The Subsurface Biogeochemical Research and Terrestrial Ecosystem Science programs within the Department of Energy’s Office of Biological and Environmental Research (BER) held their first annual joint investigators meeting May 14-15, 2013, in Potomac, Maryland, just north of Washington, D.C. Funded projects provided abstracts and posters summarizing their progress to date.

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NAME: Estella Atekwana
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ABSTRACT TITLE: Induced Polarization Signature of Biofilms in Porous Media: From Laboratory Experiments to Theoretical Developments and Validation

ABSTRACT: There is a need for appropriate techniques that can monitor the growth of microbial communities in situ and the coupled biogeochemical and biogeophysical processes. Spectral Induced Polarization (SIP), a biogeophysical technique, can play a key role. However, the triggering mechanisms of SIP responses modulation by biogeochemical activity of microbial communities (biofilm) remain poorly understood. The overarching goal of this project is to find the correlations between biogeochemical processes occurring within subsurface microbial communities and spectral induced polarization (SIP) signatures in aqueous and/or porous media. Anticipated findings will allow for the interpretation of the geophysical responses associated with the development of microbial communities in field conditions.

We investigated the SIP response of biofilm components including cells, exopolysaccharides (EPS), cells in EPS and semi-conductive particulate matter such as magnetite in EPS. Using microbial cells from three different model organisms in liquid suspension and in porous media, we observed an increase in SIP parameters with increasing cell density with a very well defined relaxation peak at a frequency of ~10 Hz which was predicted by recently developed quantitative models. However this characteristic relaxation peak was minimized in the presence of porous media. We also observed that cells suspended in EPS enhances the polarization and also shows a peak frequency at ~10 Hz.

The study of alginate gelation (representing EPS) in liquid phase and porous media in vitro revealed that solidified (gelated) alginate isolated from brown algae increased the magnitude of imaginary conductivity while real conductivity increased very moderately. In contrast, the study of the SIP response within a porous medium filled with solidified gel of alginate isolated from mucoid strain P. aeruginosa FRD-1 showed an increase in the magnitude of both imaginary and real conductivities. Further, the addition of magnetite particulates in the EPS enhanced the gelation process and the magnitude of the SIP response.

We also continued with the development of our quantitative model to investigate frequency-domain induced polarization response of suspensions of bacteria and bacteria growth in porous media. Our model results show that the growth rate and endogenous decay coefficients of bacteria in porous sand can be inferred non-intrusively from time-lapse frequency-domain induced polarization data.

Conclusions: 1) the time-lapse SIP data are applicable for the evaluation of growth rate and endogenous decay of bacteria in porous media. This finding helps to enhance the interpretation of SIP measurements from field sites; 2) SIP parameters are sensitive to the transition of an alginate from liquid to solidified stage; 3) the magnitude of SIP signatures reflects both cell density, biofilm maturity and the presence of secondary minerals; and 4) bacteria may have a characteristic low frequency relaxation peak at 10 Hz.
Our next goals include but not limited to the study of the modulation of SIP responses by field-relevant organisms *Geobacter sulfurreducens* along with its ΔpilA mutant, and *Disulfovibrio vulgaris*. Anticipated results will be useful for the development of a mechanistic model for the induced polarization response.
ABSTRACT: The subsurface is a massive reservoir of carbon compounds and hosts a large fraction of the microbial life on earth. Despite this, relatively little is known about sediment communities and their roles in carbon, nitrogen, and hydrogen cycling in the subsurface. We metagenomically sequenced unstimulated (background) sediment samples, as well as acetate stimulated sediment and planktonic communities from Rifle, CO to document microbial composition and metabolic potential. Background aquifer sediments are highly diverse; no single organism constitutes more than 1% of the community. Many of the lineages in sediment are novel, at least fifteen at the phylum level. We reconstructed a complete genome from the dominant background sediment organism, RBG-1, a representative of a previously undescribed bacterial phylum. This organism is metabolically versatile, with a large complement of respiratory machinery that likely enables dominance in sediments subjected to fluctuating redox conditions. Chloroflexi are surprisingly diverse and relatively abundant in background sediments. We reconstructed draft genomes for genomically unsampled members of the Chloroflexi, some of which are capable of carbon fixation and other biogeochemical processes. Archaea are also relatively abundant in background sediment. Among the archaea genomically characterized, we recovered a few Thaumarchaeota, and a vast clade of novel Halobacteriales.

We documented very different sediment-associated and planktonic communities, both with and without acetate amendment. From acetate-stimulated sediment, we recovered nearly 100 high quality complete and draft genomes and reconstructed organism abundance patterns within the geochemical context. The results indicate the importance of Bacteroidetes, Betaproteobacteria, and bacteria from phyla lacking (e.g., SR1, OP11, OD1, WWE3) or with very few (e.g., Ignavibacteria, Elusimicrobia) cultivated representatives. Metagenomic sequencing of acetate-stimulated groundwater filtered through a 0.2 µm filter revealed the predominance of bacteria from OP11, OD1, and WWE3 on the 0.1 µm filter. Cryo-TEM characterization demonstrated cell sizes below the theoretical minimum size for life. We also reconstructed over 1,000 complete phage genomes. In a majority of cases phage can be linked to their hosts via CRISPR spacer targeting. The planktonic community composition shifts during the biostimulation process. Notably, genomes were reconstructed for TM6 lineage bacteria from samples on the 0.2 µm filter were collected only late in biostimulation. From the same experiment, methanogens were notable by their near-absence, despite evidence for methane production.
ABSTRACT: Background: The methylation of inorganic Hg is known to be mediated by microorganisms under anoxic conditions. Previous studies have elucidated the chemical species of mercuric Hg [Hg(II)] that are bioavailable to methylating microbes. The reduced form of mercury, elemental Hg [Hg(0)], is generally considered chemically inert and unavailable for biologic uptake by anaerobic bacteria. However, the oxidation and subsequent methylation of Hg(0) by anaerobic bacteria have never been tested. In this project, we are conducting laboratory experiments to examine the anaerobic oxidation of Hg(0) and the effect of microbial Hg(0) oxidation on Hg stable isotope fractionation and MeHg production.

Hypotheses:
1: In anoxic environments, anaerobic bacteria catalyze the oxidization of Hg(0) to Hg(II).
2: Microbial oxidation of Hg(0) to Hg(II) imparts a mass-dependent fractionation of Hg stable isotopes.
3: Anaerobic Hg-methylating bacteria produce MeHg when provided with Hg(0) as the sole Hg source.

Results: We examined the Hg(0) oxidation activity of the obligate anaerobic bacteria Desulfovibrio desulfuricans ND132 (Deltaproteobacterium) and Geothrix fermentans H5 (Acidobacterium), and the facultative anaerobic bacteria Shewanella oneidensis MR-1 (Gammaproteobacterium) and Ralstonia metallidurans AE104 (Betaproteobacterium). Anoxic cultures were exposed to Hg(0) in the dark, and samples were collected and analyzed for the formation of non-purgeable Hg. We found that all four bacterial strains produced non-purgeable Hg from dissolved Hg(0) under anoxic conditions. Derivatization of the non-purgeable Hg in the cell suspensions to diethylmercury and analysis of Hg(0)-reacted bacterial cells using X-ray absorption near edge structure (XANES) spectroscopy demonstrated that cell-associated Hg was dominantly in the oxidized Hg(II) form.

The fractionation of Hg stable isotopes by microbial Hg(0) oxidation was investigated using multi-collector inductively coupled mass spectrometry. Oxidation of Hg(0) to Hg(II) by D. desulfuricans ND132 resulted in mass-dependent fractionation of Hg stable isotopes.

The production of methylmercury by D. desulfuricans ND132 was determined by distillation and ethylation-gas chromatography. When exposed to a constant source of Hg(0), D. desulfuricans ND132 produced up to 118 μg/L of methylmercury after 36 h of incubation. A major fraction of the methylated Hg was exported out of the cell and released into the culture medium. These results indicate that anaerobic Hg-methylating bacteria can catalyze the oxidization of Hg(0) to Hg(II), and produce MeHg when provided with Hg(0) as the sole Hg source.
NAME: Joel Blum  
ORGANIZATION: University of Michigan  
PROGRAM AFFILIATION: SBR  
ABSTRACT TITLE: Isotopic Characterization of Biogeochemical Pools of Mercury and Determination of Reaction Pathways for Mercury Methylation

ABSTRACT: This project utilizes stable Hg isotopes to reveal locations of Hg methylation in the East Fork Poplar Creek (EFPC) watershed, TN and to place constraints on processes that lead to methylmercury (MeHg) production, transport and degradation. During the first 16 months of the project we: 1) completed three sampling campaigns (fall, spring, and summer) in which water, sediment, and periphyton from EFPC, Hinds Creek and adjacent wetlands were collected, 2) developed protocols and collected samples of dissolved gaseous mercury (DGM) from EFPC stream water, 3) obtained samples of fish tissues from the ORNL Ecological Assessment Group, 4) obtained samples of Clinch River and EFPC sediments from TN Dept. of Environ and Conservation, 5) completed Hg and MeHg concentration analyses and Hg isotopic analyses of sediments, suspended particulates, surface waters, pore waters, and periphyton from one entire field sampling campaign, as well as fish tissues, and 6) conducted photochemical and dark oxidation experiments with EFPC water and model ligands to investigate Hg isotope fractionation.

Sediments downstream of Y-12 in EFPC had elevated THg concentrations (3.2 to 60 ug/g) compared to background sediments, and average δ²⁰²Hg of 0.11 ± 0.11‰ and Δ¹⁹⁹Hg of -0.08 ± 0.03‰ (mean ± 1s.d.; n=6). The THg concentration of sediments downstream in Poplar Creek (PC) and the Clinch River remains elevated (0.24 to 3.9 ug/g) and δ²⁰²Hg has a range between -0.82‰ and 0.07‰. Sediments in the Clinch River and Hinds Creek upstream of the confluence with PC have THg concentrations between 0.01 and 0.05 ug/g and the Hg isotopic composition is distinct from downstream sites (δ²⁰²Hg of -1.40 ± 0.06‰ and Δ¹⁹⁹Hg of -0.25 ± 0.03‰). The results demonstrate that Y-12 impacted sediments have a unique Hg isotopic composition that persists in EFPC and can be traced downstream to PC and the Clinch River.

Mercury isotopic composition within the EFPC stream corridor changes with distance from the Y-12 complex to its confluence with PC. Stream water concentrations of dissolved total mercury (THg) decreased from 66 ng/L at Station 8 within the Y-12 complex, to 17 ng/L near the confluence with PC, δ²⁰²Hg decreased from -0.11‰ to -0.19‰, and Δ¹⁹⁹Hg decreased from 0.06‰ to -0.06‰. Isotopic composition of suspended sediment also shifts from the Y-12 complex (δ²⁰²Hg = -0.60‰, Δ¹⁹⁹Hg = 0.02) to downstream reaches (δ²⁰²Hg = 0.07‰, Δ¹⁹⁹Hg = -0.12‰). The isotopic composition of DGM ranged from δ²⁰²Hg values of 0.97‰ to 1.10‰ and Δ¹⁹⁹Hg values of -1.52‰ to -0.29‰, differing significantly from that of stream water, suspended particulate matter, and sediments. Changes in mercury concentration and isotopic composition along the flow path within the EFPC can be interpreted within the context of isotopic data from laboratory Hg(0) photochemical and dark oxidation experiments.

We used fish mercury isotope composition and %MeHg to estimate the isotopic composition of the sources of inorganic Hg (IHg) and MeHg accumulating in fish. In the heavily IHg contaminated EFPC, we estimated
ABSTRACT: Hexavalent chromium (Cr$^{6+}$) is a major contaminant in the environment. As a redox-sensitive element, the fate and toxicity of chromium is controlled by reduction-oxidation (redox) reactions. Previous research has demonstrated the ability of chemically reduced smectite and aqueous Fe$^{2+}$ to reduce Cr$^{6+}$ to Cr(III) as a way of immobilization under natural conditions. However, it is still poorly known whether or not natural or biologically reduced clay minerals exhibit similar reactivity and if so, what are the kinetics and mechanisms of Cr$^{6+}$ reduction by clay-Fe(II). The objective of this study was to determine the kinetics of Cr$^{6+}$ reduction by Fe(II) associated with various common clay minerals and the nature of reduced Cr(III). The reactivity of clay minerals nontronite (NAu-2), montmorillonite (Swy-2), chlorite (CCA-2), and clay-rich sediments from the Ringold Formation of the Hanford site of Washington State towards reduction of Cr$^{6+}$ was studied. Structural Fe(III) in the clay minerals was first bioreduced to Fe(II) by Geobacter sulfurreducens with acetate as the sole electron donor and anthraquinone-2,6-disulfate (AQDS) as electron shuttle in select experiments in synthetic groundwater (pH-7). Biogenic Fe(II) was then used to reduce Cr$^{6+}$ at three different temperatures, 10°, 20° and 30°C, in order to determine the activation energy of the redox reaction between Cr$^{6+}$ and clay-Fe(II). The results showed that nontronite was the most effective in reducing Cr$^{6+}$ at all temperatures and chlorite the least. Despite a large amount of Fe(III) bioreduction in chlorite, most bio-produced Fe(II) was not reactive towards Cr$^{6+}$ reduction at 10°C, however at 30°C there was some reduction, which was likely due to released Fe$^{2+}$ from the dissolution of chlorite. For all the clay minerals, the ratio of Fe(II) oxidized to Cr$^{6+}$ reduced was close to the expected stoichiometric value of 3±0.5. Scanning electron microscopy with focused ion beam and transmission electron microscopy with electron energy loss spectroscopy (TEM-EELS) characterization revealed that reduced chromium was Cr(III) and it was closely associated with residual nontronite NAu-2 as micron-scale aggregates. This unique textural association was expected to minimize any chance of Cr(III) reoxidation upon exposure to oxidants. These kinetic results are important for our understanding of how various clay minerals may be used to reductively immobilize heavy metal contaminants such as Cr, Tc, or U from the environment.

We have also been studying the reactivity of clay-Fe(III) towards the oxidation of uraninite. These experiments have focused on the comparing the reactivity of biogenic clay-Fe(II) versus chemically reduced clay-Fe(II). Results obtained with nontronite NAu-2 suggest that only 25 to 35% of the structural clay-Fe(III) can be reduced by a variety of oxidants (including DMRB, quinones, and uraninite). Experiments are now being conducted with nontronite NAu-2, DMRB and nitroaromatic compounds (NACs) to operationally define this thermodynamic endpoint.
ABSTRACT: 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 about a dozen mineral phases that present different cation sites that are incorporation targets. 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 and quantum mechanical models to bear on the study of the local environments of incorporation.
NAME: Elizabeth Butler
ORGANIZATION: University of Oklahoma
PROGRAM AFFILIATION: SBR
ABSTRACT TITLE: Stability of Iron-Chromium Precipitates for Long Term In-Situ Chromium Immobilization

ABSTRACT: The objective of this research was to identify factors affecting the properties of Fe-Cr precipitates formed in microbial and abiotic anoxic systems. Specific objectives were to: (1) measure the rates of Cr(VI) reduction by Fe(II) minerals and microorganisms; (2) characterize the morphology, crystallinity, and composition of the formed Fe-Cr precipitates; and (3) measure oxidation rates of dissolved Cr(III) in equilibrium with these precipitates by birnessite.

Microbially-active microcosms contained hematite, aluminum substituted goethite (Al-goethite), or iron rich nontronite (NAu-2), and Desulfovibrio vulgaris strain RCH1 (Chakraborty R., http://www.ncbi.nlm.nih.gov/nuccore/CP002297.1) (RCH1). Microcosms containing hematite were prepared with a low and high dilution of the culture to yield faster (hematite/RCH1/fast) or slower (hematite/RCH1/slow) Cr(VI) removal. Abiotic microcosms contained either FeS or dithionite reduced NAu-2. Dissolved Cr(VI) was removed relatively rapidly in all microcosms, with the fastest Cr(VI) disappearance for FeS (30 minutes), and the slowest for hematite/RCH1/slow (one month). Thus, in all systems, Cr(VI) was removed from solution in the time scale of ground water transport.

After the kinetic experiments, the solids were retrieved, washed, and dried. X-ray absorption spectra indicated the presence of mixed Fe(III)-Cr(III) hydroxide phases for all solids. Although the majority of solid phase Cr was in the +III oxidation state, solid phase Cr(VI) was also identified at a relative concentration of 21-25% for RCH1/hematite (slow and fast) and RCH1/Al-goethite, 10-11% for RCH1/NAu-2 and dithionite reduced NAu-2, and 4% for FeS. Transmission electron microscopy (TEM) and scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDS) were done to further characterize the solids. The most pronounced morphological changes after reacting with Cr(VI) were observed for RCH1/hematite/fast and FeS. TEM images of RCH1/hematite/fast after reaction with Cr(VI) show jagged edges and pitting, as well as development of a very thin surface layer, suggesting dissolution of hematite and formation of a new phase. TEM and SEM images showed a possible organic film in which nanoparticles with the highest Cr:Fe ratio (approximately 1) were observed. TEM analysis of FeS after reacting with Cr(VI) indicated a poorly crystalline, essentially amorphous material with individual grains difficult to distinguish at the nanoscale. SEM/EDS of this material showed elevated Cr, increased O, and decreased S, suggesting formation of a Cr-Fe hydroxide.

Finally, each solid was equilibrated in the presence of an aqueous suspension of birnessite (along with controls containing no birnessite) for approximately two weeks. We speculated that dissolved Cr(III) in equilibrium with each Fe-Cr solid might be oxidized by the birnessite to Cr(VI), driving further Cr(III) dissolution and oxidation. Solid phase Cr(VI), present at significant quantities in some solids, could also dissolve, regardless of whether birnessite was present. All solids from the microbially-active microcosms showed only minimal release of Cr(VI), and no significant difference in dissolved Cr(VI) concentration in the presence of birnessite compared to the control. FeS and dithionite-reduced NAu-2, on the other hand, showed a steady increase in concentration of Cr(VI) over time, suggesting that these precipitates may be susceptible to re-mobilization of Cr in the subsurface.
NAME: Andres Campiglia
ORGANIZATION: Department of Chemistry, University of Central Florida
PROGRAM AFFILIATION: SBR
ABSTRACT TITLE: Field-Deployable Nanosensing Approach for Real-time Detection of Free Mercury

ABSTRACT: 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. During the past year of the project, our efforts were directed towards: (a) the development of an optically transparent nanorod-modified electrode for measurement of mercury using surface plasmon resonance (SPR) with electrochemical reduction of Hg species; and (b) the design and set-up of a portable spectrometer (prototype) for field measurements of mercury. A systematic approach was developed for the immobilization of gold nanorods (Au NR) on Indium Tin Oxide (ITO)-coated glass cover slides via covalent binding through (3-mercaptopropyl)trimethoxysilane (MPTMS). Experimental parameters were optimized for appropriate MPTMS surface coverage, CTAB removal, and ionic strength conditions that provide reproducible immobilization of commercially-available Au NRs on ITO surfaces. An application of potential controlled SPR sensing is demonstrated with Hg-Au amalgam formation at the NR surface, where HgCl₂ is reduced with applied potential at the Au NR surface, resulting in a characteristic change in SPR spectrum. The original version of the portable spectrometer was significantly improved with the implementation of numerous modifications. These included newly designed sensor head (or sensor box) and input box for fiber optic bundles. Instrument control is now possible with the aid of a laptop. An in-house LabView data acquisition program facilitates computer control of the water flow, real-time spectral acquisition, correlation of maximum wavelength shifts to mercury concentration and instrument calibration.
**NAME:** Jeffrey Catalano  
**ORGANIZATION:** Washington University in St. Louis  
**PROGRAM AFFILIATION:** SBR  
**ABSTRACT TITLE:** Dominant Mechanisms of Uranium-Phosphate Reactions in Subsurface Sediments

**ABSTRACT:** Phosphate addition is an in situ remediation approach being considered by the DOE 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 in treated systems. 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 identify the U(VI)-phosphate precipitates that form from homogeneous solutions, characterize the cooperative and competitive effects of phosphate on U(VI) adsorption and precipitation on smectite and iron oxide minerals, and then investigate how phosphate affects U(VI) speciation and fate in sediments from the Rifle and Hanford sites.

Batch experiments were performed to determine the products of U(VI)-phosphate precipitation in homogeneous systems and the resulting equilibrium solubility of those solids as a function of groundwater composition. In the absence of groundwater cations (e.g., Na⁺ and Ca²⁺) metastable chernikovite \([H_2O(UO_2)(PO_4)·3H_2O]\) forms instead of the thermodynamically stable uranyl orthophosphate \([(UO_2)_3(PO_4)·4H_2O]\). Sodium and calcium addition affect U(VI)-phosphate precipitation, with the former inducing the precipitation of Na-autunite \([Na(UO_2)·(PO_4)·3H_2O]\) whereas calcium causes U(VI) removal through both autunite \([Ca(UO_2)_2(PO_4)·11H_2O]\) precipitation and adsorption onto or incorporation into calcium phosphates.

Separate batch experiments have been conducted to investigate how phosphate affects U(VI) adsorption and precipitation in the presence of montmorillonite and goethite. For both systems, supersaturation is required before the nucleation and precipitation of Na-autunite initiates. The critical supersaturation needed for Na-autunite precipitation on goethite was twice that for montmorillonite. Before the onset of precipitation, U(VI) binding is through adsorption processes. Phosphate has a negligible effect on macroscopic U(VI) adsorption to montmorillonite at pH 4, 6, and 8 despite evidence from EXAFS and TRLFS spectroscopies for ternary complexation. In contrast, phosphate enhances U(VI) adsorption to goethite at pH 4 but suppresses adsorption at pH 8; the former is attributed to U(VI)-phosphate ternary complexation and the latter to surface site competition between U(VI) and phosphate.

Our efforts are currently transitioning to batch and column studies of U(VI)-phosphate reactions in sediments from the Rifle site in Colorado. Batch studies in artificial Rifle groundwater show negligible U(VI) sorption except above \(10^{-5}\) M initial U(VI). The addition of phosphate reduces U(VI) sorption. Studies of U(VI) speciation and the origin of this inhibitory effect are ongoing. Column experiments have also been initiated to examine the influence of phosphate on U(VI) transport, again in artificial groundwater. Following uranium loading, half of the columns then received artificial groundwater amended with phosphate and the other half received phosphate-free solutions. Flow rates were set to provide environmentally relevant seepage velocities. Periodic stopped flow events are being performed.
to assess the effects of intraparticle diffusive transport on overall U(VI) mobility. After reaction, columns will be sampled along their length and characterized to determine U speciation and micro-scale spatial distribution. Similar studies are planned for sediments from the Hanford site.
NAME: Clara Chan  
ORGANIZATION: University of Delaware  
PROGRAM AFFILIATION: SBR  
ABSTRACT TITLE: Fe-oxidizing microorganisms in microscopic model aquifer systems: toward understanding post-biostimulation permeability reduction and oxidative processes at the Rifle IFRC site

ABSTRACT: At the Rifle IFRC, injection of organic carbon stimulates the production of reduced Fe and S chemical species and also results in significant permeability decreases especially within injection wells. Due to the increase in reduced iron species, we predict that iron oxidizing microbes will further contribute to the decrease in permeability during the reoxidation of the aquifer. We first sought to verify the presence of iron oxidizing microbes in the aquifer through culture based methods. Iron sulfide gradient tube cultures inoculated with Rifle groundwater yielded two isolate strains that oxidize Fe and precipitate iron oxyhydroxides. Neither isolate was related to previously identified iron oxidizing microbes. Isolate CD03 is 98% similar to Curvibacter delicatus, and P101 #2 is 97% similar to Hydrogenophaga taeniospiralis. The isolates are not obligate iron oxidizers, but are also capable of growth on organic substrates and hydrogen. The diversity of metabolisms allow these organisms to adapt to changing geochemical conditions. Next, we designed a flow through culture chamber that allows for imaging of sediment pore space with multiphoton confocal microscopy. Our goal is to visualize the distribution of cells and biominerals in relation to the pore structure, to gain insight into Fe oxidizer controls on aquifer permeability reduction. We packed the chamber with Nafion, an optically transparent polymer which allows us to image pore spaces. Rifle isolates were inoculated into the simulated aquifer system, then growth media was injected at a constant flow rate. We will present our results demonstrating the distribution of cells and biominerals in pores/pore throats and resulting changes in pore scale architecture.
NAME: Jon Chorover
ORGANIZATION: University of Arizona
PROGRAM AFFILIATION: SBR
ABSTRACT TITLE: Uranium and strontium fate in waste-weathered sediments: Scaling of molecular processes to predict reactive transport

**ABSTRACT:** Multi-faceted lines of inquiry are needed to bridge between molecular- and field-scale information. By focusing them on a common experimental design, we seek to reveal non-linear and emergent behavior in contaminated weathering systems. Silicate weathering reactions in acidic uranium waste streams are expected to result in ripening and aging of waste-containing secondary products.

**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) Uranium speciation in legacy sediments from the U-8 and U-12 Crib sites can be reproduced in bench-scale weathering experiments conducted on unimpacted Hanford sediments; (2) 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; (3) Although field contamination and laboratory experiments differ in their diagenetic time scales (decades for field vs. months to years for lab), sediment dissolution, neophase nucleation, and crystal growth reactions that occur during the initial 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:** Geochemical transformations and transport behaviors that occur in bench-scale studies of waste-sediment interaction will eventually be compared with parallel solid-phase analyses of core sample extractions from the acid uranium waste impacted U-8 and U-12 Cribs at Hanford. Crib waste aqueous simulants are being reacted with Hanford sediments in batch and column systems. Coupling of contaminant uptake to mineral weathering is being monitored using a suite of methods both during waste-sediment interaction, and after, when waste-weathered sediments are subjected to infusion with circumneutral background pore water solutions.

**Results:** We have accomplished a set of one year bench-scale batch weathering and flow-through column experiments where Hanford Sediment was reacted with U-bearing synthetic crib waste (SCW) solutions as a function of pH, U and phosphate concentrations. PO₄ exerts strong control over U speciation at all pH with the rapid precipitation of meta-ankoleite [K(UO₂PO₄)·3H₂O] leading to near complete immobilization of U. Bolwoodite [K(UO₂)(HSiO₄)·3H₂O] increased even in PO₄-containing systems. Without PO₄, U speciation was controlled by the (pH-dependent) rate of silicate weathering. In this case, U immobilization was limited to 25-50% as precipitated becquerelite [Ca(UO₂)₆O₄(OH)₆·3H₂O] or compreignacite [K₂(UO₂)₆O₄(OH)₆·3H₂O] and bolwoodite. Mineral precipitate composition depends on initial solution pH. Carbonate dissolution buffers influent pH 3 solutions to ca. pH 7-8 and initial pH 2 solutions to ca. pH 3-4, with subsequent silicate weathering that makes Si⁴⁺ available for bolwoodite precipitation. Parallel homogeneous nucleation experiments and thermodynamic calculations
conducted to assess precipitation of reference uranium-bearing phases from supersaturated aqueous solutions confirmed the strong phosphate control over U speciation and multi-speciation of U in its absence. Geochemical modeling was further conducted to assess aqueous speciation and solubility envelopes of U-bearing solids as a function of system composition. These data streams guide follow-on desorption experiments and reactive transport modeling to understand the fate and transport of U in the Hanford subsurface.
NAME: Gary Curtis

ORGANIZATION: U.S. Geological Survey

PROGRAM AFFILIATION: SBR

ABSTRACT TITLE: Upscaling of U(VI) Desorption and Transport from Decimeter-Scale Heterogeneity to Plume-Scale Modeling

ABSTRACT: Modeling reactive transport of contaminants in groundwater is uncertain because detailed experimental laboratory data are available at the centimeter scale but field-scale transport occurs on the kilometer scale. In this project we test approaches for incorporating small-scale information into field-scale simulations using extensive datasets collected at bench-, decimeter, and tracer test scales of U(VI) at the Naturita, Colorado UMTRA site.

Laboratory batch experiments using contaminated sediments from the Naturita site indicated that U(VI) desorption was rate-limited and required on the order of 30 days to reach equilibrium. Simulations using a dual domain mass transfer model indicated that after approximately 2 weeks the temporal changes of the U(VI) concentration were driven by weathering reactions. The decimeter scale experiments were conducted in 2.5m long 2D tanks packed with two size fractions excavated from the field site. Desorption of U(VI) was simulated using an equilibrium surface complexation model calibrated to batch data and mass transfer rate parameters estimated by model calibration. Reactive transport in the more permeable fraction was limited by the desorption rate whereas equilibrium was closely approximated in the less permeable material. These results were extended in synthetic upscaling experiments where a 2D domain was successfully simulated by a 1D dual domain model using immobile porosity values estimated from the volume fraction of the less permeable facies.

Analysis and modeling of two tracer tests conducted at the Naturita field site has further demonstrated importance of rate-limited mass-transfer on U(VI) transport. Electrical resistivity measurements used to monitor a tracer experiment that had a high sodium chloride concentration demonstrated that effective parameters for mobile-immobile zone transfer could be determined both along the flowpath as well as locally at an observation well, providing insight into the spatial variability of the mass transfer processes. Results from these studies were extended to simulate U(VI) transport observed in the second tracer test where a low electrical conductivity, U(VI) free water was injected into the aquifer. The results demonstrated that the model calibrated to electrical observations coupled with U(VI) surface complexation model developed in the laboratory had significant predictive capability of field transport. A combined analysis of data from the laboratory experiments and field tracer tests is being used to evaluate upscaling approaches at the plume scale. Ultimately, plume-scale predictions of U(VI) transport for 16 years (1998-2013) will be compared with measured values in the aquifer, with upscaling of reactive transport parameters in accordance with the meter-scale studies.
NAME: Gary Curtis
ORGANIZATION: U.S. Geological Survey
PROGRAM AFFILIATION: SBR
ABSTRACT TITLE: Multiscale Assessment of Prediction Uncertainty in Coupled Reactive Transport Models

ABSTRACT: Reactive transport simulations provide a systematic framework for integrating hydrologic and biogeochemical conceptual process models into a quantitative description of subsurface behaviors, which can then be used to project future concentrations. Assessing the uncertainty of these projections is difficult, because subsurface environments are open and complex, and subject to multiple interpretations and conceptualizations. This challenge is addressed using the method of multimodel analysis, in which model predictions are based on a weighted average of predictions of multiple plausible models, each of which is associated with a model averaging weight estimated from a maximum likelihood Bayesian model averaging approach.

The model averaging approach was evaluated for uranium transport at the Naturita UMTRA site using available data at the batch, column and tracer-test scale and in synthetic 3D simulations at the plume scale. At the batch and column scales, significant uncertainty results from imperfect knowledge of the adsorption reactions that immobilize uranium at the mineral surfaces as well as auxiliary reactions such as speciation and ion exchange. At the tracer test scale and especially in the 3D plume scale, additional uncertainty results from imperfect knowledge of the distribution of the reactive facies that describe the flow, transport and reactions. Model averaging had superior predictive coverage in both the column and tracer test analyses. The results for the synthetic physically and geochemically heterogeneous 3D study showed little support for oversimplified models such as those with homogeneous layers or zonal distributions of reactive facies. Heterogeneous distributions of reactive hydrofacies generally performed well; in some but not all instances these models gave superior predictions relative to model averaging. Model averaging had superior predictive coverage by considering model uncertainty. Parametric and conceptual model uncertainty for the stimulated bioreduction of U(VI) by microbial processes at the Rifle IFRC site was evaluated using a set of synthetic observations generated by a 3D reactive transport simulation of the Big Rusty field experiment. The ability to parameterize a comprehensive reaction network using a 1D model of the simulated 3D system was evaluated using sensitivity analysis and parameter estimation methods. Evaluation of the 1D approach was based on the ability of the calibration to estimate the known parameter values used in the 3D simulation, and on the suitability of the resulting parameter uncertainty estimates used in assessing predictive uncertainty. Results indicated that, although the 1D model can provide concentrations of key constituents that are representative of the 3D system, spatial variability in groundwater flow that is unresolved in the 1D abstraction can significantly bias the estimated reaction parameters. Derived linear parameter uncertainty estimates tended to be much smaller than indicated by the known conceptual errors. Without considering the impact of these conceptual errors, predictive uncertainty could be significantly underestimated.
ABSTRACT: Evidence of dual-domain mass transfer (DDMT) includes long “tailing” behavior and concentration rebound during aquifer remediation. The lack of experimental methods to verify and measure mass transfer in-situ independently of tracer breakthrough results in significant uncertainties in estimates of controlling parameters. Our objectives are to develop geophysical strategies to measure mass-transfer parameters over a range of spatial scales, and to demonstrate these strategies to produce estimates of mass-transfer parameters for Hanford 300 Area materials. Here, we assess the utility of time-lapse electrical resistivity (ER) and complex resistivity (CR) to improve the basic and site-specific understanding of mass transfer. During our final year of funding, we focused on (1) ER and CR laboratory experiments; (2) comparison of ER and CR results to nuclear magnetic resonance (NMR) measurements of mobile and immobile porosity; and (3) pore-network modeling of the electrical signature of mass transfer. Column ER/tracer-test experiments were performed using two samples of the porous zeolite clinoptilolite, which is a known dual porosity medium. We used glass beads and well sorted sand as two reference samples in which tailing behavior is not evident. Parametric sweeps using the model STAMMT-L identified best-fit DDMT parameters for the range of samples studied.

A comparison of solute transport behavior for the two zeolites revealed contrasting DDMT characteristics. For example, longer solute residence times were evident for the finer grained zeolite, for which NMR spectra indicate greater immobile porosity. The utility of CR methods for determining DDMT characteristics is ongoing. Debye decomposition analysis of CR spectra revealed a dominant relaxation time that appears to be controlled by grain size, which may have limited value for assessing mass-transfer properties. There is, however, an observed increase in polarization associated with short relaxation times with increasing immobile porosity as measured by NMR. From this we infer that the CR spectra are sensitive, in these examples, to the intra-grain porosity and hence physical characteristics that influence mass transfer.

Pore-network models qualitatively reproduce the hysteresis observed in time-lapse ER but indicate that the bicontinuum Archie model used previously systematically over predicts the contribution of immobile porosity to bulk conductivity. Alternative petrophysical models have been developed to account for different internal connectivity for mobile and immobile domains, providing a superior fit to the pore-network simulations of the bulk-fluid conductivity relation.

Infiltration and tracer experiments were conducted to assess the DDMT properties of the vadose zone in the Hanford Integrated Field Research Challenge well field in July of 2012. Data exhibit DDMT behavior. Analysis involves calibration of models to the breakthrough data and comparison to the electrical measurements. Publications on results from zeolites and pore-network modeling are in preparation. To date, three papers from this project have been published, and a fourth is undergoing revision. Ongoing work focuses on (1) analysis of field-experimental data, (2) development of the CR-NMR spectra analysis combining additional measured physical properties (e.g. surface area) (3) additional pore-scale modeling of the geoelectrical signature of DDMT.
NAME: Robert Ewing
ORGANIZATION: Iowa State University
PROGRAM AFFILIATION: SBR
ABSTRACT TITLE: Effects of pore-scale physics on uranium geochemistry in Hanford sediments

ABSTRACT: The Hanford 300 Area sediments are mainly river cobble and gravel, with sands and fines (silt + clay) variably filling the voids in between. Fines comprise only 1.78% of the total sediment mass, but hold a disproportionately large fraction of the total U. However, the coarser sediments (e.g., >2 mm), with their larger mass fraction, may serve as a long-term U release pool despite their lower U concentration. We hypothesized that slow U release at the 300 Area is partly due to low connectivity of intra-granular pores. The objectives of this project were (1) to evaluate the U distribution in 2-8 mm size sediments, (2) to determine whether low pore connectivity affects U distribution and release from Hanford 300 Area sediments, and (3) if so, to assess its implications for long-term release. Our approach integrates laboratory experiments and pore-scale network modeling. Laboratory experiments include stirred flow cell, column, and batch sorption approaches, with the resultant samples analyzed by ICP-MS instrumentation for multiple elemental concentrations in liquid samples. In addition, laser ablation (LA)-ICP-MS was conducted for elemental mapping of 2-8 mm sized basaltic clasts. Pore-scale network modeling was also abstracted into a finite difference model that accounts for low pore connectivity effects on porosity and diffusion.

This past year (operating under a no-cost extension) we:

1) Collaborated with scientists of PNNL and Florida State University (FSU) on uncertainty analysis of an elution experiment (reported last year) measuring uranium desorption from different size fractions (<75 µm, 75-500 µm, 500-2000 µm, 2-8 mm, and a <2 mm composite). Using the experimental data collected from this project, a PhD student at FSU presented analysis at the 2013 AGU Fall Meeting, and is preparing a manuscript for publication to Water Resources Research.

2) Extended our own analysis of the elution data to consider the effect of having a range of particle sizes, rather than a single size. This analysis generalizes our earlier work published in Water Resources Research.

3) Extended our finite-difference model of diffusion from porous media with low pore connectivity, to account for non-linear sorption isotherms.
ABSTRACT: Stimulation of microbial uranium reduction in the subsurface can lead to substantial quantities of this toxic element being immobilized, but the potential for re-oxidative mobilization causes long-term stabilization to be technically challenging. Within natural environments, however, uranium often correlates with iron rather than existing as a discrete uranium oxide phase; uranium incorporation into iron oxides is a mechanism that can explain the co-occurrence of these two elements and may represent a natural attenuation pathway for uranium. Further, incorporated uranium within Fe (hydr)oxides appears stable with respect to oxidative dissolution, representing a potential means for long-term sequestration of uranium in the subsurface. Within this project we are examining the molecular mechanisms by which uranium is retrained by iron oxides and conditions optimal for its retention.

In order to examine the mechanism of uranium incorporation within iron oxides, and conditions conducive to its incorporation, we conducted a series of batch experiments coupled with spectroscopic and microscopic analysis of the reaction products. Through our experiments, we determine: (i) the oxidation state of incorporated U, (ii) the effects of calcium and carbonate (influencing U aqueous speciation) concentration, (iii) the pathway-dependence on Fe(II) and U(VI) concentrations, and, (iv) the competitiveness of U incorporation versus U reductive precipitation (to UO₂). A series of batch reactions with ferrihydrite were conducted at pH ~7, [U(VI)] from 1 to 170 μM, [Fe(II)] from 0 to 3 mM, [Ca] at 0 or 4 mM. Uranium valence state was measured using x-ray photoelectron spectroscopy, and U sequestration mechanisms were identified and quantified using extended x-ray absorption fine structure (EXAFS) spectroscopy, x-ray powder diffraction, and transmission electron microscopy (TEM). Depending on the reaction conditions, 12 to 80% of total U was incorporated into goethite. Uranium incorporation was a particularly dominant retention pathway at U concentrations ≤50 μM independent of U(VI) speciation, accounting for 70-80% of total U. With increasing U(VI) and Fe(II) concentrations, U(VI) reduction to UO₂ became more prevalent, but U incorporation remained an operative retention pathway. However, at high Fe(II) concentrations (3 mM), reduction to U(IV) and precipitation of UO₂ dominated. We further investigated the impact of mineralogical impurities common to the subsurface and found that although Al can inhibit ferrihydrite transformation, U(VI/V) incorporation occurred even at Al contents as high as 20% in Al-ferrihydrite. The combination of Ca-UO₂-CO₃ aqueous complexes and structural Al in ferrihydrite, however, resulted in limited incorporation or reduction. Our results demonstrate that U incorporation can be a major U retention pathway across a variety of aqueous phase and mineralogical conditions. Ultimately, it is critical to recognize that multiple retention processes of uranium will occur in the subsurface, which must be considered in the long-term fate of this hazardous element.
ABSTRACT: Iron oxides are a ubiquitous class of compounds that are involved in many biological, geological, and technological processes, and the Fe(III)/Fe(II) redox couple is a fundamental transformation pathway; however, the study of iron oxide surfaces in aqueous solution by powerful spectroscopic techniques has been limited due to “strong absorber problem”. In this work, atomic layer deposition (ALD) thin films of polycrystalline α-Fe_2O_3 were analyzed using the Eisenthal chi^{(3)} technique, a variant of second harmonic generation (SHG) that reports on interfacial potentials. By determining the surface charge densities at multiple pH values, the point of zero charge was found to be 5.5 ± 0.3. The interaction of aqueous Fe(II) at pH 4 and in 1 mM NaCl with ALD-prepared hematite was found to be fully reversible and to lead to about 4 times more ferrous iron ions adsorbed per square centimeter than on fused-silica surfaces under the same conditions. The data are consistent with a recently proposed conceptual model for net Fe(II) uptake or release that is underlain by a dynamic equilibrium between Fe(II) adsorbed onto hematite, electron transfer into favorable surface sites with attendant Fe(III) deposition, and electron conduction to favorable remote sites that release and replenish aqueous Fe(II). Then, the chi^{(3)} technique was used to investigate the adsorption of Cr(III) and Cr(VI) to the hematite/water interface under flow conditions at pH 4 with 10 mM NaCl. We observed partially irreversible adsorption of Cr(III), the extent of which was found to be dependent on the concentration of Cr(III) ions in solution. This result was confirmed using X-ray photoelectron spectroscopy. The interaction of Cr(III) with hematite is compared with the adsorption of Cr(III) to the silica/water interface, which is the substrate for the ALD-prepared hematite films, and found to be fully reversible under the same experimental conditions. The observed binding constant for Cr(III) interacting with the silica surface was found to be 4.0(6) × 10^3 M^{-1}, which corresponds to an adsorption free energy of −30.5(4) kJ/mol when referenced to 55.5 M water. The surface charge density at maximum metal ion surface coverage was found to be 0.005(1) C/m^2, which corresponds to 1.0 × 10^{12} ions/cm^2 assuming a +3 charge for chromium. In contrast, the observed binding constant for Cr(III) interacting reversibly with the hematite surface was calculated to be 2(2) × 10^4 M^{-1}, corresponding to an adsorption free energy of −35(2) kJ/mol when referenced to 55.5 M water. The surface charge density at maximum metal ion surface coverage was found to be 0.004(5) C/m^2 for the reversibly bound chromium species, which corresponds to 8.3 × 10^{11} reversibly bound ions per cm^2, again assuming a +3 charge of chromium. The data also allows us to estimate that about 6.7 × 10^{12} Cr(III) ions are irreversibly bound per cm^2 hematite at saturation coverage. The results of this investigation suggest that the use of hematite in permeable reactive barriers, for cost-effective chromium remediation, allows for Cr(III) remediation at very low concentrations through adsorptive and redox processes but quickly renders the barriers ineffective at high chromium concentrations due to surface saturation.
NAME: Yuri Gorby
ORGANIZATION: Rensselaer Polytechnic Institute
PROGRAM AFFILIATION: SBR
ABSTRACT TITLE: Bacterial Nanowires and Extracellular Electron Transfer to Heavy Metals and Radionuclides by Bacterial Isolates from DOE Field Research Centers

ABSTRACT: Research conducted at the University of Southern California, Montana State University, and now at Rensselaer Polytechnic Institute in Troy, New York, embraces controlled cultivation approaches for controlling and optimizing the production and activity of extracellular conductive protein filaments called ‘bacterial nanowires’ by diverse groups of microorganisms. The objective of our collaborative project is to evaluate the contribution of conductive bacterial nanowires on the fate and transport of uranium and chromium in contaminated subsurface systems.

During the past 2 years, we have developed and applied controlled-cultivation techniques for optimizing the production of conductive bacterial nanowires by 2 organisms isolated from contaminated sediments within the DOE National Lab complex. *Geobacter daltonii* FRC32T is an iron(III) and uranium(VI)-reducing bacterium isolated from uranium-contaminated subsurface sediments at the DOE ORFRC. *Desulfovibrio vulgaris* RCH-1 is a sulfate-reducing bacterium that was isolated from chromium-contaminated groundwater at the 100H Hanford Site. Both organisms produced branched extracellular appendages in response to electron acceptor limited conditions, with ferric nitrilotriacetic acid (Fe(III)-NTA) provided as electron acceptor for *G. daltonii* FRC32T and sulfate provided for *D. vulgaris* RCH-1 in chemostat and biofilm reactors, respectively. Amending the medium with amino acids to support the production of protein increased the production of extracellular filaments under electron acceptor limitation. Branched appendages from *G. daltonii* FRC32T were confirmed to be electrically conductive nanowires using conductive atomic force microscopy. Attempts to evaluate the conductive properties of *D. vulgaris* RCH-1 are ongoing.

The influence of *G. daltonii* FRC32T and *D. vulgaris* RCH-1 on fate and transport of uranium and chromium will be evaluated using 2D-flow columns designed and manufactured at RPI. Conditions within these flow columns will be manipulated to control the production of bacterial nanowires and other components of the extracellular polymeric matrix. Information gained from this research will be used to better understand the biogeochemical processes that influence contaminant transport at Hanford and the Oak Ridge Integrated Field Research Center. Understanding the components and mechanisms of charge transfer to extracellular electron acceptors by this and other environmentally-relevant organisms is an important step in realizing their full potential as tools for remediation of contaminated subsurface systems.
NAME: Kim Hayes

ORGANIZATION: University of Michigan

PROGRAM AFFILIATION: SBR

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

ABSTRACT: This UM/ASU research seeks to identify how iron-sulfide minerals can inhibit the oxidation rate of reduced U solids formed by sulfate reducing bacteria (SRB). SRB normally utilize sulfate as a terminal electron acceptor and produce sulfide that precipitates with Fe(II) to form iron-sulfide (FeS) solids. At DOE sites contaminated with U, SRB can also reduce aqueous-phase U(VI) to insoluble U(IV) solids such as uraninite ((UO₂(s)). SRB can accomplish U(VI) reduction either directly by enzymatic electron transfer processes or indirectly through chemical reduction by the sulfides species produced. 

The hypothesis of this study is that iron sulfides inhibit the kinetics of re-oxidation of U(IV) to U(VI) when oxidants such as oxygen, nitrite, or Fe(III) are introduced.

In the past year of this project, we achieved significant progress on all three major tasks: (1) evaluating the impact of a range of iron sources in biogenic FeS production under reducing conditions by Desulfovibrio vulgaris, (2) flow-through reactor studies of the inhibition of the oxidation rates of reduced U(IV) solids by FeS, and (3) packed sediment column studies for assessing the abiotic oxidation dynamics of U(IV) solids in the presence and absence of biogenic FeS under the influence of dissolved oxygen (DO) and nitrite.

Various iron sources were evaluated for biogenic FeS production by D. vulgaris in batch experiments with pyruvate as the electron donor, including soluble Fe(II) and Fe(III) and solid Fe(III) (hydr)oxides. When pyruvate was the electron donor, D. vulgaris produced distinct sulfate- and Fe(III)-reduction patterns and biogenic FeS characteristics. When soluble Fe(II) or Fe(III) was the iron source, simultaneous reduction of sulfate and soluble Fe(III) occurred along with the rapid utilization of pyruvate. Furthermore, the greater proton production during pyruvate fermentation (compared to lactate) lowered the pH, which led to more crystalline FeS formation. With solid-phase Fe(III) as the electron acceptor, complete and more rapid pyruvate consumption (compared to lactate) led to more FeS production and vivianite precipitation due to the greater accumulation of soluble Fe(II).

Continuously stirred tank reactors (CSTRs) were used for examining the inhibition mechanism and kinetics of UO₂ oxidation by mackinawite under oxic groundwater conditions. Experiments were conducted as a function of pH, calcium, FeS content, and DO concentrations to assess the impact of geochemical conditions on UO₂ oxidation kinetics. Results consistently show that FeS serves as an effective oxygen scavenger to inhibit the oxidation of UO₂. The solution pH influences UO₂ oxidative dissolution by shifting FeS oxidation mechanism. The presence of Ca²⁺ in the groundwater leads to the formation of passivation layer on UO₂ surfaces, which moderately reduces UO₂ dissolution rate. The UO₂ dissolution rate is strongly dependent on the concentration of DO and FeS solids in groundwater, with lower rate at lower DO levels and higher FeS content.

Packed column flow-through experiments were used to assess the abiotic oxidation dynamics of uraninite by nitrite and DO in natural sediments in the presence and absence of biogenic FeS. After acetate-induced bioreduction of Rifle sediments in parallel columns, one with sulfate present and one
without, gamma radiation was used to sterilize sediments before the oxidation reaction was initiated. The study shows that abiotic UO$_2$ oxidation by nitrite is essentially insignificant in comparison to oxidation by DO. Additionally, sediments containing FeS minerals significantly inhibit UO$_2$ oxidation compared to sediment without FeS present. In both columns, significant amounts of sediment-bound U remained after extensive oxidation, suggesting a U adsorption or other solid-phase incorporation mechanism related to the biologically reduced sediments.
ABSTRACT: 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. In benthic sediments and other anaerobic settings, inorganic mercury Hg(II) is typically coordinated to reduced sulfur-containing ligands including inorganic sulfide and natural organic matter. This presentation will discuss research to characterize and identify the forms of Hg(II) that are bioavailable to methylating bacteria. We performed laboratory experiments to demonstrate that the combination of Hg(II), sulfide, and dissolved organic matter (DOM) in solution results in a heterogeneous mixture of dissolved Hg(II) complexes, amorphous Hg-S-DOM nanoparticles, and crystalline HgS particles. The composition of this mixture evolves over time and is not necessarily predicted by equilibrium speciation models. Moreover, in bacterial cultures of sulfate reducers, the production of methylmercury depended on the form of mercury added. Net production of MeHg was greatest in cultures receiving dissolved Hg, while cultures receiving Hg-S-DOM nanoparticles generated 20-40% less MeHg in most cases, depending on the productivity of methylating microbes. This result was in stark contrast to the minimal production of methylmercury in all cultures amended with microcrystalline metacinnabar HgS. The production of MeHg in the nanoparticle exposures also depended on the age of the nanoparticle stock solutions. Small angle X-ray scattering and X-ray absorption spectroscopy was used to characterize the structure of the nanoparticles as they changed in their stock solution. The results indicated that organic matter was altering the rate of crystal ripening and agglomeration of the nanoparticles. These changes resulted in nanoparticles with a lower dissolution potential when added to bacterial culture media in the methylation experiments. These experimental results point to the need of a kinetics-based reaction model that links inorganic mercury speciation (including nanoscale species) and methylation potential. Overall we anticipate that this approach may enable the consideration of nanoscale geochemistry for predictions of Hg reactivity and bioavailability in the environment.
ABSTRACT: We have been conducting a long-term column experiments to study the link between biostimulation and changes in permeability. Nine columns (4.8 cm ID, 30 cm long) were built with side ports at 2 cm intervals, which allow for dissolved species sampling and pressure change measurements along their vertical axis. The experiment has been operating now for approximately 700 days. One column is equipped with electrodes to determine the induced polarization (IP) and link this to changes in permeability and to IP measurements that have been conducted at Rifle during previous biostimulation experiments. Columns were filled with Rifle background sediment and the water being pumped into the columns is Rifle groundwater that is being shipped to the laboratory at monthly intervals. The experiment is divided into an acetate biostimulation phase of 488 days and in a post biostimulation phase that has now been operating for 212 days. Columns have been sacrificed at regular intervals during the biostimulation and the post-biostimulation period to analyze for solid-species changes and total microbiological quantification. Results to date have shown that there was a small and gradual (seemingly linear) increase in pressure over time during the first 200 days of biostimulation. Then, after about 220 days of biostimulation, we see a significant change in pressure along the longitudinal axis of the columns. This pressure increase occurred approximately 50 days after sulfate was completely reduced. Effluent analyses show that by this time, methane was near solubility. Microbiological analyses have shown that for the first 200 days of the biostimulation total bacterial numbers were similar to methanogen numbers (∼ 10^8 cells/g). For columns sacrificed after 200 days of biostimulation, methanogen numbers were an order of magnitude higher than total bacteria. They have remained higher also throughout the post-biostimulation period. Although Rifle field experiments, focusing on dissolved groundwater samples have not reported significant presence of *Archaea*, these results, that focused on sediment analyses, seem to indicate that *Archaea*, in this case methanogens, might be a key organism competing for electron donors during biostimulation.

During the post biostimulation period, methane concentration in the effluent decreased within 20 days by 3 orders of magnitude, from solubility to about 10^{-6} M, and remained at that concentration without much change until this date.

In terms of permeability changes in response to biostimulation, we conclude that biogas formation has a higher impact than the precipitation of mineral phases and/or biomass buildup, and that these changes are reversible. Towards the end of the biostimulation period, the average permeability in the columns had decrease by one order of magnitude.

A conservative estimate of the acetate converted to methane over the 700-day duration of the experiment is 12% on a molar base. This is a lower-limit estimate, based on cumulative acetate consumed during the biostimulation period (488 days), and methane monitored in the effluent up to 700 days. It does not include any methane oxidation during the post stimulation phase, and there was a short period (∼ 35 days) where methane gas in the effluent was not quantified.
ABSTRACT: Discharges of uranium containing groundwaters to surface waters are a concern at several wetlands, including some at DOE facilities. Sharp biogeochemical transitions that occur near wetland plants may greatly affect the fate of many redox-sensitive metals and radionuclides, including uranium. Our research investigates how wetland plant roots affect sediment biogeochemistry and uranium mobility with respect to both short- and long-term goals.

We have formulated three primary hypotheses: (1) oxygen released by wetland plant roots oxidizes iron to Fe(III), providing favorable conditions for iron reducers that also reduce U(VI) to much less mobile U(IV), (2) microorganisms, including iron/uranium reducing microorganisms, are stimulated by organic carbon provided by wetland plants via root exudates and root turnover, and (3) U(IV) remains stable for extended time periods in wetlands that are often anaerobic and nitrate poor. Testing these hypotheses requires examining key iron and uranium reducing microorganisms, along with the spatial distribution and speciation of uranium and other important chemical species.

We have developed small-scale (700 mL) up-flow vegetated (Typha latifolia or Scirpus acutus) and non-vegetated mesocosms, and are operating them in our greenhouse in an up flow mode to simulate groundwater to surface water discharge and to test above hypotheses. The porous medium used was sand augmented with (0.2 wt-%) sediment collected from Savannah River Site (SRS) wetlands, in order to provide an appropriate microbiological seed. Siderite was supplied at the bottom of some of the microcosms for each plant treatment, in order to buffer the pH and provide an iron source since the sand had a very low iron content. To differentiate biogeochemical changes in sediments near roots and sediments that are not in direct contact with roots, several small bags (70 micron-mesh nylon) were filled with 1 to 3 grams of the sand/sediment mixture and placed at different locations in the mesocosms allowing for the retrieval of sediments from a root-exclusion zone. A modified Hoagland solution was pumped through the mesocosms at a rate of 100 to 150 mL per day. After the mesocosm plantings had been established, uranium was supplied into the influent via peristaltic pumps, resulting in an estimated 20 uM influent uranium concentration. Mesocosms were then operated for 80 days, and dissolved species were periodically sampled. The mesocosms were subsequently sacrificed and analyzed for solid-associated chemical species as well as microbiological characterization.

Results indicate differences between the mesocosms planted with Typha latifolia (common cattail), Scirpus acutus (hardstem bulrush), and no plant controls, as well as between mesocosms with and without the siderite iron source. To differentiate biogeochemical changes in sediments near roots and sediments that are not in direct contact with roots, several small bags (70 micron-mesh nylon) were filled with 1 to 3 grams of the sand/sediment mixture and placed at different locations in the mesocosms allowing for the retrieval of sediments from a root-exclusion zone. A modified Hoagland solution was pumped through the mesocosms at a rate of 100 to 150 mL per day. After the mesocosm plantings had been established, uranium was supplied into the influent via peristaltic pumps, resulting in an estimated 20 uM influent uranium concentration. Mesocosms were then operated for 80 days, and dissolved species were periodically sampled. The mesocosms were subsequently sacrificed and analyzed for solid-associated chemical species as well as microbiological characterization.

Results indicate differences between the mesocosms planted with Typha latifolia (common cattail), Scirpus acutus (hardstem bulrush), and no plant controls, as well as between mesocosms with and without the siderite iron source. Planted mesocosms showed increasing Fe concentrations in solution with elevation. Typha latifolia roots show larger proportions of Fe in the oxidized state than Scirpus acutus roots. Total bacterial populations as well as Geobacter sp. numbers were highest on roots (on a per gram basis), followed by sediments near roots, and lowest in the root-exclusion zones.
NAME: Kristina Keating  
ORGANIZATION: Rutgers University  
PROGRAM AFFILIATION: SBR  
ABSTRACT TITLE: Integrated geophysical measurements for bioremediation monitoring: combining NMR, magnetic methods and SIP

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

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

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

The second year has focused on collecting laboratory NMR, MS, and SIP measurements on columns packed with sediments from the Rifle IFRC site as the columns are amended with acetate. The column experiments are designed to simulate field conditions. As of this date, columns that are capable of being used for SIP, MS and NMR measurements have been designed and built and the experiments are being initialized. In addition to the geophysical measurements, changes in the geochemistry and pore geometry inferred from these ongoing measurements will be verified by standard, independent
ABSTRACT: The spatial distribution of clay can have a large impact on the sorption of contaminants, therefore affecting their transport and fate in the natural subsurface. In this work, the effects of illite spatial distribution on Cr(VI) adsorption was examined using column experiments and reactive transport modeling under several flow rate conditions. Three columns were set up with the same volume fraction of illite (10%): the Mixed column has illite evenly mixed within a quartz matrix; the Flow-transverse column has illite distributed in one horizontal zone in the direction that is vertical to the main flow; the Flow-parallel column has illite distributed in one cylindrical zone in the direction parallel to the main flow direction. Cr(VI) adsorption and desorption column experiments were carried out under flow velocities of 0.6, 2.9, and 14.8 m/day. Two-dimensional reactive transport modeling was used to understand the role of illite distribution in determining Cr(VI) sorption kinetics.

The difference in the sorption kinetics in the three columns varies, depending on the flow conditions. Under the slower flow rate of 0.2 ml/min, the Cr(VI) adsorption behavior was very similar for the Mixed and Flow-transverse columns. Sorption of Cr(VI) occurred immediately following the injection of an inlet solution with a pH of 4.0. The sorption occurred fast early on and reached 80% of Cr(VI) sorption capacity of the columns at about 12 residence times. Sorption slowed down after that and reached full capacity with another 30 – 40 residence times. In contrast, sorption in the Flow-parallel column occurred at a much lower rate early on, reaching about 80% sorption capacity within about 30 residence time, about 2.5 times slower than the other two columns. The 2D reactive transport model shows that the Cr(VI) sorption in this column is limited by the rate of mass transport at the illite-quartz interface. Cr(VI) was only adsorbed on the illite-quartz interface early on and gradually percolated into the illite zone over time. The transverse dispersivity, a measure of the mass transport rate, plays a key role in determining the sorption kinetics of the Flow-parallel column. At the flow velocity of 2.9 m/day, the difference among the three columns is similar to the difference at the flow rate of 0.6 m/day. At the flow rate of 14.8 m/day, the three columns show almost exactly the same sorption behavior with overlapping breakthrough curves. With increasing flow rates, the overall sorption capacity of all three columns also decreases.
ABSTRACT: Diverse groups of microorganisms and abiotic reactions affect the oxidation state of redox-active metals, and oxic-anoxic transition zones are hotspots for metal cycling. Redox transition zone processes are relevant controls for the fate of radionuclides such as uranium, which is mobile as U(VI) but only sparingly soluble in its U(IV) form. Metal-reducing bacteria adapted to life in oxic-anoxic transition zones play important roles for controlling U mobility. To explore the effects of changing redox conditions on the microbiology contributing to ferric iron and U(VI) reduction, an x-ray accessible plexiglass column with sampling and outflow ports along the length of the column has been constructed. Initial tests of the column packed with acid washed sand and FeOOH-coated sand showed that changes in the distribution and speciation of Fe resulting from the flow of dissolved sulfide can be monitored with synchrotron x-ray spectroscopy using the intact column. Another column design allows the infusion of different (e.g., oxic and anoxic) feed solutions from both ends leading to the formation of a controllable redox gradient and is being used to investigate the responses of microbes to fluctuating oxic-anoxic transition zones.

A number of microbes have been implicated in metal reduction and tools to directly measure their metal-reducing activity are desirable. We applied high precision mass spectrometry and demonstrated that a significant U isotopic fractionation occurs during microbial U(VI) reduction, with U(VI) becoming isotopically lighter with progressive amounts of reduction. Enrichment factors (ε) measured with different metal-reducing isolates (i.e. Geobacter, Anaeromyxobacter, Shewanella and Desulfitobacterium) tend to range between 0.7‰ and 1.0‰, and follow the expected inverse relationship between reaction rate and the magnitude of fractionation. Only Shewanella sp. strain NR exhibited unusual near-zero fractionation at low U concentrations, suggesting that multiple U reduction pathways with different mechanisms (and thus different isotopic fractionation) contribute to U(VI) reduction. Further, metaproteomics analysis identified specific c-type cytochromes expressed in cultures of Anaeromyxobacter dehalogenans strain 2CP-C grown with manganese oxide as electron acceptor suggesting that the presence and abundance of the specific transcripts and/or proteins provides information about the physiological status of the organism. The analysis of samples obtained from defined laboratory studies and groundwater samples collected at field sites demonstrates the value of the new tools to assess, monitor, and predict reductive processes affecting the redox speciation (i.e., mobility) of relevant metals and radionuclides.
The mechanisms for Fe(III) oxide reduction in Geobacter species are of interest because of its important role in global carbon cycling. This is also of specific interest to the Subsurface Biogeochemical Research Program because Geobacter species, growing primarily via Fe(III) oxide reduction, have been shown to be important agents in the bioremediation of uranium-contaminated groundwater. There has been substantial debate about the mechanisms for Fe(III) oxide reduction in Geobacter species. One model is that electrons are transported outside the cell along proteinaceous pili that have metallic-like conductivity and that the multi-heme c-type cytochrome OmcS, which is specifically localized along the pili, facilitates electron transfer from the pili to the Fe(III) oxide. The alternative model is that outer-surface c-type cytochromes not associated with pili are the primary electrical contacts with Fe(III) oxides. Conclusively differentiating between these two hypotheses has been difficult, in part because of the non-specific reduction of Fe(III) by many different cytochromes in vitro. Furthermore, genetic approaches have often yielded strains in which the deletion of a single gene influenced the production and/or localization of multiple proteins.

In order to investigate Fe(III) reduction mechanisms further, a strain of G. sulfurreducens, designated Aro-5, was constructed which produced pili with diminished conductivity. This was accomplished by modifying the amino acid sequence of PilA, the structural pilin protein. An alanine was substituted for each of the five aromatic amino acids in the carboxyl terminus of PilA, the region in which G. sulfurreducens PilA differs most significantly from the PilA of microorganisms incapable of long-range extracellular electron transport. Strain Aro-5 produced pili that were properly decorated with OmcS. Other abundant outer-surface c-type cytochromes, such as OmcZ, were properly expressed and localized. However, the Aro-5 pili had greatly diminished conductivity and Aro-5 cultures were severely limited in their capacity to reduce Fe(III) compared to the control strain. The capacity of the Aro-5 strain to produce electrical current with a graphite anode serving as the electron acceptor was less than 10% of the control strain and the conductivity of the Aro-5 biofilms was 10-fold lower than the control strain. These results further demonstrated that loss of pili conductivity reduced the capacity for long-range electron transport. Thus, the expression of outer-surface c-type cytochromes is insufficient for Fe(III) reduction in G. sulfurreducens. The pili of G. sulfurreducens must be conductive in order for the cells to be effective in extracellular long-range electron transport.

Previous evidence for the alternative hypothesis, that G. sulfurreducens reduces Fe(III) with outer-surface c-type cytochromes not associated with pili, was the finding that deletion of gene GSU1501, which controls production of exopolysaccharide, inhibited Fe(III) oxide reduction. However, further examination of the GSU1501-deletion mutant revealed that this strain does not properly localize OmcS on the pili. OmcS is produced and is abundant in the extracellular matrix. Thus, the phenotype of the GSU1501-deletion mutant is also consistent with the model in which long-range electron transport is along the pili, which delivers electrons to OmcS for the final electron transfer to Fe(III) oxide.

These studies rule out the possibility that outer-surface c-type cytochromes can effectively facilitate Fe(III) oxide in the absence of pili conductivity and support the concept that long-range electron transport along pili is the primary mechanism for Fe(III) oxide reduction by G. sulfurreducens.
NAME: Derek Lovley  
ORGANIZATION: University of Massachusetts  
PROGRAM AFFILIATION: SBR  
ABSTRACT TITLE: Detailed Modeling of Uranium Mobility Under a Combined Influence of Microbial Activities and Geochemical Transformations

ABSTRACT: The mechanistic model of in situ uranium bioremediation requires a detailed representation of subsurface microbial metabolism and an accurate characterization of interactions between microbial and geochemical processes. The overall objective of this project is to develop a fine-scale computational framework capable of predicting uranium mobility under a combined influence of microbial activities and geochemical transformations.

We have made progress in both biotic and abiotic processes for achieving the project goal. 1) A preliminary pan-genome-scale metabolic model for subsurface Geobacter community was developed. The unique features of the metabolism suggest more of a necessity from studies of subsurface communities than from the subsurface isolates or from the non-subsurface model species. 2) The quality of the metabolic network reconstruction for subsurface Geobacter community was improved by removing thermodynamically infeasible loops with unrealistic high fluxes. 3) Physiological studies of the sulfate reducing bacteria, Desulfobacter postgatei, were conducted to evaluate the metabolic activity under electron donor-limiting and electron acceptor-limiting conditions, respectively. A genome-scale model of Desulfobacter postgatei was developed, providing some insights into the sulfate reduction pathways. 4) An optimized genome-scale metabolic model for Anaeromyxobacter dehalogenan (a unique bacteria with diverse metabolic capabilities such as uranium reduction) was developed, and a bi-level optimization algorithm for resolving energetic model inconsistencies (REMI) was implemented to remove the reactions with unrealistic high ATP production. 5) Computational thermodynamics based on the coupled oxidation-reduction reactions for geochemical transformation was integrated into a comprehensive reactive-transport model of in-situ bioremediation of U(VI), providing predictions and insights into the role of abiotic redox reactions that occur in systems with interplay between microbiological and geochemical processes. This approach helps to address some questions of interest, such as the reason why abiotic U(VI) reduction often takes place under laboratory conditions but has not been reported to play an important role under environmental conditions. The above-mentioned recent advances in modeling biotic and abiotic processes will help to ultimately build a fine-scale predictive model for computer-aided design of field-scale uranium bioremediation.
ABSTRACT: Strategies to simply and inexpensively monitor the activity of microorganisms in soils and sediments are needed for studies on bioremediation and for assessing changes in microbial activity due to environmental perturbations, such as climate change. Current methods typically involve invasive sampling of the environment, which can alter microbial activities, and require the addition of tracers or indicators, adding complexity and expense. Furthermore, these traditional methods are not amenable to real-time monitoring of in situ activity.

We are developing a simple and inexpensive electrode-based method for monitoring rates of microbial activity in a wide diversity of anaerobic soils and sediments. Our strategy is based on the concept that the pathways for intermediary metabolism of organic carbon are similar in all anaerobic soils regardless of whether the terminal electron-accepting process is Fe(III) reduction, humics reduction, sulfate reduction, or methane production. In each type of soil, complex organic matter is fermented to simple intermediates, such as acetate and H₂, which are then consumed by the terminal respiratory processes. If a graphite electrode is inserted in anaerobic soil and connected to another electrode in contact with oxygen, then the electrode in the soil can serve as an alternative electron acceptor. Microorganisms colonizing the electrode surface will oxidize the acetate and other intermediates, produced from fermentation, with electron transfer to the electrode. The rate of this electron transfer is measurable as an electrical current. Thus, in the absence of any other complicating factors, it can be expected that the amount of current produced from an electrode in an anaerobic soil will be directly related to the rates of microbial metabolism in those soils or sediments.

This concept is being tested with a diversity soils and sediments in which different electron accepting processes predominate. Cores of freshwater methanogenic sediment were incubated at a range of temperatures to provide sediments with a range of rates of metabolism. Graphite electrodes were inserted at different depths in the sediments and electrically connected to graphite cathodes suspended in the overlying water through a resistor. Remarkably stable currents were recorded with higher currents at sediment depths or temperatures that were expected to have higher rates of microbial metabolism.

In order to directly compare current production with rates of microbial activity, the sediments near each electrode was sampled by subcoring and the subcores were injected with a [2-¹⁴C]-acetate tracer. Production of ¹⁴CH₄ and ¹⁴CO₂ were monitored over time to determine the rates of acetate metabolism in the sediments. There was a strong positive correlation between the rates of microbial metabolism determined with the traditional [2-¹⁴C]-acetate tracer techniques and the levels of current production. Similar studies were conducted on artic peat from the DOE Next-Generation Ecosystem Experiments (NGEE) Arctic project site in the Barrow Experimental Observatory near Barrow, Alaska. The correlation between rates of metabolism as determined with [2-¹⁴C]-acetate and current production fell on the same line as for the methanogenic sediments, despite the fact that in some of the artic peat sediments Fe(III) reduction appeared to be the predominant terminal electron-accepting process. Electrode deployments at the NGEE site generally yielded in situ currents consistent with the laboratory studies.
ABSTRACT: We are developing novel field-portable catalytic DNA biosensors to detect and quantify bioavailable radionuclides such as uranium, technetium, strontium, and plutonium as well as metal contaminants such as mercury and chromium. The sensors will be highly sensitive and selective, not only for different metal ions, but also for different oxidation states of the same metal ion (such as U(IV) vs. U(VI), or Cr(III) vs. Cr(VI)). To achieve the goals, we are using the combinatorial biological technique called in vitro selection to obtain catalytic DNAs that are highly specific for a given radionuclide or metal ion. We also use state-of-the-art biochemical and biophysical techniques to elucidate the structural elements responsible for high selectivity. These DNA have been transformed into fluorescent or colorimetric sensors by labeling the DNA with fluorophores or gold nanoparticles through catalytic beacon and nanoparticle assembly technologies developed in the PI’s group, with detection limit down to 11 ppt and over millions-of-fold selectivity of uranyl over other radionuclides and metal ions. These sensors have been developed into commercially available sensor products that allow detection and quantification of radionuclides and metal ions in less than 2 min.

In the past year, we have made significant progresses in three areas. First, we have developed label-free catalytic and molecular beacon containing an abasic site for sensitive fluorescent detection metal ions such as Pb²⁺.¹ The label-free method can significantly simplify the sensing method and lower its costs. Second, having obtained highly sensitive and selective sensors for radionuclides and metal ions, we have elucidated structural features and mechanism for the high sensitivity and selectivity, such as the importance of peripheral sequences in determining the metal selectivity of a catalytic DNA.² This knowledge will be quite valuable for designing new sensors and chelating agents for radionuclides and metal ions. Finally, for the first time, we have now extended the catalytic DNA sensors in detecting uranyl in living cells using catalytic DNA gold nanoparticles probe that can readily enter cells and can serve as a metal ion sensor within a cellular environment.³ Such a method can be generally applied to the detection of other radionuclides and metal ions using other catalytic DNA selected through in vitro selection.

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

NAME: Craig Lundstrom

ORGANIZATION: University of Illinois at Urbana-Champaign

PROGRAM AFFILIATION: SBR

ABSTRACT TITLE: Development of U isotope fractionation as an indicator of U(VI) reduction in U plumes

ABSTRACT: We are evaluating $^{238}\text{U}/^{235}\text{U}$ (as $\delta^{238}\text{U}$) as a tool for monitoring U(VI) reduction in the controlled field setting at the Rifle, CO IFRC site. Understanding the biogeochemical behavior of uranium in the subsurface will help to improve remedial strategies and long-term stewardship activities at DOE sites. We present $^{238}\text{U}/^{235}\text{U}$ (as $\delta^{238}\text{U}$) results for three Rifle IFRC field experiments: (1) the 2010–11 experiment, which combined bicarbonate-induced desorption of U(VI) and down-gradient acetate-induced bioreduction (21 and 25 days, respectively), (2) the subsequent 2011–12 experiment, which evaluated the impacts of prolonged bicarbonate and acetate amendments (94 and 72 days, respectively) and the impact of a second acetate injection and (3) the 2012–2013 reoxidation experiment.

Large variations in $\delta^{238}\text{U}$ accompany acetate-induced biostimulation under both iron reducing (2010–11) and sulfate reducing conditions (2011–12). In both cases, $^{238}\text{U}$ is preferentially reduced to U(IV), leaving the remaining groundwater U(VI) relatively enriched in $^{235}\text{U}$ ($\Delta^{238}\text{U} = -1.3$ and $-1.9\%$, respectively). Thus $\delta^{238}\text{U}$ in waters decreases as the U concentration drops from ~150–200 ppb U prior to the acetate injection to ~10 ppb after. For the 2010–11 experiment, groundwater U(VI) concentration and $\delta^{238}\text{U}$ quickly return in unison to background values within 113 days after the end of the acetate injection. During the post-injection phase, as concentrations rebounded, absence of $^{238}\text{U}/^{235}\text{U}$ greater than pre-injection values implies the primary source of U is advection of U(VI), rather than reoxidation of U(IV). This is particularly important as the long term success of this remediation technique depends on the stability of sequestered U(IV). Early results from the 2012–2013 reoxidation experiment suggest that as the dissolved oxygen injection proceeded, oxidation of U(IV) precipitates leads to both an increase in U concentration and $^{238}\text{U}/^{235}\text{U}$ of groundwater.

For 2010–11 samples impacted by both bicarbonate and acetate amendments, we observe an initial increase in U(VI) concentration (more than doubling) due to the bicarbonate desorption of U(VI) from mineral surfaces followed by a large drop in U(VI) concentration as acetate-induced reduction proceeds. As with the bicarbonate-only desorption experiment [1], U(VI) desorption resulted in no change in $^{238}\text{U}/^{235}\text{U}$; this ratio, however, changes with the onset of reduction with acetate amendment. $\delta^{238}\text{U}$ decreases quickly, then rebounds during the time of lowest dissolved U(VI) concentration, and finally increases slowly to background levels within 266 days from the end of the acetate injection. The much slower recovery is suggested to result from the slow exchange of newly advected U with the relatively light sorbed U (resulting from equilibration of sorption sites with light dissolved U(VI) during the biostimulation). Thus, bicarbonate-induced U desorption in combination with biostimulation increases the removal of U(VI) during biostimulation.

Our research demonstrates the potential for $^{238}\text{U}/^{235}\text{U}$ to detect U(VI) reduction in the subsurface and to distinguish between removal by reduction and relatively temporary processes such as sorption. Preliminary results from the latest experiment at Rifle suggest the $^{238}\text{U}/^{235}\text{U}$ technique can also be used to detect reoxidation of previously sequestered U.

NAME: Brian Mailloux  
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ABSTRACT TITLE: Linking As, Se, V, and Mn Behavior to Natural and Biostimulated Uranium Cycling

ABSTRACT: Biogeochemical redox cycles in groundwater systems frequently mobilize metals and metalloids that directly impact human health. These same biogeochemical cycles can also alter uranium speciation and mobility thus directly impacting groundwater quality at DOE legacy sites. Interestingly, the reducing conditions that immobilize uranium may be optimal for mobilizing metals such as As, Mn, Se, and V. These processes are occurring at the Department of Energy’s Field Research Challenge (IFRC) site near Rifle, Colorado and offer a unique opportunity to study biogeochemical redox cycles. At well U01 near the Colorado river, natural redox fluctuations occur with changing river stages. When the river stage is high the aquifer appears oxidized. When the river stage is low the aquifer becomes reducing and Mn, As, and sometimes Fe becomes mobilized. Interestingly, little change in U concentrations are observed. During biostimulation at the IFRC As concentrations dramatically increase in the groundwater. During Fe(III) reduction, As levels increase to concentrations similar to levels observed in well U01 (~1.0 µM). However under sulfate reducing conditions As concentrations can be over ten times higher. Initial speciation work by IC-ICP-MS from samples collected during the “Best Western” experiment in 2011 indicated that arsenite was the dominant species during Fe(III) reduction but thioarsenates, in particular trithioarsenate, become dominant during sulfate reduction. No thioarsenites were observed. Across the flow field arsenic species also varied with a higher proportion of thioarsenic species located near the injection well. Speciation results were in close agreement with geochemical modeling. Future work will focus on examining the interplay between organic carbon sources and arsenic speciation during natural redox fluctuations observed during changes in river stage. In addition, we will also attempt to determine the source of organic carbon for the regions of the aquifer that are naturally reducing. The goal is to better understand redox cycling and the source of organic carbon in aquifer systems.
Subsurface remediation and materials transport through soils depend on flow in porous media. Darcy’s law states that such flow is proportional to permeability, which depends in part on porosity. However, when colloidal materials (such as clays, precipitates, or microbes) form deposits, the resulting permeability cannot be accurately predicted from porosity. This is a major challenge for subsurface remediation, casting doubt on models linking biological, geological, and chemical processes. It has been hypothesized that the morphology of colloid deposits—specifically, their fractal dimension—could be a critical aspect relating deposition and permeability. Until now, determining colloid deposit morphology within porous media at the relevant spatial scale is an unsolved problem. To address this issue, we have developed a unique extension of static light scattering to measure the fractal dimension of colloid deposits within refractive index matched (i.e., transparent) porous media. The media in our flow column is Nafion, a synthetic material that becomes essentially transparent when saturated by a solution of isopropanol and water. We are studying the aggregation and deposition of colloidal polystyrene microspheres. Laser light scatters from the deposited colloids, but not from the transparent Nafion, such that the intensity of scattered light as a function of scattering angle indicates the fractal dimension.

While neither the solid nor fluid phase components are taken from natural environments, this system will permit us to study the relationship between deposit fractal dimension and flow within saturated columns under well characterized flow conditions. Currently, we are measuring how deposit fractal dimension varies with ionic strength, and how fluid velocity (i.e., shear stress) dynamically changes deposit morphology.

In complementary research aimed at generalizing these laboratory results to field settings, we are also measuring the fractal dimension of suspended colloids in groundwater collected from the Old Rifle Integrated Field Research Challenge (IFRC) site in western Colorado. Other samples, to be analyzed in Summer 2013, will provide fractal dimension of mineral precipitates and microbial biofilms collected from laboratory models prepared in collaboration with Lawrence Berkeley National Laboratory. The ultimate goal of this research is to create a clogging model linking environmental variables and colloidal phenomena to soil permeability, in order to reduce the associated uncertainty in models for subsurface remediation and materials transport.
ABSTRACT: The US Department of Energy (DOE) conducted 828 underground nuclear tests at the Nevada National Security Site (NNSS) from 1951 to 1992. The resulting cavities, sterilized by conditions sufficient to melt rock, afford an opportunity to examine microbial ecology in a zone where the juxtaposition of radioactivity with varying rock types, temperature, chemistry and hydrology enables the study of processes controlling radionuclide transport. This project focuses on the hypothesis that radiogenic substrates such as H2 at DOE sites may support the growth of microorganisms, and thus indirectly influence the mobility of redox-sensitive radionuclides. Consistent with this hypothesis were early project results (e.g. as reported at the 2012 SBR PI meeting) correlating the presence of both H2 and SSU rRNA genes nearly identical to those of candidatus Desulforudis audaxviator in radioactive fluids from the NNSS. In the South African deep subsurface, Desulforudis is believed to utilize H2 and SO4\(^{2-}\) from radiochemical reactions; and until recently, this region was thought to define the geographic limit of the genus. Thus, the detection of D. audaxviator in radioactive subsurface water at the NNSS (up to about 6% of all bacteria by pyrotag analysis) was taken to support the inferred radiochemical lifestyle for D. audaxviator. More recently, however, our group has detected Desulforudis rRNA genes (including near-perfect matches for D. audaxviator) in non-radioactive subsurface habitats on and near the NNSS; up to 1% of pyrotags in an 880 m-deep carbonate borehole near Death Valley, CA, for example. This observation, coupled with the fact that multiple bacterial and archaeal lineages appear to be shared between deep NNSS/Death Valley boreholes and very deep habitats in South Africa, suggests that the truth is more complex.

Since our previous report, we have made a concerted effort to bring some of these cosmopolitan deep microbial lineages into cultivation with the aim of determining their effects upon the mobility of DOE legacy wastes (e.g. Pu) using laboratory microcosms. In particular, energy resources and dissolved gases representative of relevant habitats have been targeted. These efforts have resulted in viable (albeit to date non-transferable) colonies of D. audaxviator. More robust cultivations of a variety of forms first detected at great depth in African mines have also been achieved. These include sulfate-reducing Firmicutes, methanogens, and especially a locally-abundant group of Thaumarchaeota (e.g. SAGMA-14) that appear to grow anaerobically in pure culture by oxidizing methane or ammonia with sulfate as an electron acceptor. These results suggest that Earth may indeed possess a recognizable deep, hard rock microbiome and that understanding the nature of this little known realm of the microbial world may be necessary for predictive understanding of radionuclide mobility at some DOE sites.
ABSTRACT: 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 carboxyl and phosphoryl functional groups to be the important metal binding groups on bacterial cell envelops. However, our XAS studies have indicated that Hg$^{2+}$ binds to sulfhydryl groups in preference to the more abundant carboxyl and phosphoryl groups on cell envelops when Hg concentration is submicromolar. The stoichiometry of these Hg-cysteine complexes on bacterial cell envelope changes as a function of the ratio of aqueous Hg concentration to bacterial cell density which has a significant impact on the solubility and bioavailability of Hg. The overall goal of our study is to provide a quantitative and mechanistic understanding of the impact of bacterial sulfhydryl groups on the uptake, speciation, transport and bioavailability of Hg in the environment.

We examined the speciation and stability of Hg complexes on the cell envelops of Bacillus subtilis, Shewanella oneidensis MR-1, and Geobacter sulfurreducens as a function of pH and aqueous Hg, in the presence and absence of organic (DOM) and inorganic (Cl$^-$) ligands. A novel technique using a fluophore was developed to characterize the concentration of reactive thiols on bacterial cell envelops. To complement fluorophore method, potentiometric titrations were performed after blocking the thiol sites. The estimated thiol density on the cell membranes of B. subtilis and S. oneidensis MR-1 obtained from these two different methods agrees well.

Hg XAS and UV-VIS luminescence spectroscopy studies indicate that Hg speciation is significantly different on methylating and non-methylating species. While the non-methylating species (S. oneidensis MR-1 and B. subtilis) form the biologically unavailable HgS$_3$ ($S= organic thiol$) complex at low nanomolar Hg concentration, the methylating species G. sulfurreducens forms the bioavailable complexes HgS$_2$ ($S= cysteine$) at nanomolar Hg concentrations. Hg speciation on G. sulfurreducens was counter-intuitive because it exhibited HgS$_2$ complexes although it has far more reactive thiols on its cell envelope when compared to S. oneidensis MR-1. Transition from thiol to carboxyl binding occurred in the concentration range when all thiol sites on cell envelope were saturated.

We are currently exploring whether the presence of Hg induces thiol release onto the cell membranes or modify the redox behavior of reduced S-groups in the cell envelope, and the role of DOM on Hg interactions with different bacteria under different pH and DOM concentrations. We are also interested in understanding the ultimate fate of Hg complexed with cell envelope after cell turnover. Mercury is a common contaminant at several DOE sites, and our study provides important insights on the understanding of the bioavailability, fate and transport of Hg at these sites.
ABSTRACT: The stability and dissolution rate of solid U(IV)-oxide phases in aquifers are important for transport of dissolved U as the mobile uranyl cation ($^{VI}$O$_2^{2+}$). Recent studies have suggested that microbially mediated, nitrate-dependent U(IV) oxidation under anaerobic conditions could complicate efforts at long-term reductive immobilization. We investigated the anaerobic, oxidative dissolution of biogenically produced U(IV)-oxide (nominally UO$_2$(s)) by either chemical oxidants (nitrate or nitrite) or by *Thiobacillus denitrificans*, a chemolitho-autotrophic bacterium that catalyzes anaerobic, nitrate-dependent U(IV) and Fe(II) oxidation. Mixtures of biogenic UO$_2$(s) and quartz with and without *T. denitrificans* were used in flow-through column experiments to examine coupled and competing oxidation-reduction processes. Abiotic oxidation of UO$_2$(s) in the presence of nitrate under anaerobic conditions was slow but faster than control experiments of non-oxidative dissolution. Abiotic UO$_2$(s) oxidation by nitrite was more rapid by several orders of magnitude. In the presence of *T. denitrificans* and dissolved nitrate, higher rates of dissolved U release were observed compared with abiotic controls, suggesting that *T. denitrificans* catalyzed the oxidative dissolution of UO$_2$(s) in addition to the abiotic oxidation pathways. X-ray spectroscopic characterization of reaction products indicated a fraction of solid-associated oxidized U(VI) that is retained in the column. Analysis of local atomic structures showed formation of U-oxo molecular moieties within or on particle surfaces that are similar but not identical to aqueous or sorbed uranyl species, suggesting mostly surface particle oxidation rather than detachment and re-adsorption of uranyl in the column. Reactive transport modeling (using Crunchflow) of column experiments incorporating thermodynamic solubility, irreversible overall abiotic and biotic kinetic reactions, and uranyl sorption on quartz was used to derive rates for overall kinetic reactions from column effluent U concentrations. Steady-state U concentrations were well simulated for a small amount of UO$_2$(s) oxidation relative to total UO$_2$(s) mass, but calculations were sensitive to particle surface area.

We investigated the enzymes involved in anaerobic, nitrate-dependent Fe(II) and U(IV) oxidation in *T. denitrificans*, as these enzymes have not yet been described for any other microorganisms that catalyze these processes. We previously reported that two c-type cytochromes, Tbd_0187 and Tbd_0146, were involved in anaerobic nitrate-dependent U(IV) oxidation in *T. denitrificans* and we hypothesized that c-type cytochromes would also catalyze nitrate-dependent Fe(II) oxidation. Studies to identify genes associated with nitrate-dependent Fe(II) oxidation included whole-genome transcriptional assays (including the use of FeCO$_3$, Fe$^{2+}$(aq), and U(IV) oxides as electron donors under denitrifying conditions), targeted insertion mutations of 26 genes of interest, and random transposon-mutagenesis studies with screening for Fe(II) oxidation. Non-defective mutants included the c$_1$-cytochrome subunit of the cytochrome bc$_1$ complex, which has relevance to a previously proposed role for this complex in nitrate-dependent Fe(II) oxidation. Of the transposon mutants defective in Fe(II) oxidation, one mutant with a disrupted gene associated with NADH:ubiquinone oxidoreductase (complex I) was >30% defective relative to the wild-type strain. Overall, our results indicate that nitrate-dependent Fe(II) oxidation in *T. denitrificans* is not catalyzed by the same c-type cytochromes involved in U(IV) oxidation, nor have other c-type cytochromes yet been implicated in the process.
NAME: Susan Pfiffner
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PROGRAM AFFILIATION: SBR
ABSTRACT TITLE: Metaproteomics of Metal and Radionuclide Bioreduction

ABSTRACT: The project goal is to utilize innovative proteomics tools to generate new information regarding microbial activity contributing to metal and U(VI) reduction, and thus enable science-based decision making for long-term site management. Current objectives are to: (1) examine c-type cytochromes in *Anaeromyxobacter dehalogenans* strain 2CP-C, *Shewanella oneidensis* strain MR-1, and *Geobacter daltonii* strain FRC-32 under different growth conditions and develop a sequence library comprised of c-type cytochromes implicated in metal reduction and other redox processes; (2) examine the proteomes of these strains and identify proteins that correlate with metal reduction; and (3) apply proteomic analyses to ORIFRC field samples to demonstrate the utility of this approach for site assessment, efficient implementation of bioremediation and long-term monitoring. We used a tiered approach that combines database mining, controlled laboratory studies, and PCR approaches to support the proteomics characterizations.

c-Type cytochromes are heme-containing proteins involved in electron transfer to oxidized metal species. The genomes of *A. dehalogenans* strain 2CP-C, *S. oneidensis* strain MR-1, and *G. daltonii* strain FRC-32 encode 69, 40, and 72 c-type cytochromes, respectively. Distinct c-type cytochrome expression patterns were observed in cells grown with the different electron acceptors. Our proteome measurements revealed that the number of c-type cytochromes identified in Fe(III) and Mn(IV) grown cells were 19 and 20 (out of 40) for strain MR-1; and 27 and 25 (out of 69) for strain 2CP-C. Proteomic characterization of c-type cytochrome expression revealed substrate-dependent responses suggesting that c-type cytochrome profiling provides information about cellular metabolic activity.

To extend the proteome approach to field samples, proteomic analysis was performed on groundwater filters from Area 2 of the ORIFRC which were collected 4 days after the emulsified vegetable oil (EVO) amendment. The altered groundwater community in EVO amended well was dominated by members of the *Betaproteobacteria* (i.e., *Dechloromonas*, *Ralstonia*, *Rhodoferax*, *Polaromonas*, *Delftia*, *Chromobacterium*) and *Firmicutes*. When metaproteomic workflows were applied to early biostimulation (4 days after EVO injection), distinct differences in protein expression were observed between groundwater collected from wells up-gradient and down-gradient of the EVO injection gallery. In the biostimulated sample the prominent proteins were categorized as being proteins involved in ammonium assimilation, nitrous oxide reduction, EVO degradation, and polyhydroxybutyrate formation. c-Type cytochromes and citrate synthase, which is a biomarker for hexavalent uranium reduction activity, were detected in low abundances suggesting that metal reduction has not commenced 4 days following EVO injection. Thus environmental metaproteomics provided valuable information and complemented nucleic acid-based approaches for identifying microbial community responses to biostimulation and elucidating active metabolic pathways.
ABSTRACT: The overarching objective of this work is to examine Pu(IV) and Pu(V) sorption to pure metal (oxyhydr)oxide minerals and sediments using variable temperature batch sorption, X-ray absorption spectroscopy, electron microscopy, and quantum-mechanical and empirical-potential calculations. The data will be compiled into a self-consistent surface complexation model. The novelty of this effort lies largely in the manner in which the information from these measurements and calculations will be combined into a model that will be used to evaluate the thermodynamics of Pu sorption reactions as well as to predict sorption of Pu to sediments from DOE sites using a component additivity approach. This study has demonstrated that sorption of Pu cannot be simply described by interactions between a single oxidation state. Rather the complex, redox sensitive nature of Pu must be fully constrained in order to obtain a sorption model which accurately predicts the observed species. This presentation will focus on the progress made in the past year examining 1) quantification of actinide sorption enthalpies, 2) examination of the mechanisms underlying surface mediated redox reactions, and 3) aging of Pu surface complexes resulting in irreversible or hysteretic sorption.

Quantification of actinide sorption enthalpies: The sorption of Eu(III), Np(V), Pu(IV), and Pu(V) to the iron oxide minerals hematite and goethite has been examined as a function of temperature. Quantifying the sorption constants allows for evaluation of the sorption enthalpy and entropy through the use of a van’t Hoff plot. Both Eu(III) sorption to hematite and Pu(IV) sorption to goethite had positive enthalpy values and entropy values. These measurements are consistent with the experimental hypothesis that removal of hydrating waters provides an entropically driven free energy of these sorption reactions. The entropy of Eu(III) and Pu(IV) were $439 \pm 26 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$ and $187 \pm 25 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$, respectively. The higher value for Eu(III) indicates the importance of the loss of at least 5 of the initial 9 hydrating water molecules upon sorption. This loss of water upon sorption and formation of a bidentate surface complex was verified using quantum mechanical modeling, extended x-ray absorption fine structure spectroscopy (EXAFS), and electron microscopy. The surface complexation models generated as part of this task have been used to successfully predict successfully sorption of Np(V) and Pu(IV/V) to Hanford 200 Area sediments.

Examination of surface mediated reduction: Using quantum mechanical modeling and dual isotope batch sorption experiments, a series of experiments has examined the reduction of Pu(V) to Pu(IV) on mineral surfaces. These experiments are vital to understanding the frequent observations of surface mediated Pu(V) reduction on non-redox active mineral surfaces such as quartz (which was verified in this work using X-ray absorption near edge structure spectroscopy, XANES). Dual isotope batch sorption tests using $^{242}$Pu and $^{238}$Pu demonstrated that processes such as generation of radiolytic byproducts of water and disproportionation of Pu at the surface were unlikely to be the cause of Pu(V) reduction. These results were supported by quantum mechanical modeling which examined the influence of various radiolytic byproducts which were co-adsorbed with Pu.

Aging of Pu surface complexes: Desorption of $^{239/240}$Pu was examined on a subsurface sediment from the Savannah River Site which had been aged 32 years. To compare the aged Pu with “fresh” Pu, $^{242}$Pu was
reacted with the aged Pu-contaminated soils for three days. Batch sorption/desorption experiments and selective extractions for total and amorphous iron demonstrated that the reversibility of sorption of Pu decreases significantly with aging time. This may be due to the transfer of Pu from amorphous to crystalline iron phases within the soil matrix or stronger binding of Pu to the solid surface based on the dehydration of the surface complex as described above.
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ABSTRACT TITLE: Scale-Dependent Fracture-Matrix Interactions And Their Impact on Radionuclide Transport

ABSTRACT: Matrix Diffusion and Adsorption within a rock matrix are important mechanisms for retarding transport of radionuclides in fractured rock. Due to computational limitations and difficulties in characterizing complex subsurface systems, diffusive exchange between a fracture network and surrounding rock matrix is often modeled using simplified conceptual representations. There is significant uncertainty in “effective” parameters used in these models, such as the “effective matrix diffusivity”. Often, these parameters are estimated by fitting sparse breakthrough data, and estimated values fall outside meaningful ranges, because simplified interpretive models do not consider complex three-dimensional flow. There is limited understanding of the relationship between the effective parameters and rock mass characteristics including network structure and matrix properties. There is also evidence for an apparent scale-dependence in “effective matrix diffusion” coefficients. These observations raise questions on whether fracture-matrix interaction parameters estimated from small-scale tracer tests can be used for predicting radionuclide fate and transport at the scale of DOE field sites.

High-resolution three-dimensional Discrete-Fracture-Network-Matrix (DFNM) models based on well-defined local scale transport equations can help to address some of these questions. Due to tremendous advances in computational technology over the last 10 years, DFNM modeling in relatively large domains is now feasible. The overarching objective of our research is to use DFNM modeling to improve fundamental understanding of how effective parameters in conceptual models are related to fracture network structure and matrix properties. An advanced three-dimensional DFNM model is being developed, which combines upscaled particle-tracking algorithms for fracture-matrix interaction and a parallel fracture-network flow simulator. The particle-tracking algorithms allow complexity in flow fields at different scales, and track transport across fracture-matrix interfaces based on rigorous local approximations to the transport equations. This modeling approach can incorporate aperture variability, multi-scale preferential flow and matrix heterogeneity. The code can handle computational domains with about 1 Billion nodes for flow and 1 Billion particles for transport. The overarching goal is to obtain insights on (i) the relationship between effective fracture-matrix interaction parameters, network structure and matrix properties and (ii) their scale dependence in different types of fractured rock environments.

We will present results obtained using “high-resolution” particle tracking algorithms in large-scale (~10,000 fracture) fracture networks in which small-scale aperture variability is explicitly represented within each fracture. Flow simulations in these fracture networks illustrate the important role of head variations along an intersection in driving flow through slow advective loops in dead-end fractures. Subsequent transport simulations demonstrate that these advective loops lead to long-tailed breakthrough curves even in the absence of true matrix diffusion. Ongoing efforts are focused on extending the network flow and transport simulations to very large scales by including recently developed “upscaled” particle-tracking algorithms, which allow use of much larger time steps. The
upscaled algorithms have been verified using the “very-high-resolution” simulation results as a benchmark, and hold significant promise as an efficient tool for field-scale simulation.

The final stage of our research will specifically target applications at the Oak Ridge Field Research Center, former nuclear test sites in Nevada (e.g. the Shoal and Bullion tests), and other field sites (e.g. Mirror Lake) where tracer tests were conducted to obtain fracture-matrix interaction parameters for site-scale transport models. We will explain the differences in behavior observed at these sites using our network model and subsequently simulate radionuclide transport at the site scale and 100+ year time scales.
ABSTRACT: 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, $^{129}$I, a major by-product of nuclear fission [1,2]. In order to assess the distribution of $^{129}$I and stable $^{127}$I in environmental systems, a sensitive and rapid method was developed which enabled us to determine isotopic ratios ($^{129}$I/$^{127}$I) and speciate I via GC-MS [3]. Results using this new method demonstrate that the mobility of $^{129}$I species greatly depends on the type of I species and its concentration [4,5], pH [6,7], and sediment redox state [7], with equilibration times taking up to 12 weeks [6,7]. For example, at ambient concentrations ($\sim 10^{-7}$ M), I$^-$ and 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), I$^-$ travels along with the water [5]. Iodate removal can also occur through incorporation into CaCO$_3$ crystal lattice, e.g., at the Hanford Site [8]. Iodide and iodate interactions with NOM leads to covalent binding of I to a limited number of aromatic carbon moieties on the particle surface [6,9,10,11]. Iodine association with NOM is important in sediments, even when organic carbon are very low (e.g., <0.2% at Hanford Site) [8,12]. Removal of iodine from the groundwater through interaction with NOM is complicated by the release of mobile organo-I species [9,10,11]. A small fraction of NOM that is bound to iodine can behave as a mobile organo-I source [9], a process that we were able to numerically simulate using kinetic Michaelis-Menton-type redox-reactions and kinetic uptake reactions [2]. Field [13] and laboratory studies evaluating the cause for steady increases in $^{129}$I concentrations (up to 1000 pCi L$^{-1}$, 3 orders of magnitude greater than drinking water limits of 1 pCi L$^{-1}$ $^{129}$I) emanating from radiological basins at SRS indicate that an increase of 0.7 pH units in groundwater over 17 years may explain the observed increased groundwater $^{129}$I concentrations [14]. Bacteria from a $^{129}$I-contaminated aerobic aquifer at the F-area of SRS can accumulate I$^-$ at environmentally relevant concentrations (10$^{-7}$ M), but account for only a minor fraction of total added iodide (0.2-2.0%) [15], indicating that bacterial I$^-$ accumulation likely does not account for the high fraction (up to 25% of total I) of measured organo-I in groundwater [4,16]. However, enzymatic oxidation of I$^-$ likely plays a greater role in iodination of NOM, and is currently being investigated further [15,17]. Iodide oxidizing activity of bacteria and organic acid produced by bacteria significantly contribute to organo-iodine formation [15,17]. Our references: [1] Kaplan et al. 2013. Crit. Rev. Environ. Sci. Technol., subm. [2] Chang et al. 2013. ES&T, subm. [3] Zhang et al. 2010. ES&T, 44, 9042. [4] Schwehr et al. 2009. ES&T 43, 7258. [5] Zhang et al. 2011. ES&T 45, 5543. [6] Xu et al. 2011. ES&T 45, 9975. [7] Emerson et al. 2013. Chem. Geol., subm. [8] Zhang et al. 2013. ES&T, in subm. [9] Xu et al. 2011. GCA 75, 5716. [10] Xu et al., 2013. STOTEN 449, 244. [11] Xu et al. 2012. GCA 97, 166. [12] Xu et al., 2013. ES&T, in subm. [13] Otosaka et al. 2011. STOTEN 409, 3857. [14] Kaplan et al. 2011. ES&T 45, 489. [15] Li et al. 2011. Appl. Environ. Microbiol. 77, 2153. [16] Li et al. 2012. p. 89-97. In: Interdisc. Stud. Environ. Chem. Vol. 6 - Environ Poll. Ecotox. Terra Sc. Publ. Comp. Tokyo. [17] Li et al. 2012. ES&T 46, 4837.
ABSTRACT: 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, $^{239,240}$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 [2,3]. The role of NOM in both immobilizing or re-mobilizing Pu thus has been demonstrated. It was found that partitioning coefficients ($K_d$) of intact humic acids (HAs) were significantly higher than those when HA was treated with HF, due to a lower number of chelating sites for Pu in the HF-treated HAs, or due to hydrophobicity differences between the two types of HAs. $K_d$s of Pu (IV) with HAs were higher at low pH (4.4) than those at high pH (7.1), in contrast to the observation of Pu sorption to most mineral phases [4], possibly caused by the increased solubility of HA under more alkaline conditions. Though the colloidal fraction of HAs (>1kDa MWCO) only accounts for a minor fraction of total OC (<5%) at pH 4.4, Pu binding to HAs accounts for 61-83% of the total added Pu, indicating that colloidal organic matter is the mobile Pu carrier in the wetland area. Lastly, $^{239,240}$Pu concentrations were found to be positively correlated to particulate hydroxamate and nitrogen contents, demonstrating Pu binding to siderophores, a NOM compound. Additional research involves the extraction and synthesis of the NOM precursor of the organic Pu carrier. In a previous study, almost all of the mobile $^{239,240}$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 (one type of siderophore compounds), and amide functionalities (both to chelate iron and Pu) [3]. To help understand Pu interactions with cutin and siderophore degradation products, a modified method [5] was used to extract the cuticle material from Western wheatgrass (Agropythi Smithii), one of the dominant vegetation species at the RFETS. The crude cuticle extract contains a significant portion of residual carbohydrates and amide functionalities. This cutin material was further purified to remove carbohydrates. Oxidation experiments were carried out to investigate the depolymerization of cutin, incorporation of carbohydrates, siderophore compounds, as well as the chelation of iron(III) (as a surrogate of Pu(IV)) during a simulated humification process. Finally, using advanced analytical tools to quantify and identify NOM moities ($^{13}$C NMR, HRMAS, ATR-FTIR, etc.), this material was compared with the naturally-occurring organic colloidal Pu carrier [3] to unravel the history of the molecular basis for the Pu vector in the environment. References: [1] Kaplan et al. (2007), ES&T 41, 7417-7423. [2] Santschi et al. ES&T 36, 3711-3719. (2002). [3] Xu et al. (2008) ES&T 42, 8211-8217. [4] Kaplan et al. (2006), ES&T 40, 5937-5942. [5] Deshmukh et al. (2003) Phytochemistry 64, 1163-1170.
ABSTRACT: 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:

1. Determine if electron transfer occurs between sorbed Fe(II) and structural Fe(III) in clays over a range of conditions and clay structures.
2. Evaluate whether Fe atom exchange occurs between aqueous Fe(II) and structural Fe(III) in clays, and natural, clay-rich sediments.
3. Assess whether Fe(II)-Fe(III)clay electron transfer and atom exchange will impact the redox cycling of U and inorganic Hg in the subsurface.

Our findings indicate that electron transfer occurs between sorbed Fe(II) and structural Fe(III) in two well-characterized smectites (NAu-1 and NAu-2). More specifically, our findings demonstrate that electron transfer to structural Fe in clay minerals can occur from Fe(II) sorbed to both basal planes and edge OH-groups. We used Mössbauer spectroscopy with selective chemical extractions to distinguish electron transfer from Fe(II) sorbed to basal planes and to edge OH-groups and observed more extensive electron transfer from edge OH-groups at pH value 7.5 compared to electron transfer from basal plane-sorbed Fe(II) at pH 4.0 and 6.0. Interestingly, the Mössbauer hyperfine parameters for the reduced structural Fe(II) in the clay mineral were distinctly different depending on whether reduction occurred from Fe(II) sorbed to basal planes or edge-OH groups. Preliminary data suggest that electron transfer results in exchange of Fe atoms between the aqueous phase and clay mineral structure as we have previously observed for Fe(III) oxides. Continuing work is underway combining enriched-57Fe tracers with monitoring natural, mass-dependent stable Fe isotope fractionation via 56Fe/54Fe to evaluate both the mechanism of atom exchange and whether Fe atom exchange may be accompanied by significant stable Fe isotope fractionation.

We also applied first principles calculations using a small polaron hopping approach and Marcus electron transfer theory to examine electron exchange mobilities in a smectite clay mineral. Our calculations provide rates of electron hopping that agree very well with values deduced from variable temperature Mössbauer data indicating a surprisingly fast electron mobility at room temperature and suggest that electron hopping within the octahedral sheet should dominant.
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ABSTRACT TITLE: Coupled biogeochemical exploration of contaminant mobility at the microbe / mineral interface

ABSTRACT: Concurrent arsenic mobilization to levels as high as 9 μM at the U.S. Department of Energy’s Integrated Field Research Challenge (IFRC) site in Rifle, Colorado highlights one of the challenges to widespread use of biostimulation for uranium immobilization. The addition of acetate to this shallow alluvial aquifer results in a shift from iron to sulfate reducing conditions. Arsenic release correlates with sulfate reduction and is characterized by a dominance of soluble arsenic-sulfur species (thioarsenics). To further explore this phenomenon, laboratory sediment columns derived from the site were seeded with Geobacter sulfurreducens and fed a synthetic groundwater containing 19 ± 2 mM acetate for ~135 days. After achieving iron-reducing conditions, columns were differentiated by three treatments: high sulfate (10 mM SO$_4^{2-}$), low sulfate (1mM SO$_4^{2-}$) and inhibition of sulfate reduction (10mM SO$_4^{2-}$ + 1.5 mM molybdate). The 'high sulfate' column is most analogous to field conditions at the Rifle IFRC, which had 8-10 mM SO$_4^{2-}$ in groundwater pre- and post-stimulation. In each case, column effluent showed an initial increase in soluble arsenic to approximately 1 μM, presumably associated with reductive dissolution of Fe- and Mn- solids, followed by a slow decline over the remainder of the experiment. While significant quantities of thioarsenic species could be formed in the laboratory by combining sulfide and arsenite at circumneutral pH in aqueous batch, arsenic breakthrough in the three flow through-columns was independent of sulfate-reduction. Similarly low concentrations of thioarsenics formed in the high sulfate, low sulfate and the sulfate-reduction-inhibition column which, by the end of the experiment, were evolving ~8,000 μM, ~300 μM, and ~10 μM sulfide, respectively. For comparison, sulfide concentrations reached ~300 μM in the field during peak sulfate reduction. Furthermore, the introduction of a pulse of soluble As(III) to the columns resulted in >85% of total arsenic being immobilized, presumably due to sorption or formation of FeS species, rather than significant thioarsenic evolution. Analysis of the microbial community by 16S rRNA gene 454 pyrosequencing indicates dominance by the orders of Clostridiales, Rhodospirillales, Burkholderiales, and Pseudomonadales with C. delftia as the most prevalent species. To further deconstruct the role of different microorganisms and geochemical conditions in these flow-through systems, work is transitioning to the mineral-microbe interface using sediment and mineral thin section (~60 um) coupons sorbed with As(V). By colonizing the coupons with axenic bacteria that are selected for iron, sulfate and arsenic respiratory processes, we can monitor for alterations in geochemical and biofilm properties associated with microbial colonization. Through ongoing biofilm visualization development pairing fluorescence in situ hybridization (FISH) with quantitative evaluation of minerals by scanning electron microscopy (QEMSCAN) new possibilities for visualizing these types of microbe-metal interactions is evolving. In addition a protocol for species-specific metallo-labeling of microbial cells for subsequent SEM and synchrotron-based micro-XAS analysis is currently being optimized in order to concurrently monitor elemental redox, mineralogical associations and microbial processes at the micron scale. This novel approach exploits the versatility and cost-efficiency of in situ hybridization-based methods without the limitations of fluorescence-based detection of cells in complex environmental samples. Collectively this work has importance in merging existing molecular and geochemical toolsets to investigate complex mineral-microbe processes relevant to metal fate and transport.
ABSTRACT: Mercury's preference for soft ligands such as thiols has been conceptualized in the theory of hard and soft acids and bases (HSAB), i.e. hard (less polarizable) metals have higher affinities for hard ligands than for soft (more polarizable) ligands, and vice versa. We have made the surprising finding that absolute affinities of Hg2+ for two anionic ligands (L-) computed in the gas phase trend in the opposite direction to those affinities computed in water. That is, the gas-phase free energy of forming HgL2 becomes more favorable with ligand hardness. In contrast, the aqueous-phase Hg2+ affinity increases with ligand softness. This switch in affinity upon hydration becomes apparent on addition of as few as two explicit water molecules and is obtained within both the chalcogenide and halide groups, in agreement with the long observed preference of Hg2+ for soft ligands in aqueous solution. Moreover, by comparing binding of one versus two anions to Hg2+, we found that the gas-phase trend for forming HgL2 arises from the enhanced reactivity of HgL+. Our approach establishes a quantitative theoretical basis for predicting Hg speciation in the biosphere.

Mercuric reductase, MerA, the key enzyme in the bacterial mercury resistance (mer) system, catalyzes the NADPH-dependent reduction of mercuric ion, Hg2+, to elemental Hg0. Each of the two monomers of the MerA homodimer contains a flavin adenine dinucleotide (FAD) cofactor that mediates electron transfer from NADPH to Hg2+ bound to the inner pair of cysteines in the active site (C136/C141 of Pseudomonas aeruginosa Tn501 MerA numbering). A second cysteine pair at the C-terminus of the other monomer (C558’/C559’) is essential for acquiring and transferring Hg2+ to the inner pair. Here, quantum mechanical/molecular mechanical (QM/MM) simulations elucidate the steps of MerA reduction, (a) equilibration between two redox states of the cofactors, NADPH/FAD and NADP+/FADH-, (b) Hg2+-transfer from the outer C-terminal cysteine pair to the inner active site cysteine pair, and (c) the reduction of the C136-C141 disulfide and the C136-S-Hg-S-C141 complex by FADH-, the two-electron reduced FAD. This analysis establishes the first atomic-level, energetic description of the MerA mechanism and contributes generally to understanding intracellular metal trafficking and catalysis by the larger family of NAD(P)-dependent flavin-disulfide oxidoreductases.
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ABSTRACT TITLE: Persistence of Microbially Facilitated Calcite Precipitation as an in situ Treatment for Strontium-90 and Other Metal Contaminant

ABSTRACT: Subsurface radionuclide and metal contaminants throughout the U.S. Department of Energy (DOE) complex pose one of DOE’s greatest challenges for long-term stewardship. One promising stabilization mechanism for divalent ions, such as the short-lived radionuclide $^{90}$Sr, is co-precipitation in calcite. We have previously found that nutrient addition can stimulate microbial ureolytic activity, that this activity accelerates calcite precipitation and co-precipitation of Sr, and that higher calcite precipitation rates can result in increased Sr partitioning. We have conducted integrated field, laboratory, and computational research to evaluate the relationships between ureolysis and calcite precipitation rates and trace metal partitioning under environmentally relevant conditions, and investigated the coupling between flow/flux manipulations and precipitate distribution.

A field experimental campaign conducted at the Integrated Field Research Challenge (IFRC) site located at Rifle, CO was based on a continuous recirculation design; water extracted from a down-gradient well was amended with urea and molasses (a carbon and electron donor) and re-injected into an up-gradient well. The goal of the recirculation design and simultaneous injection of urea and molasses was to uniformly accelerate the hydrolysis of urea and calcite precipitation over the entire inter-wellbore zone. The urea-molasses recirculation phase lasted, with brief interruptions for geophysical surveys, for 12 days followed by long-term monitoring which continued for 13 months. Following the recirculation phase we found persistent increases in urease activity (as determined from $^{14}$C labeled laboratory urea hydrolysis rates) in the upper portion of the inter-wellbore zone. We also observed an initial (first 2 weeks) increase in urea concentration associated with injection activities. After injection ceased, we observed a decreased urea concentration and increased in ammonium and dissolved inorganic carbon (DIC). Based on the loss of urea and the appearance of ammonium, a first order rate constant for urea hydrolysis of 0.18 day$^{-1}$ and an associated $R_f$ value for ammonium of 11 were estimated. The rate constant is approximately 6 times higher than estimated for previous field experiments conducted in eastern Idaho. Additionally, DIC carbon isotope ratios were measured for the groundwater. Injected urea had a $\delta^{13}$C of -40.7±0.4 ‰ compared to the background groundwater DIC $\delta^{13}$C of -16.6±0.2‰. Observed decreases in groundwater DIC $\delta^{13}$C of up to -19.8‰ followed temporal trends similar to those observed for ammonium and suggest that both the increase in ammonium and the shift in $\delta^{13}$C are the result of urea hydrolysis.

Analysis of a conservative tracer test conducted one year after the end of recirculation and comparison with a pre-injection tracer test indicated that the urea and molasses amendment resulted in preferential blocking of flow access to lower permeability zones and consequent flow focusing within the most conductive portion of the aquifer. Additionally, exchangeable ammonium was observed in the lower portion of the post-injection core. Although direct observation of calcite precipitation was not possible because of the high pre-existing calcite content in the site sediments, an observed $\delta^{13}$C decrease for solid carbonates from sediment samples collected following urea injection (compared to pre-injection values) is likely the result of the incorporation of inorganic carbon derived from urea hydrolysis into newly formed solid carbonates.
ABSTRACT: The main objective of this project is to examine the role of phosphohydrolases in naturally occurring subsurface bacteria for the purpose of promoting the immobilization of uranium through the formation of insoluble uranium phosphate minerals. Our prior work focused on pure culture and soil column studies that utilized contaminated soils from the DOE Oak Ridge Field Research Center (ORFRC) to demonstrate that microbial phosphatase activity liberated enough inorganic phosphate ($\text{PO}_4^{3-}$) from synthetic organophosphate compounds to promote uranium-phosphate mineral formation under oxic and anoxic conditions at different pH (pH 5.5 and 7). Simultaneously, addition of exogenous organophosphate was shown to promote the formation of intracellular polyphosphate. The objective of the present research was to examine the potential of utilizing phytate, a naturally-occurring and abundant organophosphate in soils, as a phosphorous source to promote U(VI)-phosphate biomineralization by natural microbial communities. While phytate hydrolysis in aerobic incubations of ORFRC soils was not evidenced at pH 7.0, complete hydrolysis was observed both with and without electron donor at pH 5.5, suggesting indigenous microorganisms express acidic phytases in these soils. The presence of uranium accelerated phytate hydrolysis but decreased the rate of hydrolysis of inositol intermediates as a result of a possible toxicity effect on the indigenous population. The abundant production of inorganic phosphate drastically decreased uranium solubility via formation of ternary sorption complexes and precipitation of U(VI)-phosphate minerals. Two phytase-positive microorganisms identified as Bradyrhizobium and Variovorax species by 16S rRNA sequencing were isolated from these soils. To determine the effect of uranium on phytate hydrolysis by ORFRC microorganisms, Variovorax sp. was exposed to increasing concentrations of uranium then incubated aerobically in pH 5.5 artificial groundwater amended with an electron donor and phytate as the sole phosphorus source. The hydrolysis of phytate by cells exposed to uranium was incomplete compared to unexposed cells, yet cells grew even in the presence of elevated uranium concentrations. Simultaneously, total inorganic phosphate production decreased linearly with increasing uranium concentrations, indicating a significant uranium toxicity effect on the cells. More importantly, exposure to increasing uranium concentrations resulted in earlier onset and more rapid accumulation of inorganic phosphate compared to identical reactors without uranium, suggesting phytate hydrolysis is activated as a uranium detoxification mechanism. Overall, the results of this study demonstrate the ability of natural microbial communities to liberate phosphate from phytate in acidic soils and the potential utility of phytate-promoted biomineralization of U(VI)-phosphate minerals as a uranium immobilization strategy.
ABSTRACT: Very effective Hg detoxifying proteins have evolved in many bacteria and archaeta. Exploiting removal of Hg$^{2+}$/MeHg$^+$ in wetlands by such naturally Hg resistant (HgR) bacteria requires knowing how these proteins work. We study HgR genes of α- and γ-proteobacteria and actinobacteria abundant in the ORR and report here on the mechanisms of the mer reductase (MerA) and demethylase (MerB) and regulatory proteins that optimize their expression. We also report on novel specific, high affinity Hg binding to natural, cellular nucleic acids.

β PROTEOBACTERIAL FUSED MerB-MerA: Ochrobactrum anthropi, a relative of α-proteobacteria found in the East Fork Poplar Creek, have a merBmerA gene encoding a novel fused MerB-MerA with demethylase and mer reductase activities. We cloned the full fusion and its separate merB and merA genes and properties of all three constructs will be presented.

γ PROTEOBACTERIAL MerA: Reduction of Hg$^{2+}$ in all MerA proteins occurs deep within the catalytic domain. Two cysteines near the C-terminus transfer Hg$^{2+}$ from dithiol complexes in solution to a pair of cysteines in the active site. Altered rates for Hg$^{2+}$ acquisition and reduction with a K449A mutation suggest K449 electrostatically controls dynamic motions of the C-terminal tail. Computational studies implicate two additional residues. Effects of mutations of these on kinetic and dynamic properties of the enzyme will be presented.

ACTINOBACTERIAL MerA/MerB: Relatives of Streptomyces lividans are also found in high Hg regions of the ORR. S. lividans MerA lacks the tethered NmerA domain found in γ-proteobacterial MerA, and its MerB lacks a cysteine of proteobacterial MerB essential for Hg$^{2+}$ transfer to NmerA, but has a distinct C-terminal cysteine pair whose role in RHg$^+$ binding and Hg$^{2+}$ release is being probed by mutagenesis.

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

PROTEOBACTERIAL REGULATION OF mer EXPRESSION: In gamma-proteobacteria, repressor-activator MerR holds RNA polymerase (RNAP) at the mer operator-promoter (MerOP) until Hg$^{2+}$ stimulates it to let RNAP transcribe. We find that Hg$^{2+}$ also binds the 38 bp MerOP DNA in the absence of thiols (as in acute Hg$^{2+}$ exposure) at 2-3 high affinity sites. The EXAFS spectrum of Hg-MerOP is indistinguishable from pure thymidine(T)+Hg and best fits two N ligands. Calorimetry reports similar high affinity sites in the upper and lower MerOP single strands and in the Hg+T interaction. Once formed, Hg-DNA adducts are only slowly removed by a two-fold excess of cysteine. T’s do not abut in B-form DNA, but MerOP may adopt a cruciform structure where two Ts abut in the lower strand loop and we are currently testing relevant mutants. Intact E.coli cells bind >3-fold more Hg$^{2+}$ than their available thiols could bind and EXAFS indicated N or O ligands are involved. MerOP-Hg binding reveals Hg binding distinct from the classic model based on slippage in AT runs, so DNA and RNA (uracil) may be an unrealized sink for Hg$^{2+}$.
with unknown turnover. We are pursuing this broader aspect of Hg biology with larger defined DNA and RNA molecules.

**ACTINOBACTERIAL REGULATION OF mer EXPRESSION:** In contrast, Actinobacteria control the *mer* operon with a simple ArsR-type repressor. The *S. lividans* MerR (SLMerR) is a distinct clade of the ArsR family which, by homology, shows a possible Hg$^{2+}$ binding site near its DNA recognition helix, very different from metal sites in other ArsR regulators. SLMerR over-expression was toxic in *E.coli* due to codon incompatibility, so we synthesized a codon-optimized *S.lividans merR* gene with a His-tagged SUMO fusion for facile purification of a tagless wildtype protein. This has worked beautifully with 100% cleavage yielding completely pure, soluble SLMerR at approximately 12-13 mg/L, ready to scale up for biochemical and biophysical studies.
NAME: Anne Summers
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PROGRAM AFFILIATION: SBR
ABSTRACT TITLE: Identifying Components of Toxic Metal Stress and Hg Methylation with Global Proteomics

ABSTRACT: Methylmercury (MeHg) and inorganic Hg directly inactivate proteins by binding to their cysteine or selenocysteine residues. As recently reported (Polacco, et al. MCP, 2011), we have devised a high-throughput global proteomics method using the 7 stable isotopes of Hg to identify proteins of *E.coli* most vulnerable to forming stable adducts of phenylmercury (PhHg; a proxy for methylmercury) and are now also using this proteomics method to identify proteins involved in Hg methylation by *Desulfovibrio* ND132. The *E.coli* dataset of ~1.62 million total observed MS2 spectra from three complete biological replicates identified 1562 of *E.coli* MG1655’s 4249 encoded proteins (37% of total) and 303 of these, in a wide variety of functional groups, had stable cysteine-Hg adducts (Zink et al, in preparation). Bulk cell properties altered by PhHg or Hg exposure include thiol homeostasis, electrolyte balance, and free iron (LaVoie et al, in preparation).

PROTEOMIC LABEL-FREE QUANTIFICATION OF Hg PEPTIDES – Quantification of unique modified peptides can afford comparison of their relative detectability as a function of different iodoacetamide (IAM) treatments after phenylmercury acetate (PMA) exposure. We devised an indirect and a direct method for quantifying peptides modified by PMA. First, since existing signal collation methods are challenged by the 7 stable isotopes of Hg, we simply used current label-free AMT methods to quantify indirectly the PMA-provoked decline in iodoacetylation modifications (CAM-mods) of 428 robustly observed peptide cysteines. PMA-exposure decreased CAM-mods in essentially all detectable cysteines by ~5-fold (average). This PMA-provoked decrease per-cysteine was sufficiently reproducible to allow ranking each cysteine for likelihood of PhHg modification. Our second approach to quantifying Hg-modified peptide cysteines was achieved by a novel computational breakthrough in LC-MS feature detection allowing use of the broad isotope envelopes of Hg peptides and, thereby, the direct quantification of 369 Hg-modified peptide cysteines. With this latter tool we found Hg-modified peptides decreased in cells treated with 20 mM IAM but not with 10 mM IAM (vs. no IAM treatment) but in this case the decrease was only 1.7-fold, a difference too small to rank cysteines as more or less likely to suffer an Hg-adduct. While it is not yet clear why the indirect metric of CAM-mods per peptide cysteine display a wider range than the direct measure of the Hg-mods themselves, this latter new method will greatly expedite robust direct assessment of the modifiability of individual peptide cysteines.

STABILITY OF Hg-PEPTIDES TO PROTEOMICS: We find the abundance of Hg-modifiable peptides is lower than their unmodified form and the same is true of the Hg-modified tripeptide, glutathione (GSH). Possible technical factors include differential stability to pre-column procedures, column conditions, and MS conditions. Initial experiments with a pure Hg-adduct peptide showed it was stable to a broad range of MS inlet temperature and voltage conditions. We are extending this analysis to a more hydrophobic peptide and additional work with Hg- and CAM-modified GSH and lysozyme.

Hg EXPOSOME OF A METHYLATING BACTERIUM: To identify proteins involved in Hg\(^{2+}\) uptake and methylation we have first done a total proteome on *Desulfovibrio* ND132 growing facultatively without Hg\(^{2+}\) to test the efficacy of our modified proteomics method on it. We saw 3008 proteins of 3455 encoded (87%; based on 66,121 distinct peptides) considerably more than reported on pyruvate or fumarate grown *Desulfovibrio* G20 (1900 identified proteins of 3258 encoded), likely due to better recovery of cysteine-containing peptides with IAM treatment. Indeed, we observed 1851 cysteine-
containing proteins of 3071 encoded (60%). Preliminary analysis of an Hg-exposed ND132 proteome done with and without IAM treatment yielded 28,622 distinct peptides corresponding to just 2,535 proteins. Of these, 3,921 distinct peptides were modified with CAM and 308 with Hg. Differences with respect to the no-Hg ND132 proteome and with respect to the *E.coli* PMA-exposure proteome will be discussed. In related work, a double mutant in the genes (*hgcAB*) encoding the newly discovered Hg(II) transmethylase is only slightly less resistant to Hg(II) than its parent wild-type strain, showing definitively that the ability to methylate Hg does not enhance resistance to Hg, as had been earlier suggested by work with unrelated methylating and non-methylating field isolates of *Desulfovibrio*. 
NAME: Martial Taillefert  
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PROGRAM AFFILIATION: SBR  
ABSTRACT TITLE: Mineral Solubility and Free Energy Controls on Microbial Reaction Kinetics: Application to Contaminant Transport in the Subsurface

ABSTRACT: Recent developments in the theoretical treatment of geomicrobial reaction processes have resulted in the formulation of kinetic models that directly link the rates of microbial respiration and growth to the corresponding thermodynamic driving forces. In this project, these kinetic models for the microbial reduction of uranium(VI) are verified and calibrated. The approach combines laboratory experiments on uranium bioreduction using pure cultures and natural samples, competition between reduction of U(VI) and iron oxides, and field-scale reactive transport modeling. Gibbs free energy yields are manipulated by varying the concentrations of U(VI), type and concentration of iron oxides, electron donor, as well as the carbonate alkalinity, and calcium concentrations. Rates of enzymatic reduction of U(VI) were measured under variable, but controlled, geochemical conditions to determine the effect of pH on bioreduction rates by *Shewanella putrefaciens*. In the presence of excess carbonates, the pH decreased over time prompting a shift in U(VI) speciation from U(VI)-carbonato complexes toward more readily-reducible U(VI)-hydroxide and increased bioreduction rates. Ca\(^{2+}\) suppressed the formation of labile U(VI) complexes and required a larger decrease in pH to achieve comparable rates. These results indicate that the main reducible fraction of U(VI) consists of hydroxide complexes, despite being the least abundant species in solution, and suggest that the pH decrease associated with U(IV) precipitation is required to promote U(VI) bioreduction in the presence of carbonates and calcium. If the pH is too low, however, it may have a toxic effect on cells. Experiments conducted at pH 7.0 in the presence of different carbonate and calcium concentrations revealed that exposure to uranyl species in these conditions for short periods of time decreased cell viability, though cells grew at the same rate once they recovered. Cell viability decreased with increasing concentration of non-carbonato complexes of U(VI) in solution, suggesting that these complexes are bioavailable yet toxic at high concentrations. 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, and calcium conditions. Overall, these findings suggest that uranyl hydroxide complexes are the main bioavailable species to *Shewanella* and potentially explain why it does typically not grow efficiently on uranium at pH < 8. Incubations are currently conducted with *Geobacter bemidjiensis* isolated from Rifle to determine whether a similar effect is observed with other metal-reducing bacteria. In addition, well-mixed retentostat reactor experiments using sterilized native Rifle sediments inoculated with *G. bemidjiensis* are underway to study microbial reaction kinetics at low to near-zero growth rates and in conditions close to thermodynamic equilibrium.
ABSTRACT: Injection of electron donors into the subsurface to stimulate soluble U(VI) reduction to less soluble U(IV) species is being tested as an approach for in situ remediation of U contamination. The success of this approach depends on the long-term stability of U(IV). The reduction of Fe and Mn oxides during U(VI) reduction produces reduced metals which may later be oxidized forming strong oxidants such as MnO₂ after the addition of electron donor has ceased and oxidizing conditions return. The primary focus of our project has been on the interactions between Mn and U and with groundwater constituents and their effects on U(IV) stability.

The effect of Mn(II) on the UO₂ dissolution rate under reducing and oxidizing conditions was investigated. Under reducing conditions, a strong inhibitory effect was observed. Solid characterization suggested that precipitation of MnCO₃ may limit the exposure of U(IV) surface sites on UO₂ to trace level oxidants. In contrast, a promotional effect was observed under oxic conditions. It is hypothesized that Mn(II) adsorbed to UO₂ can be oxidized by dissolved O₂. The oxidation of Mn(II) could form reactive Mn(III) or Mn(IV) species, which could serve as additional oxidants for UO₂ that accelerate UO₂ dissolution. As an intermediate in Mn biogeochemical cycling, soluble Mn(III) has recently been found as an important environmental oxidant in oxic-anoxic interfaces. The kinetics of oxidative UO₂ dissolution by soluble Mn(III) stabilized by pyrophosphate was quantified. Soluble Mn(III) induced rapid UO₂ dissolution at a rate higher than by a comparable concentration of dissolved O₂. Self-consistent kinetic models were derived with excellent fits of the experimental results. The quantification of the reaction kinetics of oxidative UO₂ dissolution by soluble Mn(III) may help interpret the enhanced UO₂ oxidation mediated by Mn redox cycling and inferred from field measurements at Rifle, CO.

Our previous work in diffusion-limited agarose gels has shown that microbial Mn(II) oxidation did not enhance U(IV) oxidation by oxygen but that U(VI) produced was less mobile due to its sorption onto newly formed MnO₂. This past year we probed more complex sediment systems and evaluated whether the same outcome would be observed. Duplicate columns packed with Old Rifle sediments were run in the laboratory with an influent simulating Rifle groundwater, amended with U(VI) and either containing or devoid of sulfate. After U(VI) reduction and the accumulation of U(IV) over ~75 days, replicate columns were switched to an effluent containing 50 µM Mn(II) and 5% O₂ or only 5% O₂ to simulate oxidizing conditions. While microbial Mn(II) oxidation had been reported for unaltered sediments, the presence of U(IV) or the reducing conditions prevalent in the columns precluded significant Mn oxidation in the columns. However, the oxidation of U(IV) by O₂ was evident and led to the release of U(VI) in the effluent. We examined the microscopic distribution of U, Fe, S and Ca for columns before and after O₂ oxidation. In reduced sediments U was often associated with Ca but not directly with Fe or S. This suggests Ca adsorption to the surface of UO₂ as well as the association of Ca with non-crystalline U(IV). In the oxidized sediments, U was found primarily in association with S⁰, iron sulfide minerals or CaSO₄, suggesting that the oxidation of S²⁻ by O₂ produces either S⁰ or SO₄²⁻. The implications of this work are that microbially mediated Mn(II) oxidation may be limited in reduced sediments despite the availability of O₂ and that the mechanism of redox buffering in sulfate-reducing sediments include the formation of S⁰ and CaSO₄.
ABSTRACT: Limitation/bioavailability of terminal electron acceptors (TEA) and electron donors is an environmentally relevant condition that affects the overall subsurface bioremediation process and efficiency. Microorganisms exist under "feast or famine mode" of survival, however they typically encounter famine conditions. In situ bioremediation technology uses indigenous microorganisms to treat large scale subsurface contamination. The technology relies on addition of suitable electron donors to subsurface sediments to stimulate bacterial activity. However, the high electron donor concentration conditions are artificial and transitory. Similarly TEA is also limited, we suggest that TEA-starvation conditions would evolve at injection wells after prolonged carbon addition. The work described here aims to understand the survival strategies of the anaerobe Geobacter sulfurreducens under both electron acceptor and electron donor limiting conditions.

Our results have demonstrated that G. sulfurreducens can survive under both TEA (fumarate) and electron donor (acetate) limiting conditions and displays five typical stages of growth: lag, log, stationary, death, and survival phases. The organism can sustain a stable population of \(~10^6\) cells/ml for over 2 years under TEA limiting conditions. Whereas it can sustain a higher population count of \(~10^8\) cells/ml for over 1 year under electron donor limiting conditions. Global comparative proteomic analysis was performed using iTRAQ and proteins varying in abundance with a high level of statistical significance (p<0.05) were identified. Under TEA starvation, 103 proteins were identified to be significantly up-regulated compared to 85 significantly down-regulated in survival phase cells as compared to mid-log cells. The most highly represented and significantly up-regulated proteins in the starved cells are involved in energy metabolism, cell envelope, and transport and binding functional categories. The majority of them were found to be localized in the cell membranes. The above results suggest that changes in the outer and cytoplasmic membrane are important in the survival of Geobacter under the aforesaid conditions. The cell shuts down anabolic processes and is poised, through changes in its membrane proteins, to sense nutrients in the environment, to transport nutrients into the cell and finally to detect/utilize TEAs it may encounter. Under TEA-limiting conditions, the cell is also highly reduced with minimal change in energy charge. Therefore, our proteomic and biochemical results indicates that even under TEA starvation, cells are well suited for bioremediation via reduction of radionuclides. The proteomic analysis of acetate starved cultures identified only 27 significantly up-regulated proteins compared to 196 significantly down-regulated in survival phase cells as compared to mid-log cells. Most of the up-regulated proteins belonged to energy metabolism, transport and binding, and protein fate functional categories. Most of the down-regulated proteins were ribosomal, transcription and translation proteins involved in protein synthesis, similar to TEA starvation condition. Here again, the majority of the up-regulated proteins were predicted to localized in the cytoplasmic membrane, and most of the down-regulated proteins were predicted to be localized in the cytoplasm. So far we were able to demonstrate that Geobacter can survive long-term nutrient limiting conditions and the differences in protein levels between the growing and starved cells. We will further investigate the genetic basis of the survival capacity by genomic sequencing.
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ABSTRACT TITLE: Microbiological-enhanced mixing across scales during in-situ bioreduction of metals and radionuclides at Department of Energy Sites  

ABSTRACT: Bioremediation is being investigated as an effective strategy 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 H₂ 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.

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. Selenite was chosen as a representative metal and was reduced to an insoluble precipitate inside the microfluidic reactor by *Anaeromyxobacter dehalogenes*. Precipitates were analyzed by Raman spectroscopy and energy dispersive spectroscopy to confirm the presence of reduced selenium. The density of bacteria appeared greatest on the edges of the red precipitates, suggesting that microenvironments favorable for selenite reduction and growth occur near existing crystals.

Microfluidic experiments were modeled in 2-D to predict biomass distribution and chemical mixing at the pore scale in homogenous soils. Electron donor and acceptor were delivered through the inlets and mixed occurred along the centerline by transverse diffusion. Results indicate that microbes grow along the entire centerline where mixing occurs, that shear stress mitigates growth in pore throats but promotes growth in pore bodies, and that literature parameters for growth kinetics obtained from batch reactors can be used to simulate reactive transport at the pore scale.

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.
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ABSTRACT TITLE: Multi-isotope tools for understanding interactions of nitrogen cycling with iron and uranium in the subsurface

ABSTRACT: As the most ubiquitous contaminant anion in groundwater sediments at US DOE sites, high levels of nitrate (NO$_3^-$) also co-occur with one or more priority contaminant metals or radionuclides. This project seeks to improve our understanding of subsurface biogeochemical nitrogen cycling and its multifaceted interactions with redox-active elements, in particular iron, in order to better predict the fate and transport of the widespread radionuclide uranium (U) in complex subsurface environments. In particular, bioremediation efforts focused on immobilizing U through stimulation of microbial reduction of U(VI) to U(IV) are inhibited by the presence of NO$_3^-$. Furthermore, the potential for introduction of nitrate-contaminated groundwater poses a direct and serious threat to the stability of uranium immobilization in subsurface sediments both directly, through the oxidation of U(IV) by NO$_3^-$ and/or the reductive intermediate NO$_2^-$, or indirectly by coupling of N and Fe cycling. As such, the interpretation of the results of field-scale bioremediation studies are often complicated by the fact that several competing processes occur simultaneously. Through the use of new and integrated multi-isotope tools, which represent naturally occurring spatial and temporal integrators of cycling processes, this project aims to improve the detection of biogeochemical changes in the subsurface, particularly those involving the direct and/or indirect coupling of nitrogen cycling to U(IV) oxidation and mobilization. Specifically, the use of coupled isotope systems (e.g. $^{15}$N/$^{14}$N and $^{18}$O/$^{16}$O and the ratio of their respective isotopic fractionation or $^{18}\varepsilon : {^{15}\varepsilon}$) provides an even more powerful constraint on quantifying the relative roles of simultaneously occurring cycling processes in the context of subsurface redox transformations, including those responsible for U mobilization.

Experiments were conducted to characterize the N and O kinetic isotope effects of abiotic reduction of both NO$_3^-$ and NO$_2^-$ by Fe(II) as a starting point for characterizing linkages among subsurface N, Fe and U cycling at the field-scale. Results indicate substantial reduction of both NO$_3^-$ and NO$_2^-$ by Fe(II) in both the presence or absence of iron oxyhydroxides minerals. A range of N and O kinetic isotope effects (for both NO$_3^-$ and NO$_2^-$) were observed across experimental conditions ($^{15}\varepsilon_{NO_3} = 6.8$ to $32.7\%$, $^{18}\varepsilon_{NO_3} = 3.4$ to $19.7\%$; $^{15}\varepsilon_{NO_2} \sim 11.5\%$, $^{18}\varepsilon_{NO_2} \sim 4$-$6\%$). Importantly, in all cases, abiotic reduction of either NO$_3^-$ or NO$_2^-$ by Fe(II) exhibited smaller O isotope effects than N isotope effects ($^{18}\varepsilon : {^{15}\varepsilon} = 0.4$ to $0.7$). In contrast to canonical denitrification ($^{18}\varepsilon : {^{15}\varepsilon} = 1$), values of $^{18}\varepsilon : {^{15}\varepsilon}$ in NO$_3^-$-contaminated groundwaters commonly fall between 0.5 and 0.8. As such, our results point to a potentially widespread importance of Fe (II) based N reduction in these environments.

During the next phase of characterization, a series of 17 flow-through natural sediment columns was run for ~60 days under reducing conditions. In a subset of columns, Fe reduction was allowed to occur for 30 days, followed by the addition of NO$_3^-$. Multi-isotope data showed prominent shifts in $^{18}\varepsilon : {^{15}\varepsilon}$ over the course of NO$_3^-$ addition and are interpreted as reflecting a shift from an initial Fe(II)-based reduction of NO$_3^-$ to NH$_4^+$ to the onset of biological denitrification (NO$_3^-$ reduction to N$_2$). Based on these results, we posit that tracking changes in NO$_3^-$ and NO$_2^-$ concentration and $\delta^{15}$N and $\delta^{18}$O along with aqueous and solid phase U and Fe chemistry in future experiments will allow partitioning of the specific N reducing processes directly and indirectly related to U(IV) reoxidation and mobilization under advective flow.
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ABSTRACT TITLE: Impact of viral infection of microbiota on subsurface biogeochemistry
ABSTRACT: Microbially mediated metabolisms have been identified as significant factors either directly or indirectly impacting biogeochemical cycling. To date the role that viruses play a significant role influencing microbial mortality and resulting community in terrestrial sedimentary systems is poorly understood. The objective of this project is to investigate viral infection of subsurface bacteria and the formation of contaminant-bearing viral particles and approached by examining the following hypotheses: (i) subsurface microorganisms are susceptible to viral infections, and (ii) viral surfaces will adsorb heavy metals and radionuclides.

In an effort to assess the significance of viral infection in subsurface microbial communities, the production of viral like particles in response to biostimulation was investigated by amending sediment slurries with dissolved organic carbon (\(^{13}\)C-labeled acetate) and nitrate. This stimulation resulted in the production of VLPs (Virus to Bacteria ratio ca. 480 to 2,400). Interestingly, the production of VLPs was positively correlated with acetate consumption and \(^{13}\)CO\(_2\) production; whereas changes in bacterial abundance were correlated neither with acetate consumption or \(^{13}\)CO\(_2\) production. Thus, indicating that viruses contribute to cell lysis and are sensitive indicators of microbial activity. Furthermore, the microbial community shifted from one favored by Betaproteobacteria to Gammaproteobacteria. This could simply be a result of “killing the winner” or microbial cell lysis could cross-feed the microbial community. These results suggest that viral infection could be playing a role in carbon flux thereby altering available carbon pools in subsurface environments.

The in situ addition of O\(_2\) into a reduced well field at the Old Rifle Field site, well-field Exp. Plot C, resulted in an increase in VLPs in background and stimulated wells (9.5 x 10\(^5\) – 3.1 x 10\(^6\) in CU01; 2.2 x 10\(^6\) – 3.2 x 10\(^6\) in CD01, respectively), suggesting viral response during redox shifts. VLP abundance decreased (3.1 x 10\(^6\) – 1.3 x 10\(^6\) in CU01; 3.2 x 10\(^6\) – 3.2 x 10\(^5\) in CD01) concurrent with a sharp change in geochemical parameters of the aquifer 21-28 days after injection. The VLP abundance decrease was associated with an increase in the ORP, decrease in pH and Fe(II) concentration and increase in sulfide concentrations. Viral abundance fluctuated with geochemical changes; whereas cell abundance did not similarly respond. This result is consistent our prior laboratory experiments demonstrating a correlation between carbon consumption and viral abundance, but not cell abundance. Together these results indicate that virus-to-cell ratio and viral abundance may be better indicators of biological activity than cell abundance alone as dynamic viral infection mediates cell lysis.

The production of VLP’s in groundwater has implications for nanoparticulate carbon or metal transport. Initial experiments indicate that metals (Zn\(^{2+}\)) will adsorb to the surface of Escherichia coli phage T4. Interestingly, as Zn\(^{2+}\) concentrations increased (ca.60 µM), infectivity increased. Results demonstrated an increase of plaque forming units (PFUs) for viruses incubated with Zn\(^{2+}\) relative to negative controls. Thus, indicating that Zn\(^{2+}\) enhances the infectivity of phage T4. Together, the results suggest that the sorption of metals to the surface of viruses could not only contribute to nanoparticulate transport but could also enhance infectivity further contributing to cell lysis. It is therefore necessary to establish potential relationship(s) between viruses, cells, carbon, and metals/radionuclides to provide sufficient scientific understanding to incorporate coupled physical, chemical, and biological processes into agent based and reactive transport models.
Subsurface environmental systems are open and complex, in which intricate biogeochemical processes interact across multiple spatial and temporal scales. Understanding and predicting system responses to natural forces and human activities is indispensable for environmental management and protection. However, predictions of the subsurface system are inherently uncertain, and uncertainty is one of the greatest obstacles in groundwater reactive transport modeling. The goal of this project is two-fold: (1) developing new computational and mathematical methods for quantification of predictive uncertainty, and (2) using the developed methods as the basis to develop new methods of experimental design and data collection for reduction of predictive uncertainty. The proposed computational Bayesian framework is expected to be general and applicable to gain insights into subsurface biogeochemical processes that occur across a wide range of field sites and environmental conditions.

In the first year of the project, we are focused on attaining the first object to develop a computational Bayesian framework for uncertainty quantification. The framework considers various sources of uncertainty in data, model structures, model parameters, and driving forces (e.g., natural changes related to climate change and human-induced engineering remediation). The framework is implemented using Bayesian network. In the network, model components (deterministic and stochastic) are separated into network nodes, and relations between the components are represented by network edges, which are also pathways of uncertainty propagation. To evaluate the developed method, a Bayesian network was developed for model scenarios of flooding and precipitation, groundwater flow and reactive transport problems. Contribution of different uncertainty sources to predictive uncertainty was quantified.

Uncertainty quantification within the network is conducted using the state-of-the-art sparse grid methods, which are computationally efficient and can be integrated with the Bayesian network seamlessly. The sparse grid methods can alleviate the problem of curse-of-the-dimensionality of conventional Monte Carlo methods by selecting a small number of sparse grid points (in parameter space) to evaluate statistical moments of quantities of interest. On the other hand, the sparse grid methods can also be used to develop a surrogate of the original model, a polynomial-like interpolation that is fast to evaluate. We have applied the sparse grid methods to groundwater flow and reactive transport problems. Since reactive transport problems are more challenging because of model nonlinearity, more advanced sparse grid methods are necessary such as high-order stochastic collocation method with adaptive schemes.

To thoroughly evaluate the developed methods, a synthetic problem of hexavalent uranium (U(VI)) reactive transport was developed based on data and information at the Naturita UMTRA site. The synthetic problem considers three geological settings, three kinds of boundary conditions, and nine geochemical reactions. We are conducting uncertainty quantification using conventional methods, and the results will be used to evaluate computational efficiency and accuracy of the sparse grid methods. The computational tool will be used to the real-world modeling at Naturita to gain insights into groundwater reactive transport modeling.