tannins, and other condensed aromatics. The abundance of such a lipid-rich OC in high Arctic soils may be attributed to the moss, algae and fungal (or lichen) mats, whereas lignin and other aromatic-rich vascular plants are the common sources of OC in other climatic regions. Such a contrasting composition of OC in Arctic soils when compared to others suggests that these soils respond differently to temperature changes, and also interact differently with major and trace elements. We are evaluating how these different pools of OC modifies the cycling of nutrients, such as Fe, Mn, and the solubility and speciation of contaminants, such as Hg. A discussion on the contrasting forms of OC found in soils of different climates and their role in elemental cycles will be presented.

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

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The overarching objective of this work is to develop a thermochemical model of Pu sorption to minerals and sediments that incorporates aqueous and solid phase speciation, redox reactions, and the influence of organic ligands. This has been done using a suite of techniques including variable temperature batch sorption, x-ray absorption spectroscopy, quantum mechanical modeling, and isothermal titration calorimetry.

Quantification of actinide sorption enthalpies: Ongoing experiments have demonstrated that sorption of Eu(III), Th(IV), Pu(IV), Pu(V), and Np(V) to goethite (and hematite for Eu) increases with increasing pH and increasing temperature. The data indicate sorption is endothermic and that the Gibbs free energy increases with temperature. Thus, in all cases the endothermic reaction must be balanced by a positive entropy to yield a negative Gibbs free energy ( $\Delta G = \Delta H - T\Delta S$ ). These measurements are consistent with the experimental hypothesis that removal of hydrating waters provides an entropically driven free energy of these sorption reactions. The increase in sorption as a function of temperature follows the trend, Th(IV) > U(VI) > Eu(III) > Np(V) which also corresponds to the effective ion charges and hydration energies of the given actinide oxidation states.

Examination of Pu redox speciation at solid water interfaces: Batch sorption and x-ray absorption near edge spectroscopy (XANES) starting with either Pu(III), Pu(IV), or Pu(V/VI) have demonstrated that Pu(IV) becomes the dominant oxidation state on quartz and hematite surfaces regardless of the initial oxidation state. These experiments have been coupled with quantum mechanical calculations investigating the potential role of alpha radiolysis products, such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radical (•OH), in the reduction of Pu(V/VI) to Pu(IV). Preliminary results indicate that the OH radical readily reacts with water in the first hydration sphere instead of proceeding toward the electron density of the plutonyl. We have inferred that this reaction with a hydrating water transfers a hydrogen, creating a new •OH closer to the plutonyl. Ongoing work is examining the potential role of surface catalysis.

Examination of Pu aging and mineral incorporation: Desorption of <sup>239/240</sup>Pu aged 30+ years on SRS sediment was examined and found to be essentially irreversible. A “fresh” <sup>242</sup>Pu tracer added during the desorption step did not exhibit the same behavior. Electron microscope and microprobe studies are examining the spatial distribution of Pu within these samples. The results from these experimental measurements are being compared with quantum mechanical calculations of actinide incorporation into pure mineral phases.

## **Using mercury-resistant bacteria to examine the effects of thiol complexation and thiol and Mer transporters on the microbial bioavailability of inorganic Hg and methylmercury**

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Mercury bioavailability experiments were conducted using broad spectrum mercury-resistant strains of the bacteria *Pseudomonas stutzeri* and *Escherichia coli* that are capable of converting MeHg into inorganic Hg(II) and methane, and subsequently reducing Hg(II) to elemental mercury. These strains allowed us to quantify intracellular accumulation of Hg by monitoring the loss of volatile Hg(0) over short-term (hours) incubations. We used these strains to examine the bioavailability of thiol complexes of inorganic Hg and MeHg and the roles of thiol and Hg transporters in their uptake. Thiol complexation generally lowered intracellular uptake of inorganic and MeHg, but they were still bioavailable. We measured higher levels of Hg uptake in an *E. coli* strain with Hg transport proteins (MerT, MerE, and MerP) than in the same strain in which the genes for these transporters had been knocked out. However, there was no difference in Hg uptake rates for strains with or without functioning glutathione transporters. These results indicate that thiol-bound Hg is available to mercury-resistant bacteria and its uptake is facilitated by Mer transporters, but that glutathione transporters are not involved in the uptake of the Hg(GSH)<sub>2</sub> complex in *E. coli*.

Thiol complexes of MeHg (MeHg-cysteine and MeHg-glutathione) were bioavailable in mercury-resistant strains of *P. stutzeri* and *E. coli*. As was the case for inorganic Hg, when exposed to thiol complexes of MeHg, higher rates of intracellular accumulation of MeHg were observed in an *E. coli* strain with a functioning Mer transport system than in a strain in which genes for all three Mer transport proteins had been deleted. A similar level of suppression of MeHg uptake was observed in strains in which only the periplasmic component of the Mer transport system, merP, was knocked out, indicating a critical role for this protein in modulating the transport of thiolated-Hg into the cell. We conclude that the complexes MeHgOH and MeHg-cysteine are equally bioavailable to mercury-resistant bacteria, and that Mer transporters facilitate the uptake of MeHg.

## Plutonium Immobilization and Mobilization by Soil Organic Matter

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Pu is believed to be essentially immobile due to its low solubility and high particle reactivity to mineral phase or natural organic matter (NOM). For example, in sediments collected from a region of SRS, close to a wetland and a groundwater plume,  $^{239,240}\text{Pu}$  concentrations suggest immobilization by NOM compounds, as Pu correlate with NOM contents. Micro-SXRF data indicate, however, that Pu does not correlate with Fe. However, previous studies reported Pu can be transported several kilometers in surface water systems, in the form of a colloidal organic matter carrier, through wind/water interactions. The role of NOM in both immobilizing or re-mobilizing Pu thus has been demonstrated. Our results indicate that more Pu (IV) than (V) was bound to soil colloidal organic matter (COM), amended at far-field concentrations. Contrary to expectations, the presence of NOM in the F-Area soil did not enhance Pu fixation to the organic-rich soil, when compared to the organic-poor soil or the mineral phase from the same soil source, due to the formation of COM-bound Pu. Most importantly, Pu uptake by organic-rich soil decreased with increasing pH because more NOM in the colloidal size desorbed from the particulate fraction at elevated pH, resulting in greater amounts of Pu associated with the COM fraction. This is in contrast to previous observations with low-NOM sediments or minerals, which showed increased Pu uptake with increasing pH levels. This demonstrates that despite Pu immobilization by NOM, COM can convert Pu into a more mobile form.

$^{239,240}\text{Pu}$  concentrations are positively correlated to particulate hydroxamate (a type of siderophore) and nitrogen contents. In a previous study, almost all of the mobile  $^{239,240}\text{Pu}$  in a soil sampled from near the contaminated 903 Pad in the Rocky Flats Environmental Technology Site (RFETS) was found to be associated with a macromolecule having cutin degradation products as the backbone, crosslinked to some hydrophilic moieties such as polysaccharides (to increase its solubility and thus mobility), hydroxamate, and amide functionalities (to chelate both Fe and Pu). The cuticle material from Western wheatgrass (*Agropythi Smithii*), one of the dominant vegetation species at the RFETS, was extracted, and carbohydrates removed from the crude cuticle extract. Oxidation and depolymerization experiments of cutin, incorporation of carbohydrates, siderophore compounds, as well as the chelation of Fe(III) (as a surrogate of Pu(IV)) during a simulated humification process, are assessed by  $^{13}\text{C}$  NMR, HRMAS, ATR-FTIR, etc.).

## **Collaborative Research: The Importance of Organo-Iodine and Iodate in Iodine-127,129 Speciation, Mobility, and Microbial Activity in Groundwater at DOE Sites**

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Iodine occurs in multiple oxidation states in aquatic systems in the form of organic and inorganic species (iodide and iodate). This fact leads to complex biogeochemical cycling of I and its long-lived isotope,  $^{129}\text{I}$ , a major by-product of nuclear fission. In order to assess the distribution of  $^{129}\text{I}$  and stable  $^{127}\text{I}$  in environmental systems, a sensitive and rapid method was developed which enabled us to determine isotopic ratios ( $^{129}\text{I}/^{127}\text{I}$ ) and speciate I via GC-MS and AMS. Results using this new method demonstrate that the mobility of  $^{129}\text{I}$  species greatly depends on the type of I species and its concentration, pH, and sediment redox state, with equilibration times taking up to 12 weeks. At ambient concentrations ( $\sim 10^{-7}$  M),  $\text{I}^-$  and  $\text{IO}_3^-$  are significantly retarded by sorption to mineral surfaces and covalent binding to natural organic matter (NOM), while at concentrations traditionally examined in sorption studies (i.e.,  $10^{-4}$  M or higher),  $\text{I}^-$  travels along with the water. Iodate removal can also occur through incorporation into  $\text{CaCO}_3$  crystal lattice, e.g., at the Hanford Site. Iodide and iodate interactions with NOM lead to covalent binding of I to a limited number of aromatic carbon moieties on the particle surface. Iodine association with NOM is important in sediments, even when organic carbon content is very low (e.g.,  $<0.2\%$  at Hanford Site). A small fraction of NOM that is bound to iodine can behave as a mobile organo-I source, a process that we were able to numerically simulate using kinetic Michaelis-Menton-type redox-reactions and kinetic uptake reactions. Field and laboratory studies evaluating the cause for steady increases in  $^{129}\text{I}$  concentrations (up to  $1000 \text{ pCi L}^{-1}$ , 3 orders of magnitude greater than drinking water limits of  $1 \text{ pCi L}^{-1}$   $^{129}\text{I}$ ) emanating from radiological basins at the Savannah River Site (SRS) indicate that an increase of 0.7 pH units in groundwater over 17 years may explain the observed increased groundwater  $^{129}\text{I}$  concentrations. Bacteria from a  $^{129}\text{I}$ -contaminated aerobic aquifer at SRS were found to accumulate  $\text{I}^-$  at environmentally relevant concentrations ( $10^{-7}$  M), but likely do not account for the high fraction (up to 25%) of measured organo-I in groundwater. However, we found that microbial production of peroxy-carboxylic acids and superoxide radical facilitate iodide oxidation, forming reactive species (i.e. molecular iodine, hypo-iodous acid and tri-iodine) that could readily iodinate soil organic matter. A geochemical model accounting for iodine speciation transformations, NOM partitioning, and sediment sorption in the subsurface was developed.

## **A comparison of Hg(II) uptake and accumulation between mercury methylating and non-methylating bacteria**

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Hg(II) uptake is a key first step in the methylation of Hg(II) by anaerobic bacteria and thus, conditions affecting its transport greatly influence the amount of methylmercury which accumulates. Experiments with mercury methylating and non-methylating bacteria support a common active Hg(II) uptake mechanism inhibited by Zn(II) and Cd(II) but not other divalent metals. Thus, Hg uptake appears to be a result of accidental import during the acquisition of essential trace metals, such as Zn(II). While the mechanism of uptake is similar in methylating and non-methylating bacteria, the fate of the Hg(II) following its uptake is quite different. In the Hg methylating strains, *Geobacter sulfurreducens* and *Desulfovibrio* sp. ND132, methylmercury is produced in the cytosol and rapidly exported out of the cell. Thus the accumulation of Hg(II) in methylating organisms is offset by the export of methylmercury. In contrast, *Shewanella oneidensis* does not produce methylmercury, and this is reflected in higher Hg(II) accumulation rates and yields as compared to other methylating strains. Despite the lack of methylation, however, cells of *S. oneidensis* appear to export Hg(II) possibly as a complex with a thiol such as glutathione. The export of Hg(II) or methylmercury in these different bacteria may represent metal homeostasis mechanisms to deal with the accidental uptake of toxic heavy metals such as Hg(II) at sub-toxic concentrations, where the mer-mediated Hg resistance pathway is not necessary.

## Electron Transfer and Atom Exchange Between Fe(II) and Structural Fe(III) in Clays

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Fe-bearing clay minerals serve as an important source and sink for electrons in redox reactions in various subsurface geochemical environments. Heterogeneous reactions between aqueous Fe(II) and Fe(III) oxides have been extensively studied, leading to a new conceptual framework which includes electron transfer between Fe(II) and structural Fe(III), bulk electron conduction, and Fe(II)-Fe(III)oxide atom exchange. Reactions of aqueous Fe(II) with clay minerals have received much less attention and are often described in terms of surface reactions including ion exchange, surface complexation, and/or surface precipitation. Our objectives here are to:

3. Determine if electron transfer occurs between sorbed Fe(II) and structural Fe(III) in clays over a range of conditions and clay structures.
4. Evaluate whether Fe atom exchange occurs between aqueous Fe(II) and structural Fe(III) in clays, and natural, clay-rich sediments.

We have investigated Fe electron transfer and atom exchange in clay minerals via selective chemical extractions, Fe isotope experiments and computational molecular modeling. Our findings indicate that structural Fe(III) in two nontronite clay minerals (NAu-1 and NAu-2), as well as a montmorillonite clay mineral (SWy-2) is reduced by aqueous Fe(II) and that electron transfer occurs when Fe(II) is sorbed to either basal planes and edge OH-groups of clay mineral NAu-1. Significant electron transfer occurred from edge OH-group bound Fe(II) at pH 7.5. At pH 4.0 and 6.0, where Fe(II) is sorbed primarily to the basal planes, electron transfer still occurred but to a much lower extent than from edge-bound Fe(II). Interestingly, the greatest extent of reduction was observed in the SWy-2 montmorillonite which contains the lowest % by weight Fe (2.8%). Findings from highly enriched isotope experiments suggest that between 5 and 20% Fe atom exchange is occurring between aqueous Fe(II) and structural Fe in a clay mineral. Experiments with higher precision isotope measurements using the multi-collector ICP-MS at Wisconsin confirm these findings. First principles calculations using a small polaron hopping approach suggest surprisingly fast electron mobility at room temperature in nontronite consistent with temperature dependent Mössbauer data. Electron conductivity of bulk nontronite is predicted to be highest through the octahedral sheet, as opposed to the tetrahedral sheet or from the tetrahedral to the octahedral sheet.

## **Geochemical zonation linked to microbial spatial distribution and microbe-mineral interactions in metal-immobilizing systems**

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Recent developments in microbial community sequencing and visualization techniques enable analyses of biogeochemical zonation and microbe-metal interactions at an unprecedented detail when combined with advanced geochemical analysis and redox chemistry mapping techniques. To this end, a series of SRBRs containing solid organic substrate at different ratios have been established to query the biogeochemical zonation of microbe-mineral interactions associated with zinc immobilization. Our approach, which integrates Illumina sequencing, species-specific metallo-labeling of microbial cells and detailed synchrotron and microscopic geochemical analyses, was used to establish correlations between microbial populations and geochemical reactions.

Examination of the sulfate-reducing clades in pilot-scale systems that effectively immobilized soluble Zn revealed that the genera *Desulfosporosinus* and *Desulfurispora* coincided with high Zn-removal. In contrast, systems where the major sulfate-reducers were related to *Desulfosporomusa* consistently removed less Zn. Preliminary data also suggest that a similar pattern wherein phylogenetic affiliations serve as indicators for metal immobilization may exist for key cellulose degraders, such as *Dysgonomonas* that were negatively correlated with Zn-removal in these systems.

After approximately eight months of operation, Zn-removal was situated in the upper portions of the down-flow columns followed by a trend of downstream migration after twelve months. Canonical correspondence analysis revealed that Zn-removal was positively correlated with organic substrates containing alfalfa hay, whereas the opposite was shown for columns containing woodchips. A combined approach using scanning-electron microscopy and energy dispersive X-ray spectrometry to analyse column substrates revealed preferential associations of Zn and S in those that more effectively removed Zn over a period of one year. Further investigation employing synchrotron-based X-ray absorption spectroscopy ( $\mu$ XAS) is underway in order to analyze sulfur speciation and provide 2-D redox mapping of precipitates on beamline 14-3 at the Stanford Synchrotron Radiation Lightsource.

Metallo-labeling of specific microbial genera using biotinylated 16S rRNA targeted DNA-probes, followed by incubation with a streptavidin-nanogold conjugate and subsequent gold-enhancement (Gold-FISH) has been improved by targeting multiple ribosomal sites, thus significantly increasing the signal to noise ratio without severely affecting the redox chemistry of the microbe-mineral interface. We have confirmed that our improved protocol works well for *Escherichia coli*, *Shewanella oneidensis* and *S. putrefaciens*, the latter two are all known metal-reducers and thus highly relevant in a contaminant mobility context. Efforts are currently focused on visualization of the microbe-metal interface through Gold-FISH labeling of *Shewanella* cells in the presence of manganese oxide (Birnessite) and subsequent  $\mu$ XAS analysis.

## 238U/235U as an indicator of re-oxidation of U(IV) in the subsurface of remediated sites

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At the controlled field setting at the Rifle, CO IFRC site, acetate-induced biostimulated reduction of U has been demonstrated to be accompanied by large variations in 238U/235U (discussed as  $\delta^{238}\text{U}$ ). Under both iron reducing and sulfate reducing conditions, 238U is preferentially reduced to U(IV), leaving the remaining groundwater U(VI) relatively enriched in 235U with shifts in the  $\delta^{238}\text{U}$  of  $\sim 1.3$  and  $-1.9\text{‰}$ , respectively, for a  $>90\%$  apparent U(VI) loss.

The long-term success of biostimulation as a remediation technique depends on the stability of sequestered U(IV). Uranium is readily oxidized by dissolved oxygen (DO) and other oxidants, such as nitrate,  $\text{MnO}_2$ , and ferrihydrite, posing a problem for long-term bioremediation efficacy. Because reduction of U(VI) deposits isotopically heavy U(IV), remobilization of this U(IV) has the potential to be detected when this isotopically heavy U is added back into the dissolved U(VI) pool. To assess changes in U concentration and 238U/235U accompanying re-oxidation, we present the results for two Rifle IFRC field re-oxidation experiments with dissolved oxygen (DO) and nitrate as the oxidants.

In both experiments, an oxidant (i.e., DO or nitrate) was injected into a previously biostimulated plot. Both DO and nitrate injections led to U concentration increases (up to 270 and 558 ppb, respectively), well above that of inflowing upgradient groundwater ( $\sim 175$  ppb). For the DO injection, U concentration increases were accompanied by a significant increase in  $\delta^{238}\text{U}$  from  $-0.60\text{‰}$  to  $0.00\text{‰}$ , possibly indicative of re-oxidation of isotopically heavy U(IV). However, despite the clear increase in the  $\delta^{238}\text{U}$ , re-oxidized U(IV) cannot be identified definitively as the source because the DO injection experiment began during the time the experimental plot was recovering from acetate-induced reduction (i.e., the  $\delta^{238}\text{U}$  was still isotopically lighter than upgradient groundwater). However, the nitrate injection reveals for the first time a significant increase in  $\delta^{238}\text{U}$  from  $\sim 0.00\text{‰}$  to  $0.50\text{‰}$ , well above that of upgradient water ( $0.02\text{‰}$ ). This clearly indicates re-oxidation of isotopically heavy U(IV) as hypothesized.

Our research demonstrates the potential for 238U/235U measurements to detect re-oxidation of previously sequestered isotopically heavy U(IV) in the subsurface and to distinguish between U(VI) additions from re-oxidation and other processes such as desorption. These results contribute to the development of 238U/235U as a powerful tool in the assessment of the effectiveness of U bioremediation efforts.

## Identifying Components of Toxic Metal Stress and Hg Methylation with Global Proteomics

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We study *in vivo* and *in vitro* modifications of proteins by organic and inorganic mercury compounds, specifically:

**INORGANIC Hg(II) EXPOSOME OF A NON-METHYLATING GAMMA PROTEOBACTERIUM:** The inorganic-Hg(II) exposome of the model bacterium *E. coli*, reveals a large decrease in modifiable protein cysteines as measured by iodoacetamide (IAM)-induced carboxyamidomethyl (CAM)-modifications (also seen previously in organo-Hg exposure). Surprisingly, few proteins had detectable Hg(II)-adducts and all that did had at least two cysteines in the same peptide, affording strong chelation of Hg(II). Hg(II) exposure produced more unmodified cysteines than PMA exposure, suggesting biologically-formed Hg(II)-adducts may have been lost during MS analysis. Regardless of whether this differential observation of organic- and inorganic-Hg adducts occurs biologically or during analysis, it reflects a chemical difference in these ligands with potential biological implications. We're pursuing this question in well defined pure proteins and peptides (below).

**INORGANIC Hg(II) EXPOSOME OF A METHYLATING SULFATE-REDUCING BACTERIUM:** *Desulfovibrio* ND132 growing facultatively with or without Hg(II) exhibited 54% of 3455 encoded proteins. Unlike the *E. coli* observations, we saw no change in (CAM)-modifications or unmodified protein cysteines with or without Hg(II). Remarkably, we observed no Hg(II)-modified proteins with or without IAM, perhaps because some of the 1 mM cysteine in the medium was metabolized to H<sub>2</sub>S, thus sequestering Hg(II) as insoluble HgS. Since ND132 methylates Hg(II) under these conditions, HgS may be the direct substrate for HgcAB or unidentified proteins may transfer Hg(II) from HgS to HgcAB. Possible candidate metallochaperones are being screened genetically.

**LC-MS/MS OBSERVATION OF RHg- VERSUS Hg(II)-MODIFICATIONS:** Differences in proteomic detection of Hg- and RHg-adducts could arise from differences in formation during cell exposure or in stability to LC-MS/MS processing. Such differences could underlie documented differential toxicity and bioaccumulation of these compounds, impacting remediation and stewardship. We found a pure synthetic peptide PMA-adduct stable over a range of MS inlet temperatures and voltages but its signal was 10-fold less than the unmodified peptide. With two well defined cysteine-rich proteins [the secreted enzyme lysozyme, (8 cysteines as disulfides) and the metalloregulator MerR, (8 cysteines as thiols)], we found that: (a) the most readily reduced lysozyme disulfides are highly modified by PMA or Hg(II); (b) either modification occurs less than CAM-adducts in the same peptide; and (c) PMA yields more adducts than Hg(II). These simple systems mimic proteomic results and will aid in defining the differential detectability/stability of organic and inorganic Hg-protein adducts.

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

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Traditional kinetic and thermodynamic models incorporating microbial processes are typically developed using data from batch reactors in nutrient rich media characterized by rapid microbial growth, high respiration rates, and high cell densities. In the subsurface, however, microbial communities survive under energy and nutrient limiting conditions resulting in slow to zero growth conditions that cannot be reproduced by conventional kinetics. In this project, new rate laws able to predict microbial respiration in natural conditions are developed using two approaches. First, microbial growth under bioenergetic limitations is simulated using a novel flow-through bioreactor designed to include a retention filtration unit (retentostat) and accumulate biomass over time. Maintenance energy requirements for dissimilatory reduction of nitrate, a common groundwater contaminant, were determined by running the retentostat under limited conditions with lactate as electron donor. Initial rates of ammonium production by *Shewanella oneidensis* as model microorganism were similar in the bioreactor and otherwise identical incubations conducted in batch reactors. ATP measurements, however, showed slow yet increasing microbial growth over time in the retentostat while cells in the batch reactors did not grow significantly and died within 2 weeks of inoculation. In a second approach, rates of enzymatic U(VI) reduction were measured under variable, but controlled, geochemical conditions to determine the effect of uranyl speciation on bioreduction rates by *S. putrefaciens* as model metal-reducing microorganism. High pH, DIC, Ca<sup>2+</sup>, and Mg<sup>2+</sup> suppressed the formation of labile U(VI) complexes and retarded uranium bioreduction. These results indicate that the main reducible fraction of U(VI) consists of non-carbonate complexes despite being the least abundant species in solution. At elevated concentrations of bioavailable uranyl species, however, bioreduction was inhibited by the toxicity of uranium to cells. Viability assays confirmed that the 'free' uranyl ion is responsible for uranium toxicity to *S. putrefaciens*, in agreement with the biotic ligand model of metal toxicity. A U(VI) bioreduction rate law that accounts for the speciation of U(VI) species is able to reproduce bioreduction rates in all pH, carbonate, calcium, and magnesium conditions. Future retentostat experiments will be designed to examine the thermodynamic controls on the bioreduction of non-carbonate U(VI) species in the presence of alternative electron acceptors. The kinetic and thermodynamic parameters determined in the current study will be directly applicable in reactive transport models to predict the rates of microbial respiration in both natural and contaminated subsurfaces.

## **Survival During Long Term Starvation: Global Proteomics Analysis of *Geobacter sulfurreducens* Under Prolong Electron Acceptor and Donor Limitation**

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The dissimilatory iron-reducing anaerobe *Geobacter sulfurreducens* is used as a model organism for understanding bioremediation at contaminated subsurface sites. In our studies, *G. sulfurreducens* has been shown to exhibit a GASP phenotype in the laboratory, and to revive from long-term starvation (>500 days) when inoculated into fresh medium. Upon revival, the cells are capable of reducing Fe(III) in Fe citrate. These studies have been aimed at elucidating the mechanisms employed by *G. sulfurreducens* for such long-term survival, under both electron donor and terminal electron acceptor (TEA) conditions, the latter of which could exist after prolonged carbon addition at bioremediation sites.

To investigate potential changes in protein/enzyme expression under nutrient-limited conditions, global comparative proteomics analyses were carried out for *G. sulfurreducens* cultures in mid-log and survival phases. Cultures were grown under both acetate (electron donor)-limited and fumarate (TEA)-limited conditions, and proteins were harvested at mid-log and long-term survival time points. The proteins were analyzed by LC/MS-based iTRAQ methods, and those peptides which exhibited changes in abundance between mid-log and survival phases at a significance level  $p < 0.05$  were further analyzed to determine function and cellular location. Under both electron donor- and TEA-limited conditions, many of the proteins up-regulated in survival phase vs. mid-log phase were involved in energy metabolism, and were membrane-associated. In contrast, many proteins involved in anabolic processes were down-regulated under nutrient-limited conditions. Other up-regulated proteins included those involved in protein transport, indicating that the cells are poised to sense nutrients in the environment as they become available.

Changes in acetate, fumarate, and succinate were monitored by HPLC under both acetate (electron donor)-limited and fumarate (TEA)-limited conditions to document points at which the cultures became nutrient-limited. Under acetate-limited conditions (5.5 mM acetate/30 mM fumarate), acetate was completely depleted within 3 days, while fumarate depleted rapidly within 3 days to ~12 mM, then continued to decrease gradually over ~75 days, with concomitant accumulation of succinate to ~25 mM. Under fumarate-limited conditions (30 mM acetate/50 mM fumarate), fumarate was depleted by day 7, during which time succinate concentrations increased then leveled off at ~50 mM, while acetate concentrations decreased to ~15 mM by day 3 and did not change thereafter. These results indicate that when the cells are electron-donor (acetate) limited, they may utilize other, less favorable intracellular or environmental electron donors as necessary. However, when TEA-limited, the cells survive in a highly reduced state until new electron acceptors become available.

## **Microbiological-enhanced mixing across scales during in-situ bioreduction of metals and radionuclides at Department of Energy Sites**

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Bioremediation is being investigated for long-term management of DOE sites contaminated by metals and radionuclides. Bioremediation typically requires injection of chemicals into the subsurface which mix at varying scales with the contaminant to stimulate the growth of dissimilatory metal reducing bacteria (DMRB). These bacteria couple the oxidation of injected chemicals to the reduction of contaminants as they mix in the groundwater. Syntrophic interactions with other bacterial species may also be exploited to supply DMRB with higher quality electron donors such as H<sub>2</sub> that are otherwise difficult to deliver to bacteria. Evidence from DOE field experiments suggests that mixing limitations of substrates at all scales may affect biological growth and activity for reduction. We are investigating this phenomenon using batch and microfluidic experiments and mathematical modeling.

In order to elaborate and investigate the energy transfer from an obligate symbiont, syntroph, to a partnering metal-reducing organism, we set up a two-species culture of *Syntrophobacter wolinii* DB and *Geobacter sulfurreducens*. Two strains of *Geobacter sulfurreducens* were successful partners: PCA (type strain) and KN400 (strain producing more nanowires than average). In contrast, a *G. sulfurreducens* PCA hydrogenase mutant could not serve as a partner. Therefore, hydrogen likely served as an electron shuttle between *S. wolinii* DB and *G. sulfurreducens*.

We successfully fabricated nanofluidic reactors designed to investigate the ability of bacterially produced conductive pili, or 'nanowires', to enhance the zone of mixing beyond what is possible by advection and dispersion alone. Our microfluidic experiments have identified realistic flow parameters and growth conditions amenable for growth and attachment inside these reactors that will be used in nanofluidic experiments.

A microfluidic reactor with an idealized porous medium geometry was used to study the metal reduction and biofilm grown at the pore scale. Selenite (a representative metal and an electron acceptor) and propionate (an electron donor) were delivered through the inlets and mixed along the centerline by transverse diffusion. Selenite was biologically converted to three different precipitates (monoclinic selenium, trigonal selenium, and selenium sulfide), and were segregated in the mixing zone. A continuum-scale model was developed and explained the segregation.

Our future work focuses on evaluating electron transfer across a nanoporous barrier using the two-species syntroph / *Geobacter* culture in order to elucidate electron transfer mechanisms, and on modeling this process in more realistic pore-scale geometries. We are also developing a hybrid multi-scale modeling framework to allow accurate representation of localized biofilm reactions in field-scale models.

## Long-term uranium sequestration as nitrate invades a previously reduced zone - DOE Oak Ridge IFRC site

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W.-M. Wu, C. Criddle- Stanford University, D. Watson, T. Mehlhorn, K.Lowe, J. Phillips, J. Earles, G.-P. Tang- Oak Ridge National Laboratory, B. Li, Y.-Q. Chao, T. Zhang- The University of Hong Kong, D. H. Phillips- Queen's University of Belfast, S.D. Kelly-EXAFS Analysis, P.-S.Li, H.-C.Tao-Peking University Shenzhen Graduate School, Z.-B. Chen- Dalian Nationalities University. A long term pilot-scale study of in-situ U(VI) sequestration was conducted at a site at the U.S. DOE Y-12 National Security Complex, Oak Ridge, TN. The contaminated sediments contained up to 5-6% of iron and 700-1000 mg U/kg dry weight with groundwater U of ~20-40 mg/L. U concentrations lower than the US EPA MCL (0.03 mg/L) were achieved through bioreduction treatments using intermittent injection of ethanol. Since the end of the bioreduction study, we have examined the long-term effect of exposure of the bioreduced sediments to nitrate from the natural influx of contaminated groundwater for > 1,500 days. Vertical and horizontal spatial differences in the increase (recovery) of U concentration and biogeochemical response to the influx of groundwater were observed. In general, the nitrate concentrations in the previously bioreduced area increased gradually from near zero to ~50-300 mM and then stabilized. The pH declined from the bioreduced levels of 6.2-6.7 to below 5.0. U concentrations in the bioreduced zone typically rebounded, declined and then rebounded again. The U(IV) in sediments was reoxidized to U(VI) species according to XANES analysis but the Uranium content in the sediment remained as high as previously identified. SEM-EDX analysis of reoxidized sediment samples revealed clusters of U containing carbonate precipitates (~1-2% U, w/w) that also contain high amounts of Fe, Al and Si, indicating that at least some U was still sequestered in-situ after the reintroduction of contaminated oxic groundwater. Significant levels (>100000 ppmv) of N<sub>2</sub>O was found in groundwater after reoxidation. The impact of geochemical change on subsurface microbiology was investigated by 454 pyrosequencing analysis of the sediment samples. Significant community shifts occurred after the re-oxidation. The bioreduced communities dominated by sulfate-reducing (*Desulfovibrio*) and iron reducing (*Geobacter* and *Geothrix*) bacteria shifted to denitrifying *Rhodanobacter*, iron(II) oxidizing *Phizomicrobium* as well as denitrifying *Castellaniella* under more oxic conditions. However, the U(VI)-reducing, spore-forming sulfate reducing bacteria, *Desulfosporosinus* remained abundant under the oxic conditions. These observations indicate significant U sequestration continued after the oxidative agent nitrate invaded the bioreduced area.

## Hg(0) oxidation by Anaerobic Bacteria

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Background and Objectives: Redox cycling between elemental [Hg(0)] and divalent [Hg(II)] mercury is a key control on the fate and transport of Hg in groundwater systems. Whereas dissolved gaseous elemental mercury [Hg(0)] is mobile in groundwater, while oxidized mercuric mercury [Hg(II)] readily sorbs onto mineral surfaces and organic matter. The objectives of this study were: 1) to determine if anaerobic bacteria catalyzes the oxidization of Hg(0) to Hg(II); and 2) to determine anaerobic Hg-methylating bacteria produce MeHg when provided with Hg(0) as the sole Hg source.

Research Methods: Hg(0) oxidation experiments were carried out with the obligate anaerobic bacteria *Geothrix fermentans* H5 and *Desulfovibrio desulfuricans* ND132 and the facultative anaerobic bacteria *Shewanella oneidensis* MR-1 and *Cupriavidus metallidurans* AE104. To demonstrate the formation of Hg(II), we performed ethylation experiments and X-ray absorption near edge structure (XANES) spectroscopy on Hg(0)-reacted cell. Finally, samples from experiments conducted with the methylating bacterium strain ND132 were analyzed for the production of MeHg.

Results: All four bacterial strains reacted with dissolved gaseous Hg(0) to form non-purgeable Hg. Derivatization of non-purgeable Hg to diethylmercury and the Hg LIII-edge position of the XANES spectra demonstrated that the Hg(0)-reacted bacterial samples had formed oxidized Hg(II). XANES analysis also revealed that cell-associated Hg(II) was covalently bound to bacterial functional groups, most likely to thiol moieties. Experiments with metabolically active and heat-inactivated cells indicated that both live and dead cells oxidized Hg(0) to Hg(II). MeHg analyses showed that live cells of *D. desulfuricans* ND132 produced large quantities of methylmercury. The results of this work demonstrate a potentially important pathway in the mercury cycle, whereby anaerobic bacteria produce MeHg when provided with dissolved Hg(0) as their sole Hg source.