Subsurface Biosphere Initiative
Research and education focused on life below Earth's surface.

Subsurface Biosphere Initiative and IGERT Workshop

June 15-17, 2008
Newport, Oregon

Agenda
List of Poster Presentations
Abstracts

Subsurface Biosphere
IGERT Program

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Sunday Evening, June 15

7:15 **Lew Semprini, SBI Executive Committee Chair**
Introductory talk about SBI and Workshop

7:30 **Keynote Speaker: Eric Triplett, Professor and Chair, Department of Microbiology and Cell Science, University of Florida**
Some Grand Challenges for the Future of Soil Microbial Ecology

8:30 **Campfire Social**

Monday Morning, June 16

8:00 **Jennifer Pett-Ridge, NanoSIMS group, Chemical Sciences Division, Lawrence Livermore National Laboratory**
Visualizing Single Cell Biogeochemistry: NanoSIMS Studies of Microbial Ecology

8:45 **Laurel Kluber, Oregon State University IGERT Student**
Do Mats Matter: The Role of Ectomycorrhizal Mats in Structuring Microbial Communities in Old-Growth Forest Soils

9:15 **Break**

9:45 **Radu Popa, Portland State University**
Nitrogen Bio-Cycling in Astrobiology and Early Life Evolution

10:30 **Break for lunch**

Monday Afternoon, June 17

1:00 **Dave Myrold: Update from the IGERT Director**

1:15 **Maria Dragila, Oregon State University**
Movement of Micro-Bodies in Porous Media: Micromodel Visualization Experiments

2:00 **Hollie Oakes-Miller, Portland State University IGERT Student**
Streamer Forming Microbial Communities and Biosignature Preservation in Phototrophic Streamer Mats from a Silica Depositing Hot Spring, Queens Laundry, Yellowstone National Park

2:30 **Break**

2:45 **2008 IGERT Group Process Training**
Gilberto E. Flores, Dannie Jansik, Humberto Nation, Ellen Swogger and Rick Colwell
Interdisciplinary Science and the Subsurface Biosphere: Future Directions

4:00 **Free Time and Dinner Break**

Monday Evening, June 18

7:00 **Student PowerPoint Poster Presentations and Open Bar**

Tuesday Morning, June 19

8:00 **Harry Beller, Lawrence Berkeley National Laboratory**
Genome-Enabled Studies of Uranium Biogeochemistry: New Insights into Anaerobic U(IV) Oxidation

9:00 **Sean Sandborgh, Oregon State University IGERT Student**
Physiological and Transcriptional Response of Nitrosomonas europaea *to Inhibition by Chlorobenzene*

9:30 **Break**

10:00 **Tyler Radniecki**, **Oregon State University**
Inhibition of Nitrifying Bacteria by Heavy Metals; Physiological and Transcriptional Responses upon Exposure to Cu^{2+}, Zn^{2+} *and* Cd^{2+}

10:30 **Jim Laidler, Portland State University IGERT Student**
Silicification of Viruses in a Simulated Hydrothermal Environment ("Virus under Glass")

11:00 **Other business**

12:00 **Checkout**

Poster Presentations

Subsurface Biosphere Initiative and IGERT Workshop

Newport, Oregon

Azizian, Mohammad
Comparison of Lactate and Formate as Hydrogen Donors for the Reductive Dehalogenation of Trichloroethene in a Continuous-Flow Column

Brewer, Elizabeth A.
Estimating Nitrogen Transformation Rates in Long-Term Soil Organic Matter Manipulation Plots

Caldwell, Sara
Iron Reduction and Oxidation in Aquificales *from Hydrothermal Systems*

Eberly, Jed
Analysis of Thermophilic Cyanobacteria for Biosolar Hydrogen Production

Flores, Gilberto E.
Initial Investigations into the Ecology of Thermoacidophilic Microorganisms Inhabiting Deep-sea Hydrothermal Chimneys

Jansik, Danielle
Spatial Distribution of Biofilms in Porous Media

Keiluweit, Marco
The Role of Electron Donor-Acceptor Complexes in Organo-Mineral Interactions

Kraft, Erika L.
Multi-Spectral Signal Collection in Porous Media for Observing Transport at the Meso Scale

Mustafa, Nizar
Numerical Simulation of the Anaerobic Transformation of Chlorinated Aliphatic Hydrocarbons in a Continuous Flow Column

Nielsen, Mark
Investigating the Mechanism of Current Production in Benthic Microbial Fuel Cells
A Novel Device to Controls Fuel Cell Voltage and Step It Up to a Level Appropriate for Powering Sensors in Aquatic Environments

Sabalowsky, Andrew R.
The Effect of Formate Vs. Lactate on Performance and Community Evolution in Dechlorinating Consortia Grown in Chemostats Treating TCE-Saturated Media

Saini, Gaurav
Determining the Effect of Operational Parameters on Cell Surface Hydrophobicity Measurements by Microbial Adhesion to Hydrocarbons (MATH) Test

Taylor, Anne
Physiology of Alkene Metabolism in VC-Utilizing Nocardioides *Strain JS614*

Abstracts of Talks

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In Agenda Order

Some Grand Challenges for the Future of Soil Microbial Ecology

Eric Triplett, Professor and Chair, Department of Microbiology and Cell Science, University of Florida

Soils are considered the most biologically diverse habitat on Earth. The tremendous amount of surface area available on soil particles permits many habitats for soil microbes in a small volume. Estimates of the number of bacterial species in a single gram of soil vary between 2,000 and 8 million. The most important genetic marker for prokaryotic diversity in any environment is the highly conserved small subunit of the ribosome, the 16S rRNA gene. This gene is easily amplified with universal primers and the amplified products sequenced directly through 454 pyrosequencing. Our analysis to date suggests that there are about 20,000 bacterial "species" in a gram of soil and that the four soils analyzed differ greatly in bacterial and archaeal diversity (Roesch et al. 2007). Of the 130,479 bacterial sequences obtained from four soils, 65.3% could be classified to the genus level and 36.6% were members of the ten most abundant genera in these samples. Over 400,000 sequences have now been obtained from a soil in Florida in an attempt to directly test our ability to predict the number of prokaryotic organisms in a gram of soil.

The identity of the organisms we discovered in soil through this analysis was intriguing. We have found very few organisms in common among the four soils tested (Fulthorpe et al. 2008). The identity of the dominant organisms found in each soil was surprising. Differences in archaea among the four soils have suggested a mode for the regulation of archaeal diversity and abundance in agroecosystems.

Our seemingly random choice of four soils for our initial high throughput 16S rRNA analysis has led to some interesting future questions in soil as well as other environments. The project has also inspired people in my lab to learn more about analyzing data. Hence, this work has been a terrific educational journey for all of us.

Visualizing Single Cell Biogeochemistry: NanoSIMS Studies of Microbial Ecology

Jennifer Pett-Ridge, NanoSIMS group, Chemical Sciences Division, Lawrence Livermore National Laboratory

Linking phylogenetic information to function in microbial communities is a key challenge for microbial ecology. Isotope-labeling experiments provide a useful means to investigate the ecophysiology of microbial populations and cells in the environment and allow measurement of nutrient transfers between cell types, symbionts and consortia. The combination of Nano-Secondary Ion Mass Spectrometry (NanoSIMS) analysis, in situ labeling and high resolution microscopy allows isotopic analysis to be linked to phylogeny and morphology and holds great promise for fine-scale studies of microbial systems. In NanoSIMS analysis, samples are sputtered with an energetic primary beam (Cs^+, O^-) liberating secondary ions that are separated by the mass spectrometer and detected in a suite of electron multipliers. Five isotopic species may be analyzed concurrently with spatial resolution as fine as 50nm. A high sensitivity isotope ratio 'map' can then be generated for the analyzed area.

NanoSIMS images of ^{13}C, ^{15}N and Mo (a nitrogenase co-factor) localization in diazotrophic cyanobacteria show how cells differentially allocate resources within filaments and allow calculation of nutrient uptake rates on a cell by cell basis. Images of AM fungal hyphae-root and cyanobacteria-rhizobia associations indicate the mobilization and sharing (stealing?) of newly fixed C and N. In a related technique, "El-FISH," stable isotope labeled biomass is probed with oligonucleotide-elemental labels and then imaged by NanoSIMS. In microbial consortia and cyanobacterial mats, this technique helps link microbial structure and function simultaneously even in systems with unknown and uncultivated microbes. Finally, the combination of re-engineered universal 16S oligonucleotide microarrays with NanoSIMS analyses may allow microbial identity to be linked to functional roles in complex systems such as mats and cellulose degrading hindgut communities. These newly developed methods provide correlated oligonucleotide, functional enzyme and metabolic image data and should help unravel the metabolic processes of complex microbial communities in soils, biofilms and aquatic systems.

Do Mats Matter: The Role of Ectomycorrhizal Mats in Structuring Microbial Communities in Old-Growth Forest Soils

Laurel Kluber, Oregon State University

The HJ Andrews Microbial Observatory is devoted to studying the microbial communities and activities associated with ectomycorrhizal (EcM) mats. Mats formed by EcM fungi are prominent features in Douglas-fir forest ecosystems, covering up to 49% of the forest floor and contributing up to 40% of the soil microbial biomass. Previous characterization of mat diversity was based primarily on morphological features however, through advances in molecular techniques we are able to better identify EcM mat formers and the microbial communities associated with them. A survey of biological and chemical properties in mat and non-mat soils showed increased oxalate, decreased pH, and unique enzyme profiles in the mat soils. These early results set the groundwork for a

temporal study focusing on rhizomorphic Piloderma mats, the most frequently encountered EcM mat, aiming to elucidate seasonal dynamics of the mats and their associated microbial communities. The potential for chitin degradation was determined using N-acetylglucosaminidase (NAGase) activity, and fungal and bacterial community profiles were compared using T-RFLP analysis. Piloderma mats had consistently greater NAGase activity, averaging ~60% higher than non-mats across all dates with the highest activity occurring in the spring. Fungal community profiles revealed peaks consistent with Piloderma to be dominant in mat samples, contributing up to 60% of the total fluorescence. Multivariate analysis revealed significant grouping of mat fungal communities and strong fungal indicator species for both mat and non-mat samples. Bacterial communities had significant clustering of mats and non-mats on the site level although patterns were not consistent across sites. Although shifts in both the fungal and bacterial communities occurred on a temporal scale, no significant trends were revealed across sites.

Nitrogen Bio-Cycling In Astrobiology and Early Life Evolution

Radu Popa, Portland State University

To astrobiologists and paleoecologists nitrogen is a very unique element. Its properties allow building high molecular diversity, which makes nitrogen desirable for constructing complex molecular networks. In nature the properties of nitrogen compounds favor its accumulation in fluids. Dinitrogen is stable and tends to accumulate in a planetary atmosphere, while ammonium being very soluble in water will accumulate in the hydrosphere. Only very few minerals accommodate nitrogen in their lattice and therefore geochemical cycling tends to strip this element from the lithosphere. These features and more promote nitrogen as a prime target for exploring other planets for life, and help drawing the evolution of early life's ecophysiology. The presence of microscopic nitrogen hotspots is viewed as a potential signature for life. The invasion of land by plants may not have been possible without the prior origin of nitrogen-fixing bacteria. The most important outcome of human civilization may actually be the footprint we leave on the global nitrogen cycle.

Movement of Micro-Bodies in Porous Media: Micromodel Visualization Experiments

Maria Dragila, Oregon State University

With increasing regulations on irrigation runoff and higher costs associated with the use of water, a greater number of agricultural producers and nursery operators are opting to recapture and reuse their irrigation water. Even though this practice is beneficial from the perspective of water conservation, it poses a potentially more costly problem. Plant pathogenic fungi, and in particular the water mold Phytophthora, have been frequently found in recirculated water, significantly increasing the risk of disease introduction via irrigation. Phytophthora species, including the quarantined pathogen P. ramorum, are responsible for several serious plant diseases that cause crop losses and reduction in the quality of nursery stock. Although several water treatment methods are available for

disinfecting irrigation water, these treatment methods are not always effective at eliminating Phytophthora.

The goal of this research is to optimize physical (i.e., non-biological) treatment of plant pathogen-infested irrigation water via slow sand and granular media filtration. We undertook this investigation because little is understood about how physical properties such as media grain shape and pore geometry influence governing filtration mechanisms.

P. ramorum zoospores are 5-15 μm in size (larger than typical colloids). Very little is known of the behavior of this size range in porous media transport. For colloids, Colloid Filtration Theory (CFT) currently provides the primary framework for modeling colloid retention and transport in porous media. Underlying CFT is the conceptualization of porous media as an assembly of ideal spherical collectors (unit spheres). However, a number of recent investigations have shown the importance of pore geometry on transport, including the characteristic early breakthrough of colloids. Hence, our investigation is developing a mechanistic conceptualization for the behavior of micron-sized bodies within a 'unit pore' (in contrast to the 'unit spheres' of CFT).

Based on a series of direct visualization studies of micro-bodies passing through an "ideal pore" constructed of glass spheres, we have identified three natural hydrodynamic domains within pores associated with distinct transport behaviors: (1) A central fast flow zone contributing to the majority of transport; (2) A very slow zone near grain-to-grain contacts from which micro-bodies are almost always excluded; and (3) A zone near grain walls along which a very small fraction of "trapped" micro-bodies are translocated possibly by sliding or rolling. Data from this investigation is being used to test theories related to the mechanism responsible for the early breakthrough of colloids, and to estimate the proportion of micro-bodies that can be trapped within the media.

Results from this study should lead to an improved understanding of pore-scale filtration phenomena and lay the foundation for development of water filtration methods for agricultural systems.

Biosignature Preservation in Phototrophic Streamer Mats from a Silica Depositing Hot Spring, Queens Laundry, Yellowstone National Park

Hollie Oakes-Miller[1], Linda L. Jahnke[2], Mary. N. Parenteau[2], Michael D. Kubo[2], and Sherry L. Cady[1]

[1]Department of Geology, Portland State University, Portland, OR
[2]NASA Astrobiology Institute, Ames Research Center, Moffett Field, CA

Mid-temperature streamer mats from Queens Laundry were analyzed to assess biosignature preservation during the early stages of silica mineralization. Lipid biomarker analyses were used to characterize the microbial community, and in some cases identify organisms, inhabiting the site. Compound-specific stable carbon isotope analyses were performed to characterize carbon flow as a function of silicification. The samples, which included streamer mats, silicified streamer mats, and a sinter that displays a streamer biofabric, were also studied by way of an optical light microscope (OLM), scanning electron microscope (SEM), and powder X-ray diffraction (XRD) analysis. The diagnostic lipid biomarkers for cyanobacteria (n-heptadecane, mid-chain branched methylalkanes, 2-methyl-bacteriohopanepolyols) and Chloroflexus (wax esters with carbon chain lengths of 31-36), were observed at each stage of silicification. The isotopic

fractionation of the lipid biomarkers relative to dissolved inorganic carbon was 27-31‰, 21-24‰, and 16-20‰ for the alkanes, wax esters, and bulk samples, respectively. OLM and SEM analysis revealed that filamentous organisms (Phormidium and Chloroflexus) were the dominant morphotype in all but the green layer of the mat which was dominated by Synechococcus. This study demonstrates that phototrophic lipid biomarkers are preserved during the initial stages of silica mineralization and, should they become encased early within a relatively impermeable silica matrix, could persist in the geologic record. The characterization of biosignature preservation in modern silica sinters will aid in our interpretation of ancient sinters on Earth, as well as putative hydrothermal amorphous silica deposits on Mars.

Interdisciplinary Science and the Subsurface Biosphere: Future Directions

2008 IGERT Group Process Training
Gilberto E. Flores, Dannie Jansik, Humberto Nation, Ellen Swogger and Rick Colwell

Academic disciplines evolve to address challenging scientific and societal issues. Often the paradigms and techniques of traditional disciplines are inadequate to address these issues forcing the emergence of new subdisciplines that hybridize at the boundaries of learning. One such subdiscipline that has emerged in the Earth Sciences over the past twenty years is the study of the subsurface biosphere. A premise of our IGERT program has been that research of the subsurface biosphere has grown out of the need to address problems of immediate practical concern to society and also basic scientific questions about life in the deep earth. Sometimes, only new disciplines can address complex scientific concepts that might include, in our field, the coupling of earth processes or the inherent heterogeneity of subsurface systems. At the same time that problems may force the creation of a new discipline complex research topics may only be assailable once special tools or facilities become available to a community of researchers who have lacked the means by which they can even address a question. Teams of researchers then congregate or network, even informally, and the problems can become tractable issues. Our goal for this workshop is to obtain your input on what we consider to be the basic elements that can push a science like ours forward. Collectively, we'd like you to help us envision the path whereby subsurface microbiology can best address the needs of science and society. To achieve this goal, we invite you to consider three questions, the answers to which we believe are essential to the evolution and maturation of research of the subsurface biosphere:

1) What societal or basic research "drivers" push our science?
2) What tools or facilities are available or required in order to address these drivers?
3) What research networks will permit scientists from multiple disciplines to make substantive progress in addressing the drivers that already exist or can be conceived?

Genome-Enabled Studies of Uranium Biogeochemistry: New Insights into Anaerobic U(IV) Oxidation

Harry Beller, Lawrence Berkeley National Laboratory

Anaerobic, nitrate-dependent U(IV) oxidation has considerable relevance to the bioremediation of uranium-contaminated aquifers and also represents a novel bacterial metabolic capability of fundamental scientific interest. The U.S. Department of Energy (DOE) is currently responsible for remediating 1.7 trillion gallons of contaminated groundwater and 40 million cubic meters of contaminated soil at its facilities, a number of which are highly contaminated with uranium as well as nitrate. DOE is strongly considering remediating these sites by in situ reductive immobilization, a process by which anaerobic bacteria reduce water-soluble U(VI) complexes to the poorly soluble U(IV) mineral uraninite. The discovery that Thiobacillus denitrificans, Geobacter metallireducens, and other bacteria can anaerobically re-oxidize, and thus, re-mobilize, uranium in groundwater highlights a process that could compromise the efficiency of DOE's bioremediation approach. While microbial U(VI) reduction has been the subject of extensive research, far less is known about anaerobic U(IV) re-oxidation; in fact, other than the results being presented here, the genetic and biochemical basis of this metabolism is completely unknown.

I will discuss our efforts to identify the genes/proteins that are key to nitrate-dependent U(IV) oxidation in T. denitrificans. These efforts included: (1) detailed analysis of the T. denitrificans genome, which was sequenced in 2005, (2) whole-genome transcriptional analyses of T. denitrificans with high-density, oligonucleotide microarrays, (3) proteomic studies of membrane-associated, c-type cytochromes in T. denitrificans, and (4) development of a genetic system in T. denitrificans, which enabled us to knock out specific genes putatively associated with U(IV) oxidation and test whether the knockout mutants were indeed defective in this metabolic activity. I will report on our identification of two genes critical to anaerobic U(IV) oxidation in T. denitrificans and discuss this discovery in the context of understanding dynamic redox cycling of uranium in situ.

Physiological and Transcriptional Response of *Nitrosomonas europaea* to Inhibition by Chlorobenzene

Sean Sandborgh and Mark Dolan, Oregon State University

Nitrification by autotrophic ammonia-oxidizing bacteria, such as *Nitrosomonas europaea,* is a key step in the global nitrogen cycle and is widely used for the removal of ammonia and cellular nitrogen in wastewater treatment facilities. The enzyme responsible for the initial step of nitrification, ammonia monooxygenase (AMO), which oxidizes ammonia to hydroxylamine, is a reasonably non-specific enzyme that acts on a variety of different substrates. This non-specificity allows for AMO to have inhibitory effects from a wide variety of compounds, including chlorobenzene and chlorophenols. The work presented here is one portion of a larger study aimed at identifying sentinel genes in *N. europaea,* genes that are significantly up- or down-regulated as a result of the presence of specific nitrification inhibitors. In this study, chlorobenzene concentrations of 4 μM

inhibited nitrite production in batch cultures of *N. europaea* by approximately 50% at 60 minutes of continuous exposure. Inhibition of nitrification increased with increased exposure time and coincided with the cessation of chlorobenzene transformation to chlorophenols. Chlorophenol inhibition studies indicated that the primary oxidized product of chlorobenzene, 4-chlorophenol, was the primary inhibitory compound acting on AMO. Based on oxygen uptake, 4-chlorophenol was found to inhibit AMO activity, while not significantly inhibiting the downstream process of hydroxylamine oxidation to nitrite. Whole genome microarrays were hybridized to labeled cDNA produced from mRNA harvested from batch culture experiments and analyzed for differential gene expression. Transcription results indicated a total of 30 statistically significant up-regulated sequences and 3 statistically significant down-regulated sequences in response to chlorobenzene exposure. Real-time quantitative PCR verification of some of these sequences was performed to verify the microarray data. Experiments were also performed to show the transcriptional response to increased inhibition to 4-chlorophenol as well as the time dependence of the response and the results are presented herein.

Inhibition of Nitrifying Bacteria by Heavy Metals; Physiological and Transcriptional Responses Upon Exposure to Cu^{2+}, Zn^{2+} and Cd^{2}

Tyler Radniecki, Oregon State University

The removal of NH_3 from wastewater treatment plant (WWTP) influent is critical in preventing the eutrophication of the receiving water bodies. The eutrophication of receiving water bodies can lead to massive fish kills and affect human health (blue baby syndrome). The preferred method of NH_3 removal from WWTPs is biological nitrogen removal (BNR). Ammonia oxidizing bacteria (AOB) play a critical role in the global nitrogen cycle and catalyze the first step of BNR from WWTPs by oxidizing NH_3 to NO_2^-. However, AOB are extremely sensitive to common WWTP influent components such as heavy metals. Such components can lead to BNR failure and result in regulatory fines and diminishes the quality of the receiving water body. Early warning devices (EWDs) are needed to warn WWTP operators of when a BNR failure is about to happen so preventative measures can be taken. Current monitoring methods do not identify the source of the inhibition making preventative measures difficult to impossible. This work proposes to create EWDs that are biosensors based on the gene expression of AOB. This work identifies genes that are increased in expression upon exposure to various heavy metals (Zn^{2+}, Cu^{2+} and Cd^{2+}) and may be suitable for biosensor applications. The gene expression of both sequenced (*Nitrosomonas europaea*) and unsequenced (*Nitrosococcus mobilis*) AOB were examined using Affymetrix microarrays, shotgun DNA microarrays and reverse transcriptase quantitative PCR (RT-qPCR). Correlations between observed gene expression patterns and observed physiological responses in both batch and chemostat experiments are made in an effort to further characterize the possible inhibition mechanisms of heavy metals and the possible defense mechanisms employed by AOB to these inhibitors.

Silicification of Viruses in a Simulated Hydrothermal Environment

Jim Laidler, Portland State University

One of the many roles that viruses play in the environment is the role of predator, recycling nutrients and maintaining microbial diversity. In some ecosystems, such as oligotrophic or hydrothermal environments, they are the primary predator. In these environments, where eukaryotic microbial grazers cannot function, anything that affects the ability of viruses to function can have a significant impact on microbial diversity.

Recent data from hydrothermal springs in Yellowstone National Park has suggested that the silica content of the springs may be having an adverse effect the ability of viruses to act as microbial predators. Laboratory studies have confirmed that, under silica-depositing conditions, viruses can become encased in amorphous silica and rendered non-infectious. This work has also shown that the rate and character of the silica deposition varies with pH and surface characteristics.

Since silicification in ancient hydrothermal springs led to preservation of microbial fossils as old as 3.6 billion years, it is reasonable to assume that viruses of those ancient microbes – if they existed – should also have been silicified and preserved. Energy dispersive X-ray spectroscopy (EDS) analysis of silicified viruses indicates that these fossilized viruses may be detectable though their relatively high phosphorus content.

Poster Abstracts

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In Alphabetical Order by Presenter

Comparison of Lactate and Formate as Hydrogen Donors for the Reductive Dehalogenation Of Trichloroethene in a Continuous-Flow Column

Azizian, Mohammad, Mark Dolan and Lewis Semprini, School of Chemical, Biological, and Environmental Engineering, Oregon State University

A continuous-flow anaerobic column experiment was conducted to evaluate the reductive dechlorination of trichloroethene (TCE) in aquifer material containing high iron concentrations when lactate or formate were used as electron donors. The aquifer material was obtained from the Hanford DOE site and was bioaugmented with the Point Mugu (PM) culture, which is capable of completely transforming TCE to ethene. Comparative studies were carried out with the continuous injection of 0.17mM TCE fed to the column at a rate of 0.1mL/min, while either lactate or formate substrates were injected at concentrations between 0.68-1.0 mM and 1.0 to 4.02 mM, respectively. Formate was found to be a more effective dehalogenating substrate than lactate, with a greater percentage (13-16%) of electron reducing equivalents going towards dehalogenation reactions, compared to the 4-6% donated by lactate. Sulfate reduction was found to be a competing electron acceptor reaction for both lactate and formate dehalogenating reactions. Additionally, iron reduction, based on dissolved ferrous ion in the column effluent, represented 10-15% of the reducing equivalents when formate was used as an electron donor, compared to the 33-36% when lactate was used. This demonstrates the importance of electron donor selection in the design of remediation technologies, as competitive reactions will lower the efficiency of donor usage for dehalogenation reactions, and will result in the production of greater amounts of unwanted products; such is ferrous iron in solution.

Estimating Nitrogen Transformation Rates in Long-Term Soil Organic Matter Manipulation Plots

Brewer, Elizabeth A. and David D. Myrold, Department of Crop and Soil Science, Oregon State University

The 10th anniversary of the Detritus Inputs and Removals Treatments (DIRT) experiment at the H.J. Andrews Experimental Forest was commemorated in the summer of 2007. This long-term manipulation experiment examines how the quality and quantity of soil organic matter (SOM) impacts SOM chemistry, nutrient availability and turnover. Using isotope dilution methods, gross N mineralization and nitrification rates were estimated across six treatments that controlled the quality and rate of SOM inputs (control, double wood, double litter, no roots, no litter and no inputs). Treatments

receiving an annual doubling of above-ground woody debris had decreased NH4+ production and consumption compared to the control. Ammonium production and consumption were not impacted by doubling litter or removing belowground inputs. Gross nitrification was measured in only the double wood treatment, suggesting that NH4+ consumption in all other treatments occurred by immobilization. Gross organic N turnover was estimated using double labeled (13C and 15N) glutamic acid in the control, no roots and no inputs treatments. Organic N turnover estimates show that substrate consumption or soluble organic N production or both, occur at a faster rate in the control plots. Immobilization and fast turnover of organic N suggests that the microbial communities in these plots are likely N limited, even after 10 years of manipulating C inputs.

Iron Reduction and Oxidation in *Aquificales* from Hydrothermal Systems

Caldwell, Sara, and Anna-Louise Reysenbach, Department of Biology, Portland State University

Iron cycling in certain near-neutral geothermal systems appears to be mediated, in part, by thermophilic bacteria, such as the *Aquificales,* that contribute significantly to low-oxygen energy fluxes and biomineralization of organic matter, and affect a wide range of other biogeochemical processes. Terrestrial representatives of this group, *Sulfurihydrogenibium subterraneum* and *S. azorense*, have been reported to be capable of both Fe (III)-reduction and Fe (II)-oxidation under laboratory conditions. However, growth of these cultures under Fe (III)-reducing or Fe (II)-oxidizing conditions has not been fully characterized or reproduced. Recent analyses of genome sequences from a deep-sea hydrothermal vent isolate, *Persephonella marina*, revealed the presence of numerous cytochromes which may play a role in electron transfer during iron-associated respiration. Results of *P. marina* cultivation experiments in this study confirm that this culture reduces Fe (III) (as ferrihydrite) using H_2 as the electron donor and oxidizes Fe (II) (as $FeCl_2$) using O_2 as the electron acceptor at 70°C, pH 6. Based on these results, whole genome microarrays and quantitative PCR will be used to identify and evaluate the expression or up-regulation of potential genes expressed during iron metabolism in these cultures.

Analysis of *Thermophilic Cyanobacteria* for Biosolar Hydrogen Production

Eberly, Jed and Roger Ely, Department of Biological and Ecological Engineering, Oregon State University

Biosolar hydrogen production offers great promise as a sustainable energy solution. Current research has focused primarily on mesophilic organisms however, solar-supported energy devices, including those using microorganisms or purified enzymes, must tolerate temperatures up to at least 50-60 ºC. Both cyanobacteria and green algae contain Photosystem I (PS I), PS II, and hydrogenase enzymes, necessary components for biophotolysis of water and subsequent production of H_2, but no thermotolerant organisms possessing all of these capabilities have been described in the literature. *Thermosynechococcus elongatus* is an ideal model for these studies because it has been

sequenced and is naturally transformable. A comprehensive BLAST search of *T. elongatus* failed to identify any genes coding for Ni-Fe or Fe-only hydrogenases that are typically found in cyanobacteria. However several candidate genes for hydrogen production have been identified. Preliminary screening using a newly developed hydrogen screening assay has shown a low level of hydrogen production from *T. elongatus*. The source of this hydrogen is unknown but may be related to one of the candidate genes. Ongoing work is focused on creating knockout mutants and analyzing them for hydrogen production in addition to evaluating the effects of various culture conditions (N, S, or P limitation; temperature; light/dark; pH; exogenous organic carbon) on H_2 production.

Spatial Distribution of Biofilms in Porous Media

Jansik, Danielle and Dorthe Wildenschild, School of Chemical, Biological, and Environmental Engineering, Oregon State University

Current understanding of subsurface microbial biofilm formation and their impact on fluid hydrodynamics is limited by our ability to observe the in situ microscale geometry of developed biofilms. Biomass distribution in porous media has been observed previously in only two dimensional systems; currently, no high-resolution 3-dimensional datasets exist that give sufficient information about microbial distribution such that the impact on flow and transport at the microscale can be directly computed. Three dimensional biofilms can significantly alter pore flow velocities and overall mass transfer between the aqueous and biological phases. We are currently developing new methods to resolve high-resolution 3-dimensional tomographic images of biofilms in porous media using synchrotron based x-ray microtomography. Imaging biofilms without disturbing their natural spatial arrangement has been a challenging task, primarily because most conventional dopants that typically dissolved in water also easily diffuse into biofilms. One method that we have developed to overcome this problem is the addition of silver nanoparticles to the fluid phase. Using this approach, we have been able to differentiate between the biomass filled pore space and fluid filled pore space. To date, the images that we have collected have yielded good representations of the geometry and qualitative information about structures of biomass. Ultimately, we intend to combine this kind of experimental measurement with upscaling (via volume averaging) to determine how biofilms might alter the physical properties of the porous media. Ultimately, by quantifying the spatial distribution of biofilms we will gain a greater understanding of how changes in physical parameters may impact the rate at which microbes degrade contaminants or produce products, and therefore this research has applications to bioremediation and bioprocessing.

Initial Investigations into the Ecology of Thermoacidophilic Microorganisms Inhabiting Deep-sea Hydrothermal Chimneys

Flores, Gilberto E. and Anna-Louise Reysenbach, Portland State University

Investigation into the ecology of thermoacidophilic microorganisms inhabiting deep-sea hydrothermal vent chimneys is underway. Initial studies have utilized the sole described member of the ubiquitous deep-sea hydrothermal vent Euryarchaeota group 2 (DHVE2), *Aciduliprofundum boonei*, as a model organism. These studies included whole genome analysis and optimization of fluorescent in-situ hybridization (FISH) protocols

that will allow visualization of the DHVE2 in native chimney samples. Genomic analysis of *A. boonei* revealed several distinguishing features, including the absence of obvious sugar transporters, various amino acids/peptide fermentation pathways and a unique arrangement of flagella genes. For FISH, a group specific probe was validated and optimized by altering formamide concentrations and hybridization times. Permeabilization of the cell was also optimized in order to use CARD-FISH (catalyzed reporter deposition), a modification of the FISH procedure which reduces autofluorescence of sulfide minerals while enhancing the fluorescent signal of labeled cells. The CARD-FISH protocol in conjunction with quantitative PCR (QPCR) will be used to determine the distribution and relative abundance of the DHVE2 from sulfide chimneys collected from around the globe. The first of three scheduled cruises for this project is heading out to the Mid-Atlantic Ridge next month.

The Role of Electron Donor-Acceptor Complexes in Organo-Mineral Interactions

Keiluweit, Marco and Markus Kleber, Department of Crop and Soil Science, Oregon State University

A long tradition divides the organic materials that occur in terrestrial environments into hydrophilic and hydrophobic compounds. Often, inference about natural organic matter (NOM) retention and/or contaminant behavior is largely based on these qualifications, assigning weak bonding/retention mechanisms to hydrophobic moieties and assuming energetically stronger interactions between hydrophilic substances and other, charged or hydroxylated surfaces. It appears that some progress in our mechanistic understanding of mineral-organic interactions would be possible if we were able to achieve a more specific characterization of bonding processes at the organo-mineral interface. Consequently, this contribution reviews the significance of electron-donor acceptor (EDA) complexes between natural or xenobiotic organic compounds and phyllosilicates as retention mechanisms in soils and subsurface environments. A large fraction of organic compounds present in these environments carry electron- deficient aromatic structures that may serve as π-acceptors. Prominent examples are quinone structures in NOM as well as notorious and emerging contaminants such as nitroaromatic compounds (NACs) and fullerenes (e.g. C60), respectively. The high affinity of NAC for layer silicates has long been attributed to n-π EDA complexation of the electron-deficient π-system and siloxaneoxygens. More recently, however, the importance of EDA interactions as a relevant mechanism for NAC retention in soils and sediments has become a matter of debate. New experimental approaches and computational studies indicate that, particularly for phyllosilicates with a low layer charge (montmorillonite), other mechanisms such as specific NAC-cation interactions and non-specific dispersive forces play the predominant role in the retention of NACs. We combine these new results with existing knowledge of the dominant adsorption processes and provide a mechanistic definition and evaluation of the proposed EDA complex formation between organic compounds and phyllosilicate surfaces. Explicit conclusions on both the existence and the potential contribution of specific EDA complexes for NAC stabilization cannot be drawn based on current evidence. Further research is needed to investigate the nature of NAC adsorption as well as the capability of organic electron-acceptors other than NAC to undergo EDA interactions with mineral surfaces.

Colloid transport in heterogeneous media observed using the light-transmission technique

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Colloid transport dynamics are critical to the prediction of contaminant fate and transport in groundwater, both with respect to the binding of molecules to geologic particles, and with respect to biological migration. This project utilizes an imaging technique to observe in time and spatially in two dimensions the concentration of model colloids and water-conservative tracer dye, and water content during flow through porous media. The physical size of this system is suited for addressing transport and deposition processes which are a result of physical and chemical heterogeneities found at the field-scale. Improvements to the imaging system have increased the signal to noise ratio resulting in a detection range of five orders of magnitude for colloids and seven orders of magnitude for fluorescein; their detection limits are currently 1 part per billion and 1 part per trillion respectively. This sensitivity to trace concentrations of dye makes possible the evaluation of potentially critical tailing effects in both saturated and unsaturated media. The implications of processed images are analyzed using pH, electrical conductivity, and fluorescence data that are collected in-line and recorded as fluid leaves the experimental chamber.

Numerical Simulation of the Anaerobic Transformation of Chlorinated Aliphatic Hydrocarbons in a Continuous Flow Column

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The anaerobic reductive dechlorination of tetrachloroethene (PCE) to cis-dichloroethene (c-DCE) in a laboratory column study was numerically simulated and compared with experimental observations. The column study was conducted with continuous flow and injection of PCE in synthetic groundwater. The column was packed with aquifer solids from the Hanford DOE site and bioaugmented with the Evanite (EV) dechlorinating enrichment culture. After the column was bioaugmented and fed lactate as an electron donor, c-DCE concentrations in the column effluent exceeded the influent PCE concentration. This high c-DCE concentration resulted from enhanced PCE desorption and transformation. A 1-D reactive transport model was developed that included the processes of dispersion, advection, rate-limited sorption and desorption, reductive dechlorination kinetics with competitive inhibition and microbial growth and decay. The model was validated by mass balances, comparisons with analytical solutions and batch kinetic models. Previously determined kinetic and inhibition constants for the EV culture of Yu and Semprini (2004) were input into the model simulations. Initial biomass concentration was assumed to be exponentially distributed along the column. The sorption parameters including the aquifer: water distribution coefficients (Kd_s) and first-order mass transfer coefficients for PCE, trichloroethene (TCE), and c-DCE were determined in batch laboratory studies. The system of model equations was solved numerically using COMSOL 3.3, which employs finite-element methods. The reactive transport model successfully simulated the initial results of continuous flow column experiment. The increase in c-DCE above the influent PCE concentration was simulated

and TCE was shown not to accumulate in the column effluent. The simulations showed that microbial kinetic values generated in previous studies and the sorption parameters generated in batch tests, when used in a transport model, did a reasonable job estimating PCE, TCE, and c-DCE concentration histories in the column effluent. Currently a sensitivity analysis is being performed to better understand why some differences, such as the more rapid breakthrough of c-DCE, was observed in the experimental data, but not in the model simulations.

Investigating the Mechanism of Current Production in Benthic Microbial Fuel Cells

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Benthic microbial fuel cells produce electricity by bridging the natural redox gradient across the sediment water interface. Microbes play a variety of roles including establishing (and maintaining) the redox gradient, processing organic material and producing electrochemically active products (e.g. sulfate reduction to sulfide), and catalyzing electron transfer to and from electrodes. We describe a laboratory experiment designed to investigate the mechanism of electron transfer to the anode and the relative contributions of sediment-hosted microorganisms and attached and unattached microbes in the anode chamber.

Six model benthic microbial fuel cells were constructed and operated for approximately one year. Three were regularly supplemented with lactate and three used only natural fuels contained in sediment and 0.2-micron filtered seawater. The conclusion of the experiment will be to remove the anodes from the open systems and conduct batch experiments in which we can constrain the efficiency of substrate utilization. Batch experiments will include three treatments: lactate utilization with attached and unattached microbes, lactate utilization with only attached microbes and lactate utilization with molybdate (to inhibit sulfate reduction). Our hypotheses include: 1) supplemented fuel cells will have a microbial community better adapted to utilizing lactate, 2) attached cells play the dominant role in the conversion of soluble electron donors to electrical current and 3) there are a variety of electron donors in the sediment that will be eliminated in the batch experiments resulting in lower current production. A better understanding of the importance of the various microbial communities and mechanism for electron transfer will inform future fuel cell experiments and optimization.

A Novel Device to Controls Fuel Cell Voltage and Step It Up to a Level Appropriate for Powering Sensors in Aquatic Environments

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Benthic microbial fuel cells are being developed as power supplies for sensors in aquatic environments. Chemical, microbiological and environmental factors generally receive the most attention in experimental studies. However, there are power management challenges that must be met for the practical application of this technology.

These challenges include: 1) conversion of low voltage to higher voltage useful for powering instruments, 2) storage of energy and the ability to meet variable demands, and 3) balancing current draw with environmental processes that control delivery of fuel to the fuel cell. This presentation provides background on benthic microbial fuel cells, summarizes environmental factors that affect how a fuel cell delivers power and describes the design of a device that addresses the above challenges in a single integrated package. The device is a combination potentiostat and DC to DC converter that charges a Li-ion rechargeable battery, which, in turn, provides power output for a sensor. We will present laboratory calibrations that show the efficiency of the DC to DC conversion is approximately 50% and examples of field deployments in which the converter was used to power an acoustic receiver.

The Effect of Formate vs. Lactate on Performance and Community Evolution in Dechlorinating Consortia Grown in Chemostats Treating TCE-Saturated Media

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Trichloroethene (TCE) is a ubiquitous groundwater contaminant. It, and the lesser chlorinated degradation products cis-1,2-dichloroethene (cDCE) and vinyl chloride (VC) are all known to be toxic and/or carcinogenic, and are EPA priority pollutants. Bioremediation via anaerobic reductive dechlorination is a common and desirable solution because of relative ease, cost efficiency, and ubiquity of organisms capable of this process. Reductive dechlorination has been thoroughly studied on a variety of systems, electron donors, and cultures. Few studies, however, have compared different electron donors in chemostats. The primary objective of this study was to evaluate performance and mixed culture evolution in two chemostats receiving TCE-saturated media (~7.5 mM) with approximately 2X the stoichiometric electron donor requirement, as lactate or formate, to completely dechlorinate TCE to ethene. Results from this study show that, with a 12.5-day retention time and 2X the electron donor requirement, formate is a superior electron donor in terms of completeness and longevity of dechlorination activity, and electron donor efficiency devoted to dechlorination with the Evanite mixed culture. However, performance in the lactate-fed system was more stable and less prone to perturbations than the formate-fed system. Frequent batch assays on harvested cells under non-limiting conditions revealed what culture potential was independent of extant conditions, and showed how the cultures evolved differently as a function of electron donor source and availability. In the proportions added to this consortium, much lactate was fermented to unused organic acids, and electron donor limitations appeared to be the cause of lost dechlorination activity. Frequent idealized batch assays proved to be an effective tool in not only evaluating culture potential, but in explaining what proportions of biomass were devoted to each dechlorination step, what factors limited dechlorination, and what processes did or did not compete for molecular hydrogen.

Determining the effect of Operational Parameters on Cell surface hydrophobicity measurements by Microbial Adhesion to Hydrocarbons (MATH) test

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Microbial adhesion to hydrocarbons (MATH) test is a frequently used technique for assessing cell surface hydrophobicity. Applications include cell starvation studies, oil recovery, food microbiology, oral microbiology, etc. Although this method is known to be sensitive to operating conditions, no attempt to date has been made to explicitly determine the effect of various operational parameters.

This study aims to determine the effect of hydrocarbon volume, phase separation period, vortex duration, and adsorption wavelength, on MATH test results. Specifically, n-dodecane is used as the preferred hydrocarbon phase. Three different strains of *Escherichia coli* namely JM 109, D 21, and *E. coli* D21f2 (all K-12 mutants), having progressively truncated LPS, have been used with the aim of distinguishing the consequence of LPS length on cell hydrophobicity. *Shewanella oneidensis* MR-1 is used in the current study as a representative Dissimilatory Metal Reducing Bacteria (DMRB). An attempt is also made to determine the differences in the results between cells suspended in Growth versus non-growth (starved) substrate.

The MATH test results showed significant hydrophobicity for *S. oneidensis* MR-1. As expected, the most hydrophilic strain *E. coli* JM 109 having full LPS, reported very low values of cell hydrophobicity and insignificant changes in MATH test results with variations in operating conditions. Interestingly, the strain with smallest LPS, *E. coli* D21f2, also resulted in low cell surface hydrophobicity. In addition, growth cultures showed lower hydrophobicity values than starved cultures and this could be attributed to growth or aggregation of bacteria during test duration. Adsorption wavelength was seen to cause a definite increase in cell hydrophobicity and a significant drop in test results at about 600 nm. The results of this study are expected to yield a better protocol for performing the MATH test as well as give better insights into the effect of LPS length on cell surface hydrophobicity.

Physiology of Alkene Metabolism in VC-Utilizing *Nocardioides* Strain JS614

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One of the characteristics of growth by the VC-utilizing *Nocardioides* strain JS614 is the long lag before growth begins when coming out of a repressed state. Acetate grown cells will induce and grow between 70 and 100h when ethene (Eth) is provided as a growth substrate. Supplemental ethylene oxide (Eto), which is the product of alkene monooxygenase (AkMO) oxidation of Eth, shortens the lag phase before growth begins. When propene is provided as a growth substrate for repressed cells, growth does not occur, but the addition of supplemental Eto allows acetate grown cells to utilize propene.

The generation time on propene is 8x longer than when Eth is provided as the growth substrate, and growth is linear and never achieves exponential phase. Prox accumulates in the growth media at a rate of ~ 60% of propene transformation. Supplemental propylene oxide (Prox) does not stimulate growth on propene, and inhibits growth on Eth. Evaluation of expression of the first two genes in the alkene metabolic pathway, AkMO and epoxyalkane cofactor M transferase (EaCoMT) by rt-qPCR showed that both propene/Prox and Eth/Eto induce gene expression to similar levels within 8h. We considered that there may be other enzymes in the metabolic pathway that are not co-regulated with AkMO and EaCoMT. Growth on propene requires the presence of CO_2 and is sensitive to bromoethane sulfonic acid (BES) an analogue for cofactor M, indicating the involvement of a carboxylase, while growth on Eth is not limited by lack of CO_2 or presence of BES. By observing ^{14}C incorporation we found that cells incubated with Eto had Prox-dependent carboxylase activity an order of magnitude greater than cells incubated with Prox. Collectively, these results indicate that lack of growth on propene by repressed cells is not simply a matter of lack gene induction but may be due to the negative effects of Prox accumulation, or kinetic limitations of energy yielding steps that cannot meet the reductant needs of the energy-requiring steps of the alkene metabolic pathway.