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University Abstracts: SBR Oriented

Mercury stable isotope exchange between dissolved Hg(0) and Hg(II)-thiol complexes

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Background: Mercury (Hg) stable isotopes have been employed to trace Hg sources and chemical transformations in the environment. In anoxic waters, dissolved Hg(0) and Hg(II)-complexes often coexist but the isotope exchange between mercury species is poorly understood. In this study, we carried out laboratory experiments to examine Hg stable isotope exchange between dissolved Hg(0)-Hg(II)-thiol complexes.

Research Methods: Experiments were performed to determine the rate at which isotopes are exchanged and isotopic equilibrium between dissolved Hg(0) and thiol-complexed Hg(II) under anoxic conditions. The dissolved Hg(0) pool was generated by a metallic Hg bead in a silicone tube, equilibrated with water. The Hg(0) in solution was then partially oxidized to form Hg(II). The Hg(0)/Hg(II) solution was reacted with either mercaptoacetic acid (MCA), mercaptopropionic acid (2-MPA), cysteine, or humic acids. The aqueous Hg(II) was allowed to complex with the thiol ligand. Once chemical equilibrium was reached, reactors were periodically sacrificed for isotopic analysis. For mass spectrometry, samples were diluted, then spiked with a calibrated ²⁰⁴Hg/¹⁹⁶Hg double spike. Isotopic compositions (¹⁹⁹Hg/¹⁹⁸Hg, ²⁰⁰Hg/¹⁹⁸Hg, ²⁰¹Hg/¹⁹⁸Hg, ²⁰²Hg/¹⁹⁸Hg) were measured on a Nu Plasma multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS).

Results: At the beginning of the experiment, the Hg(0)/Hg(II) solution showed a $\delta^{202}\text{Hg}$ difference between the Hg(II) pool and the dissolved Hg(0) pool of 2.4‰. Addition of MCA caused Hg(II) to complex with thiol groups, and initially the Hg(II) retained its isotopic composition. Isotopic exchange between the Hg(II)-thiol complexes and the Hg(0) occurred within the first 12 hours, driving both toward isotopic equilibrium. After 48 hours, the equilibrium isotopic fractionation between Hg(0) and thiol-Hg(II) was approximately 0.7 ‰. The results suggest that Hg isotope ratios can be affected without chemical transformations taking place. In our presentation, we will discuss the implications of our findings for interpreting isotopic data collected for groundwater containing both Hg(0) and Hg(II) species.

Fate of Uranium in Wetlands: Impact of Drought followed by Re-flooding

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Uranium contamination in groundwater can be mitigated in anoxic zones by iron-reducing bacteria that reduce soluble U(VI) to insoluble U(IV) and by uranium immobilization through complexation and sorption. Wetlands often link ground and surface-waters, making them strategic systems for potentially limiting migration of uranium contamination of water resources. Little is known about how drought periods that result in the drying of wetland soils, and consequent redox changes, affect uranium fate and transport in wetlands. In order to better understand the fate and stability of immobilized uranium in wetland soils, and how dry periods affect the uranium stability, we dosed saturated wetland mesocosms planted with *Scirpus acutus* with low levels of uranyl-acetate for 5 months before imposing a 9-day drying period followed by a 13-day rewetting period. Concentrations of uranium in mesocosm effluent increased after rewetting, but the cumulative amount of uranium released in the 13 days following the drying constituted less than 1% of the uranium immobilized in the soil during the 5 months prior to the drought. This low level of remobilization suggests that the uranium immobilized in these soils was not primarily bioreduced U(IV), which could have been oxidized to soluble U(VI) during the drought and released in the effluent during the subsequent flood. XANES analyses confirm that most of the uranium immobilized in the mesocosms was U(VI). Compared to mesocosms that did not experience drying or rewetting, mesocosms that were sacrificed immediately after drying and after 13 days of rewetting had less uranium in soil near roots and more uranium on root surfaces. Mapping of uranium and iron on root samples before and after drying has been performed to understand whether this migration of uranium is attributable to coprecipitation or sorption of dislodged uranium on roots' iron oxide coatings. Results show that short periods of drought conditions in a wetland may impact uranium distribution, but these conditions may not cause large losses of immobilized uranium from the wetland. Further experiments investigated the possibility of links between uranium immobilization and nitrogen cycling in wetlands. A recently identified *Acidimicrobiaceae* bacterium (A6) derives energy from ammonium oxidation coupled with iron reduction. This bacterium has been found in uranium-contaminated wetland sediments at the Savannah River Site. Lab experiments have demonstrated that A6 can use U(VI) as an electron acceptor in the presence of ammonium, suggesting that natural sites of active ammonium oxidation by A6 could be hotspots of uranium immobilization.

Seeing What a Microbe Sees: Real-Time Geochemical Monitoring with ‘Geo-Sense’
Toshiyuki Ueki, Dawn E. Holmes, David Walker, Kelly P. Nevin, and Derek R. Lovley

A limitation in biogeochemical modeling is a lack of real-time data on the *in situ* geochemical environment that directly impacts on microbial activity. Traditional analytical methods are often unable to distinguish speciation and/or complexation of molecules that have important influences on the interaction of those molecules with microbial enzymes. Furthermore, invasive sampling techniques, as well as the associated labor and analytical costs, prevent real-time analyses and limit the extent to which environmental heterogeneities and perturbations can be explored. We previously described SMART (Subsurface Microbial Activity in Real Time) technology, which provides real-time, continuous estimates of the overall rates of microbial activity in anaerobic soils and sediments. SMART takes advantage of the fact that the current-producing activity of microbes that colonize electrodes inserted into anaerobic soils reflects the activity of microbes in the nearby surrounding soil. In order to better understand the factors that control these rates of microbial activity, we are developing ‘Geo-Sense’ real-time biological sensors. Geo-Sense biosensors are designed to continuously report on the concentrations of important geochemical constituents that bacteria actually sense. The biosensors are constructed with strains of *Geobacter sulfurreducens* that specifically produce an electrical current in response to chemicals that interact with engineered genetic regulatory circuits. In this manner, the electrical response is to the biologically available fraction of nutrients or inhibitory compounds, providing a more microbiologically relevant analysis compared with bulk chemical analysis. Although biological sensors based on current-producing microorganisms have been described previously, those sensors relied on controlling the expression of proteins necessary for electron transfer to electrodes. This approach yields slow response times and lacks resolution. Geo-Sense biosensors utilize a different approach to control the current output. Proof-of-concept studies with either isopropyl β -D-1-thiogalactopyranoside (IPTG) or anhydrotetracycline as the inducer yielded promising results and Geo-Sense strains have now been produced for the detection of iron, arsenic, or mercury. The Geo-Sense platform offers the potential for construction of a wide diversity of chemicals for which a microbial sensor system can be identified. To date Geo-Sense has only been tested in controlled laboratory conditions. However, a strategy for field deployment based on our previously patented microbial fuel cell technology has been developed. It is expected that coupling Geo-Sense and SMART technologies will make it feasible to inexpensively and remotely collect real-time data not only on rates of microbial activity but important geochemical parameters influencing microbial activity in a diversity of soils and sediments.

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

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Clay minerals are ubiquitous in soils, sediments, and aquatic environments, and contain a significant proportion of the iron present in these environments. Fe-bearing clay minerals serve as an important source and sink for electrons in redox reactions in various subsurface geochemical environments. The 2:1 tetrahedral-to-octahedral smectite clay minerals contain a wide range of Fe contents from trace (e.g. montmorillonites) to 30% of their mass (e.g. nontronites). Here we focus on the redox reaction between aqueous $\text{Fe(II)}_{\text{aq}}$ and structural $\text{Fe(III)}_{\text{clay}}$ in smectites. We isolate the reduction of $\text{Fe(III)}_{\text{clay}}$ by $\text{Fe(II)}_{\text{aq}}$ using the isotope specificity of ^{57}Fe -Mössbauer spectroscopy. In experiments with Mössbauer-inactive $^{56}\text{Fe(II)}$, we observe more relative reduction of $\text{Fe(III)}_{\text{clay}}$ by $\text{Fe(II)}_{\text{aq}}$ for the low-Fe clay mineral SWy-2 (67% reduction) than for high-Fe containing nontronites NAu-1 and NAu-2 (10-20% reduction). Mössbauer spectra suggest that electrons are localized in SWy-2 as distinct $\text{Fe(II)}_{\text{clay}}$ species, which contrasts with the observed hopping of electrons between $\text{Fe(II)}_{\text{clay}}$ and $\text{Fe(III)}_{\text{clay}}$ for the nontronites. Our observations are consistent with clustering of Fe atoms in the nontronites but not SWy-2.

We also use enriched isotope tracers to track movement of Fe atoms between solution and the structure of the clay minerals. Our results indicate up to nearly 20% exchange between $\text{Fe(II)}_{\text{aq}}$ and NAu-2 over 6 months in pH 7.5 suspension, with less, but still significant amounts of exchange at pH 6.0. The clay minerals SWy-2 and NAu-1 exchange approximately 7 to 10% of their Fe with solution. Our calculations indicate that these levels of exchange require mixing of at least 2-3 unit cells deep, and suggest that a significant amount of Fe in the clay mineral structure is susceptible to reduction and atom exchange.

Radiocarbon Signature of Microbial DNA and RNA from a Reducing Zone of a Floodplain aquifer, Rifle Colorado

University-Led Research, SBR

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

Mailloux, B.J., Trembath-Reichert, E., Cheung, J., Watson, M., Stute, M., Freyer, G.A., Ferguson, A.S., Ahmed, K.M., Alam, M.J., Buchholz, B.A., Thomas, J., Layton, A.C., Zheng, Y., Bostick, B.C., van Geen, A., 2013. Advection of surface-derived organic carbon fuels microbial reduction in Bangladesh groundwater. PNAS 110, 5331-5335.

Competitive Complexation of Mercury with Thiols from Natural Organic Matter and Bacterial Cell Envelopes

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Thiols are known to control the speciation and bioavailability of Hg by 1) providing high-affinity binding sites, 2) mediating redox transformations, and 3) controlling precipitation and colloidal formation. Natural organic matter (NOM) is considered the main sources of thiols in aquatic environments, and is believed to control the fate of Hg. Our studies have shown that bacterial cell envelopes are an additional source of thiols in aquatic and terrestrial systems. Complexation of Hg with thiols associated with NOM is similar to that of cell envelopes in many regards. The discovery of Hg-thiol binding on bacterial cell envelopes raises the question of the relative importance of NOM compared to cells in controlling the overall fate and transport of Hg. Using Suwanee River Fulvic acid (FA) as a proxy for NOM, we conducted Hg and S X-ray Absorption Spectroscopy (XAS) experiments to evaluate the chemical reactivity and stability of Hg complexed with NOM and bacterial cell envelopes.

Hg XANES and EXAFS spectroscopic results indicate that Hg binds predominantly to the high-affinity thiol groups on bacterial cell envelopes in the presence and absence of FA. Hg binding mechanisms with the bacterial biomass do not change in the presence of FA, ruling out the possibility of the formation of ternary complexes. Additionally, pH does not affect the binding mechanism of Hg onto biomass in the presence of FA. Hg XAS results suggest that thiols on *Shewanella oneidensis* MR-1 cell envelopes out-compete thiols in FA for Hg binding, and similarly S XANES results suggest that, on an average Hg binding to FA appears weaker than Hg binding to bacterial biomass. S XANES measurements of *S. oneidensis* MR-1 show that nearly the entire S budget of the biomass is present as reduced S groups, a fraction of which is known to form strong bonds with Hg. However, FA has a range of reduced and oxidized S species.

In summary, the speciation and distribution of Hg bound to NOM or bacteria is highly sensitive to their relative concentrations and to the specific make-up of thiol within each complexant. We have conducted further studies to understand the nature and behavior of Hg complexation with several NOM and DOM of varying age and composition. Our studies illustrate weakening of the binding strength of Hg complexation with DOM as a function of age, probably due to slow oxidation of thiols in DOM. Clearly, further studies are required to understand the reactivity and stability of Hg bound to NOM and bacterial thiol sites.

***Anaeromyxobacter dehalogenans* Strain 2CP-C Employs Distinct Metabolic Activities for Growth with Metal vs. Non-metal Electron Acceptors**

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Anaeromyxobacter dehalogenans strain 2CP-C is a facultative Gram-negative Gamma-proteobacterium related to the *Myxobacteria*. Unlike other members of *Myxobacteria*, which were considered obligate aerobes, *A. dehalogenans* is capable of growth and survival in anoxic and suboxic environments. Studies of anaerobic growth of strain 2CP-C revealed tremendous respiratory versatility, as evidenced by the ability to utilize a range of electron donors (e.g. acetate, hydrogen, pyruvate, lactate, succinate, formate) and electron acceptors (e.g. nitrate, fumarate, halogenated phenols, ferric iron, nitrous oxide, etc.). In particular, the dissimilatory metabolic reduction of metals, including radionuclides, by strain 2CP-C spurred interest for *in situ* bioremediation of contaminated soils and sediments.

In order to understand the cellular mechanisms that enable this microbe to survive under different environmental conditions, a mass spectrometry-based proteomics approach was implemented to characterize the proteome profiles of strain 2CP-C grown with various electron acceptors. A total of eight growth conditions were tested, providing a global survey of the proteome-wide responses to different electron acceptors. The pan-proteome consists of 2,846 proteins, representing 65% of predicted open reading frames. The results also revealed a core proteome of 710 proteins that comprise the fundamental cellular machinery needed regardless of varying electron-accepting environments. To visualize proteins significantly changed between growth conditions, differentially abundant proteins were mapped to metabolic pathways in KEGG database using iPath 2.0. Significantly abundant proteins in metal electron acceptor growth mapped to metabolic pathways participating in the TCA cycle, and amino acid, nucleotide and carbohydrate metabolism, whereas significantly abundant proteins in non-metal electron acceptor growth mostly involved in regulatory pathways for translation and cell motility. Metabolic pathways mapping clearly indicated elevated expression of energy production pathways in growth with metal electron acceptors. Amino acid and nucleotide metabolism also demonstrated significant higher abundance levels in metal electron acceptor- growth, which could be a result of overall higher expression of energy-generating pathways in cells grown with metal electron acceptors.

Taken together, the ANOVA analysis and pathway mapping results demonstrated the distinct effects of metal and non-metal growth conditions on the proteome expression of *A. dehalogenans* strain 2CP-C. In particular, growth with metal electron acceptor resulted in elevated abundances for proteins involved in energy production compared to cells using non-metal electron acceptors.

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Radionuclide Waste Disposal: Development of Multi-scale Experimental and Modeling Capabilities *University-Led Research*

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This project is a Department of Energy, Experimental Program to Stimulate Competitive Research (EPSCoR) Implementation Grant. The experimental and modeling efforts of this project are guided by the overarching scientific question:

What are the major molecular level chemical, biological, and microbial interactions that control the mobility of radionuclides in natural and engineered systems and how can these molecular and pore scale processes be properly defined and quantified for incorporation into larger scale, coupled experimental systems and reactive transport modeling efforts?

The key issues to be addressed include identifying source terms for contaminants in geologic disposal scenarios, determining the chemical speciation of risk-driving radionuclides (*e.g.*, Np, Tc, Cs, U, I) within engineered waste forms and natural subsurface environments, delineating the biogeochemical and physical processes through which contaminant transport is manifested, and predicting contaminant mobility across wide temporal and spatial scales. The project is undertaken by an interdisciplinary team from three South Carolina universities. The project is divided into four major tasks as well as the development of a new imaging facility capable of monitoring the 2D and 3D transport of radionuclides through engineered waste forms and natural soils.

Major accomplishments of the project to date include:

- Testing of Single Photon Emission Computed Tomography (SPECT) imaging systems using a column with an idealized soil structure.
- Design and groundbreaking on a field lysimeter facility used to monitor transport of U, Tc, Np, I, and Cs under natural conditions.
- Experimental and quantum-mechanical modeling studies of hollandite compositions of the form $Ba_xCs_{2.32-2x}Ga_{2.32}Ti_{5.68}O_{16}$ are underway with varying A site (Ba/Cs) composition.
- Characterization of Tc behavior in reducing grout/cementitious waste forms under controlled laboratory and field conditions have examined the rate of Tc(IV) oxidation to Tc_2S_7 and TcO_4^- . Complimentary studies are underway to examine the use of graphitic nanoreinforcements to alter strength and porosity of the waste form.
- Component additivity surface complexation models describing Tc, U, and Cs sorption to a Savannah River Site sandy loam soil have been developed.
- A kinetic model describing competitive ion sorption processes has been developed which can account for competition between weakly and strongly sorbing ions across a wide range of concentrations. A kinetic rather than equilibrium model is needed to account for the expected disequilibrium within pore waters.

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

University-Led Research

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

This work has taken a detailed approach to understanding the reactivity of Pu and other actinides with goethite, hematite, and kaolinite surfaces and describe the data using a surface complexation modeling approach. The specific surface complexes in these models have been constrained to the extent possible by direct (x-ray absorption spectroscopy, XAS) and indirect (solvent extraction) methods of determining the Pu oxidation state and chemical species. For example, batch sorption and x-ray absorption near edge spectroscopy (XANES) starting with either Pu(III), Pu(IV), or Pu(V/VI) have demonstrated that Pu(IV) becomes the dominant oxidation state on quartz and hematite surfaces regardless of the initial oxidation state. Furthermore, variable temperature batch sorption experiments have demonstrated sorption of Eu(III), Th(IV), Pu(IV), Pu(V), and Np(V) to goethite (and hematite for Eu) increases with increasing pH and increasing temperature. The data indicate sorption is endothermic and that the increase in sorption with temperature is driven by a positive entropy. These measurements are consistent with the experimental hypothesis that removal of hydrating waters provides an entropically driven free energy of these sorption reactions. This behavior has been supported using XAS and quantum mechanical modeling.

The surface complexation models described above have been used to describe Pu sorption and redox reactions with soils from the Savannah River Site and the Hanford 200 Area using a component additivity approach which accounts for specific mineral phases within each soil. The models are capable of predicting the degree of sorption observed on relatively short time scales. However, observations of Pu desorption from a SRS soil which has been contaminated with Pu for 30+ years indicate that desorption is either drastically kinetically hindered or irreversible. Thus, there appears to be an aging effect that is not currently accounted for in our thermochemically based modeling approach. The results from these experimental measurements are being compared with quantum mechanical calculations of actinide interactions with pure mineral phases. Current experimental efforts are focused on characterizing changes in Pu(IV) and Pu(V) source materials used in a new set of field lysimeter experiments called the Radiological Field Lysimeter Experiment (RadFLEX) at the SRS.

Plutonium Immobilization and Mobilization by Soil Organic Matter

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Pu is believed to be essentially immobile in groundwater due to its low solubility and high particle reactivity to mineral phase or natural organic matter (NOM). However, previous studies reported Pu can be transported several kilometers in surface water systems, in the form of a colloidal organic matter carrier, through wind/water interactions. The role of NOM in both immobilizing or re-mobilizing Pu thus has been demonstrated. For example, in sediments collected from SRS, close to a wetland and a groundwater plume, ^{239,240}Pu concentrations are more closely correlated with organic nitrogen content than the bulk organic carbon content, suggesting ^{239,240}Pu immobilization by nitrogen-containing organic compounds. More intriguingly, hydroxamate siderophores, which are a strong iron-complexing ligand produced by microbes, are shown to be one class of the nitrogen-containing compounds (0.15-5.51% of total organic nitrogen) and are thought to be responsible for scavenging and fixing Pu in the sediment, due to its high binding constants. Additional supporting evidence that nitrogen-containing organic compounds control Pu transport was obtained from surface soil cores from three distinct soil types: paddy soil, deciduous soil and coniferous soil in the Fukushima Prefecture after the FDNPP accident. Although Pu was believed to be mostly contributed by global fallout, atmospheric fallout from 1963-1979, and atomic bomb events prior to the FDNPP accident, we found that Pu was strongly associated with organic nitrogen in these sediments, too. Electrospray Ionization-Fourier Transform Ion Cyclotron Mass Spectrometry (ESI-FTICRMS) was applied to investigate the Pu enriched fraction, which was collected from a surface sediment soil of the SRS by several separation and purification steps: 1) soil leaching by artificial groundwater; 2) filtration and ultrafiltration to obtain the 0.45 μ m to 1 kDa fraction; 3) isoelectric focusing electrophoresis (IEF) experiment to obtain the Pu-enriched fraction; 4) ultrafiltration to rinse off the detergent and buffer. Pu-enriched macromolecules mostly belong to the class of lipids, but are also contributed by lignin, proteins, unsaturated hydrocarbons and condensed hydrocarbons. Formulas of the nitrogen-containing compounds overlap with the bulk formulas. A comparison of the mass spectra of DFO-B and Pu-enriched macromolecules confirm the presence of siderophores in this Pu organic carrier phase. It is thus very likely that hydroxamate siderophores, with a molecule weight of less than 1 kDa, together with other unknown nitrogen-containing compounds, were once produced by microbes and then incorporated into a large macromolecule. This provides a novel mechanism for surface Pu migration in organic-rich terrestrial systems.

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

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Hg(II) uptake is a key first step in the methylation of Hg(II) by anaerobic bacteria and thus, conditions affecting its transport greatly influence the amount of methylmercury which accumulates. Experiments with mercury methylating and non-methylating bacteria support a common active Hg(II) uptake mechanism inhibited by Zn(II) and Cd(II) but not other divalent metals. Thus, Hg(II) uptake appears to be a result of accidental import during the acquisition of essential trace metals, such as Zn(II). While the mechanism and rate of uptake is remarkably similar in methylating and non-methylating bacteria, differences have been observed in Hg bioavailability to organisms living at different redox zones. For instance, a fermentative firmicute, *Ethanoligenes harbiense*, had low affinity for Hg(II) and exhibited the lowest accumulation rates relative to other organisms tested. The iron-reducing bacteria, *Shewanella oneidensis* and *Geobacter sulfurreducens* both showed similar initial uptake rates and specificities for Hg-thiol complexes with some thiol ligands, such as cysteine, enhancing uptake and other thiols such as penicillamine or glutathione inhibiting uptake. In contrast, the sulfate-reducing bacterium, *Desulfovibrio* sp. ND132, isolated from highly sulfidic estuarine waters, had the highest affinity for Hg thiol complexes and was able to take up Hg bound to a variety of thiols, including both penicillamine and glutathione, neither of which support Hg uptake in iron-reducing bacteria. These results demonstrate differences in Hg bioavailability across taxa and respiratory guild. Data obtained from this study is critical for understanding how different populations respond to changing Hg speciation in order to better predict methylmercury accumulation in the environment.

Genomic and synchrotron based investigation of metal immobilization during fermentation-supported sulfate reduction

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University-Led Research

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Subsurface and bioreactor systems containing an abundance of solid organic substrates offer a rich environment in which to study the influence of microbial ecology on sulfide-driven metal immobilization. These syntrophic systems rely on cellulolytic and saccharolytic fermenters to metabolize lignocellulosic materials into small organic acids and alcohols. Resulting soluble products are in turn utilized as electron donors by sulfate-reducing bacteria (SRB) who produce sulfide that can react with soluble contaminants such as zinc, copper and nickel to form insoluble metal-sulfides. In this research, we employed seven ex situ pilot-scale and six lab-scale sulfate-reducing bioreactors amended with different solid substrate permutations that received circumneutral, Zn-laden mining-impacted water. Next generation sequencing was used in conjunction with multivariate statistics to identify temporal and spatial phylogenetic trends with the ability to correlate to metabolic drivers of community composition. Despite conservation in the overall organoheterotrophic community across substrate permutations (varying percentages of woodchips and alfalfa) results indicated more recalcitrant lignocellulosic substrates selected for higher ratios of bacteroidetes to firmicutes as well as less pronounced sulfate reduction and Zn immobilization. *Ruminococcus sp.* and *Dysgonomonas sp.*, frequently linked to cellulose and cellobiose fermentation, were present in all columns regardless of substrate, although *Ruminococcus sp.* was preferentially correlated with woodchip content. In contrast, *Treponema sp.* was predominantly encountered in alfalfa-containing columns. Synchrotron-based analysis revealed geochemical patterns where Zn and Ni correlated spatially with S, indicative of sulfide-driven metal immobilization. S-speciation analysis was also used to differentiate between metal-bound sulfide, sulfate associated with metals, and organically bound sulfate. In addition, metallo-labeling of specific microbial genera using biotinylated 16S rRNA targeted DNA-probes, followed by incubation with a streptavidin-nanogold conjugate and subsequent gold-enhancement (Gold-FISH) was improved by targeting multiple ribosomal sites, thus significantly increasing the signal to noise ratio without severely affecting the redox chemistry of the microbe-mineral interface.

The natural environment of *Geobacter sulfurreducens* is oligotrophic and described as limiting in both electron donors and terminal electron acceptors (TEA). We examine here long-term batch cultures under limiting electron donors or the TEAs. The microorganism survived under long-term electron donor (acetate) starvation, maintaining a stable population of $\sim 1-2 \times 10^8$ cells mL⁻¹ for >650 days. Proteins that varied in abundance with a high level of statistical significance ($p < 0.05$) for stages between mid-log to survival phase (acetate starved) were identified using mass spectroscopy. The most highly represented proteins that significantly increased in level in the survival phase cells are generally membrane-associated and are involved in energy metabolism and protein fate. These results document that changes in the outer and cytoplasmic membranes help *G. sulfurreducens* survive during starvation through detection and transport of nutrients into the cell. A sizeable portion of the identified proteins with unknown or hypothetical function further suggest that much of the biological process involved in survival have yet to be fully understood. *G. sulfurreducens* was also able to survive under long-term TEA-starvation conditions with iron citrate as TEA and maintained a stable population of $1.5-3 \times 10^7$ cells mL⁻¹ for >650 days. We also found that survival phase cells from fumarate-limiting conditions were able to quickly resuscitate and reduce metal such as ferric iron as compared to the mid-log phase cells.