

Subsurface Biosphere Initiative

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

Subsurface Biosphere Initiative and IGERT Workshop

June 17-19, 2007

Newport, Oregon

Agenda

List of Poster Presentations

Abstracts



Subsurface Biosphere Initiative and IGERT Workshop
June 17-19, 2007
Newport, Oregon

Sunday Evening, June 17

- 7:15** **Lew Semprini, SBI Executive Committee Chair**
Introductory talk about SBI and Workshop
- 7:30** **Keynote Speaker: Mary Firestone, Professor,**
Environmental Science, Policy and Management Division--Ecosystem
Sciences, University of California, Berkeley
Can Molecular Microbial Ecology Increase Our Understanding Of
Subsurface Processes?
- 8:30** **Campfire Social**

Monday Morning, June 18

- 8:00** **Markus Kleber, Oregon State University**
The Reactivity of Biogeochemical Interfaces
- 9:00** **Peter Nico, Advanced Light Source (ALS) at the Lawrence Berkeley**
National Lab
Synchrotron Spectromicroscopy and Biogeochemical Interfaces
- 10:00** **Break**
- 10:15** **Stephanie Boyle, Recent IGERT PhD Graduate**
Bacterial and Fungal Contributions to Soil Nitrogen Cycling
- 10:45** **Shawn Starkenburg, Oregon State University IGERT Student**
New Insights into Carbon Metabolism in the Nitrite Oxidizing
Bacterium, Nitrobacter hamburgensis X14
- 11:15** **Break for lunch**

Monday Afternoon, June 18

- 1:15** **Lew Semprini**
Update from the SBI Executive Committee Chair
- 1:35** **Dave Myrold**
Update from the IGERT Director
- 1:45** **Dorthe Wildenschild, Oregon State University**
Non-Destructive 3D Imaging of Biofilm Architecture with X-Ray
Tomography
- 2:15** **Brian Wood, Oregon State University**
Biological Processes in Porous Media: from the Pore Scale to the Field

- 2:45** **Break**
- 3:00** **Andy Sabalowsky, Oregon State University IGERT Student**
*Comparison of Attached vs. Suspended Growth for Anaerobic Reductive
Dechlorination of High TCE Concentrations*
- 3:30** **2007 IGERT Group Process Training Students**
*Anaerobic Oxidation of Methane and GPT: Highlights and Lessons
Learned*
- 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** **Tommy Phelps, Oak Ridge National Lab**
Life and Times in the Deep Subsurface
- 9:00** **Jessica Goin, Portland State University IGERT Student**
*Biosedimentology of Thermal Features of the Uzon Caldera,
Kamchatka, Russia*
- 9:30** **Check Out and Bring Luggage to Cars/Conference Room**
- 10:00** **Rebecca Poulson, Oregon State University IGERT Student**
*Trace Metal Cycling in Continental Margin Sediments: Insights from
Molybdenum Isotopes*
- 10:30** **Mark Nielsen, Oregon State University IGERT Student, Hatfield
Marine Science Center**
Bug Juice: Microbial Fuel Cells in Marine Sediments
- 11:00** **Other business**

Poster Presentations
Subsurface Biosphere Initiative and IGERT Workshop
Newport, Oregon

Azizian, Mohammad, Mark E. Dolan, and Lewis Semprini

Trichloroethene Dechlorinators and Dissimilatory Fe(III) Reducers Competition for Lactate and Acetate as the Electron Donor in a Continuous Flow Column Study

Brewer, Elizabeth A., T.W. Boutton, and D.D. Myrold

Woody Plant Encroachment into Grassland Alters the Composition of Soil Microbial Communities.

Briggs, Brandon

Characterizing Biofilm Formation in Acidiphilium cryptum JF-5

Cakin, Defne and James Ingle Jr.

Methodology of Low Volume Sampling for Monitoring Oxygen and Reduced Species in Anaerobic Systems

Caldwell, Sara, Y. Liu, T. Beveridge, I. Ferrera, A.-L. Reysenbach

“Thiominerva volcanii” gen. nov., sp. nov., a new genus within the Aquificales from a terrestrial hot spring, Costa Rica

Eberly, Jed and Roger Ely

Thermophilic Hydrogenases: Properties and Applications in Biological Hydrogen Production

Flores, Gilbert and Anna-Louise Reysenbach

Ecology of Thermoacidophilic Microorganisms Inhabiting Deep-Sea Hydrothermal Vent Chimneys

Goin, Jessica

Modeling Seasonal Silica Deposition

Harrington, Stephanie

Dispersion through Highly Heterogeneous Porous Media

Jansik, Danielle

Spatial Distribution of Biofilms in Porous Media

Koch, Corey, Vincent T. Remcho, and James D. Ingle.

Microfluidics in the Subsurface: Towards In-Situ Analysis of Microliter Volumes in a Miniaturized Package

Laidler, Jim, Kenneth Stedman, Sherry Cady

Viral Mineralization in a High-Silicate Environment

Lee, Iisu and Lew Semprini, Oregon State University

Effect of the CSTR Hydraulic Retention Time on Tetrachloroethene Dehalogenation and the Lactate Fermentation Pathway

Mustafa, Nizar, M. Azizian, M. Dolan and Lewis Semprini

Numerical Simulation of the Anaerobic Transformation of Tetrachloroethene (PCE) to Ethene in a Continuous Flow Aquifer Column

Nation, Huberto

Dispersal and Degradative Mechanisms of Selenium, Arsenic and Organic Compounds from Sediments of the Colorado River Delta in the Upper Gulf of California by Physico-Chemical processes

Nielson, Mark and Clare Reimers

Resistance is Inevitable: Seeking the Relationship between Permeability and Formation Factor

Radniecki, Tyler, Mark E. Dolan and Lew Semprini

*Zinc Inhibition of *Nitrosomonas europaea* in a Continuous Culture*

Saini, Gaurav

Microbial Adhesion and Transport

Sandborgh, Sean, Tyler Radniecki, and Mark Dolan

*Effect of Chlorobenzene on *Nitrosomonas europaea* Physiology, Proteomics and Genetic Expression*

Smith, Kiara, Angela Bice, Jack Istok, and Hap Pritchard

Single Well Push-Pull Tests for Determining In-Situ Rates of Anaerobic Naphthalene Biodegradation

Swogger, Ellen

*A Method for Biofilm Growth of *Nitrosomonas europaea**

Taylor, Anne, Peter J. Bottomley, Mark E. Dolan, and Lewis Semprini

Utilization of Fluoroethene as a Surrogate for Aerobic Vinyl Chloride Degradation

Abstracts of Talks

Subsurface Biosphere Initiative and IGERT Workshop Newport, Oregon June 17-19, 2007

In Agenda Order

Introductory Talk About SBI and Workshop

Low Semprini, Daniel Arp, Peter Bottomley, Martin Fisk, and David Myrold, Oregon State University

The Subsurface Biosphere Initiative (SBI), supports several themes of OSU's Strategic Plan including the: I) Understanding of the dynamics, and sustainability of the Earth and its resources; II) Realizing fundamental contributions in the life sciences and optimizing health and well-being of the public; and III) Managing natural resources that contribute to Oregon's quality of life and growing and sustaining natural resource based industries. The specific goals of the SBI are to: 1) Promote the development a Center of Excellence for Subsurface Biosphere Education and Research; 2) Continue to develop Top Tier multidisciplinary graduate education relevant to Subsurface Biosphere; 3) Foster the development of externally funded Research Centers and Programs and large interdisciplinary research grants; and 4) Support undergraduate and graduate research experiences that promote student diversity. The three focus areas are: Global Biogeochemical Cycles, Sustainable Natural Resources, and Engineered Environmental Processes.

The Initiative involves twenty-eight faculty members in five OSU Colleges (Agricultural Sciences, Engineering, Forestry, Oceanic and Atmospheric Science, and Science). Over the past year SBI supported the hiring of three extremely talented faculty members into the colleges of Agricultural Sciences, Ocean and Atmospheric Sciences, and Engineering. These faculty members bring new expertise in geomicrobiology (Rick Colwell, Professor, COAS), transport and physical processes in the subsurface (Dorthe Wildenschild, Environmental Engineering, COE), and organic biochemistry (Marcus Kleber, Crop and Soil Science, COA). These hires bridge gaps in our technical expertise and position OSU to become a nationally known center for subsurface biosphere education and research.

The SBI website continues to develop, and provides information on the many activities of the SBI, including funding opportunities, seminar announcements, and internships. We also have initiated a monthly newsletter, sent to OSU SBI faculty, students and other interested subscribers, that includes a feature research article on a subsurface biosphere topic of interest.

Our summer internship program for undergraduate students was open for the second year and expanded to support 11 undergraduate internships, with faculty mentors from five OSU colleges. The internships culminated with a student poster presentation last September. The internship program will continue this summer.

This year's SBI workshop brings together OSU and PSU faculty and students and will showcase research from the SBI and the Earth's Subsurface Biosphere Research

IGERT Programs. The workshop includes talks from three internationally known researchers, as well as talks from faculty members and IGERT students. The poster session will include research from all the graduate students attending the meeting.

Can Molecular Microbial Ecology Increase Our Understanding Of Subsurface Processes?

Mary Firestone, Professor, Department of Environmental Science, Policy and Management--Ecosystem Sciences Division, and Eric Dubinsky (co-author), University of California, Berkeley

Terrestrial ecosystem function results primarily from integrated environmental control of biotic processes. While biotic function ultimately results from genomic control of the organism's physiology, multiple levels of biological organization are involved. Microorganisms (here bacteria and fungi) mediate processes that are centrally important to the functioning of ecosystems (e.g. nitrogen-cycling, decomposition, sulfur-cycling). Information about and understanding of the genomics and community ecology of terrestrial bacteria and fungi has increased massively over the past few years. Will this emerging understanding of the genomic basis of microbial community composition and function enhance our knowledge of biogeochemical processes? Can we enhance our ability to predict ecosystem response to environmental change by evaluating key components of the genomic response of primary biotic mediators? Can genomic, transcriptomic, and bioinformatic information be used to identify and evaluate macromolecular information central to ecosystem function? Huge bioinformatic databases describing both the phylogenetic identities of microbial community members and functional capacities are beginning to accrue. The question of whether knowledge of microbial population ecology and community ecology is useful in understanding or predicting the function of ecosystems is a fundamentally important and interesting question that has emerged over the past decade.

The Reactivity of Biogeochemical Interfaces

Markus Kleber, Oregon State University

Biogeochemical cycling can be envisioned as the result of processes that happen at interfaces, especially where transformations of (organic) matter are involved. Of particular importance is the solid-solution interface in terrestrial ecosystems, because a) adsorption of organic materials on mineral surfaces is a key process in the stabilization of organic matter against biodegradation while b) microbial adhesion at mineral surfaces precedes the establishment of ecologically functional microbial associations.

The role of mineral surfaces has been much debated in this context over the past years. It is becoming increasingly apparent that "Intimate, and seemingly protective, associations of organic substances with inorganic particles are now evident. Such challenging substrates as kerogens, coals and humus are subject to oxic degradation so severe that preservation must result from highly unusual structures or environmental conditions. As such recalcitrant substrates are destroyed, nitrogen-rich aliphatic remains of microorganisms are produced and preserved in association with fine grained minerals. Within this scenario, the interactions of organic remains with minerals in soils and

sediments take on a new, less passive role." (Hedges and Oades, *Organic Geochemistry*, 1997, 27: 319-361).

Clearly, a better understanding of the mechanisms of mineral-organic interactions is required if we want to improve the quality of simulation models that attempt to predict carbon and nitrogen cycling in the biosphere.

Synchrotron Spectromicroscopy and Biogeochemical Interfaces

Peter Nico, Advanced Light Source (ALS) at the Lawrence Berkeley National Lab

Synchrotron based spectroscopic and spectromicroscopic techniques offer unique ways to probe the fundamental mechanisms of important biogeochemical processes. These techniques offer a variety of chemical and physical information across a spatial scale that ranges from nanometers to centimeters. The basics of four different techniques will be presented along with some examples of applicable research results. Scanning Transmission X-ray Microscopy (STXM) yields chemical and electronic structure information on light elements (e.g. C and N) as well as heavier elements (e.g. Fe and U) with 30 to 50 nanometer spatial resolution. Similarly, X-ray absorption spectroscopy (XAS), both bulk and microfocused, provides information on oxidation state as well as local structural environment for slightly heavier elements (first row transition metals and larger). Synchrotron Fourier Transform Infrared (sFTIR) spectromicroscopy probes the vibrational structure of molecules in exactly the same way as laboratory based FTIR except with an order of magnitude greater spatial resolution (~5-10 microns). Lastly, X-ray microtomography yields the structural information crucial to understanding flow paths and pore structure in solid media. Taken individually or together these techniques can be applied to research questions such as soil carbon-mineral interactions, Fe biomineralization pathways, uranium sequestration, and many other important biogeochemical pathways.

Bacterial and Fungal Contributions to Soil Nitrogen Cycling

Stephanie Boyle, Recent IGERT PhD Graduate

A study was conducted to examine microbial community composition and N cycling in soils from 20-year-old experimental tree plantations with pure stands of Douglas fir (*Pseudotsuga menziesii*) and red alder (*Alnus rubra*) in a high- (Cascade Head Experimental Forest) and a low- (H.J. Andrews Experimental Forest) productivity forest. Population sizes of bacteria, fungi, and archaea were determined by Q-PCR. Results showed that fungal:bacterial ratios were significantly higher in H.J. Andrews soils and were lowest in red alder soils at Cascade Head. Community composition was assessed using terminal restriction fragment length polymorphism (T-RFLP) profiles and sequencing targeting 16S bacterial, 16S archaeal, and ITS fungal DNA. Results showed that fungal and archaeal community composition varied between sites and tree types, but bacterial composition only varied between sites. ¹⁵N isotope dilution was combined with antibiotics to assess the roles of bacteria and fungi in N mineralization and nitrification. Data showed that nitrification was a major sink for NH₄⁺ in all soil types and bacteria were the primary nitrifiers. Increased ammonification following antibiotic additions suggested that organic N may be important for the growth of heterotrophic bacteria and fungi. Community compositions of ammonia-oxidizing bacteria and archaea were

assessed by targeting bacterial and archaeal ammonia-monooxygenase (*amoA*) genes. The composition and population size of ammonia-oxidizing bacteria differed between Douglas fir and red alder and tended to group with *Nitrosospira* clusters 2 and 4. Archaeal *amoA* was only amplified from Cascade Head (high-productivity site) and grouped with other archaeal clones from soil and from estuary sediments.

New Insights into Carbon Metabolism in the Nitrite Oxidizing Bacterium, *Nitrobacter hamburgensis* X14

Shawn Starckenburg, Oregon State University IGERT Student

Nitrification, the microbiological process by which ammonia is converted to nitrate, is a major component of the global cycling of nitrogen. Alphaproteobacteria in the Genus *Nitrobacter* participate in nitrification by converting nitrite to nitrate, conserving energy in the process. Sequencing and analysis of the *Nitrobacter hamburgensis* X14 genome revealed four replicons comprised of 1 chromosome (4.4 Mbp) and three plasmids (294, 188, and 121 kbp, respectively). Surprisingly, many genes important for carbon metabolism and energy conservation are located on the two largest plasmids, including key Calvin cycle enzymes, two non-paralogous copies of RuBisCO and the only genes for carboxysome formation. Whole genome comparisons were conducted between *N. hamburgensis* and the finished and drafted genome sequences of *Nitrobacter winogradskyi* Nb-255 and *Nitrobacter* sp. Nb311A, respectively. Novel genes for inorganic and organic carbon utilization were uniquely identified in *N. hamburgensis*, including putative D- and L-lactate dehydrogenase genes. Growth experiments revealed that *N. hamburgensis* can use D- but not L-lactate as a sole carbon and energy source. When D-lactate was added to cultures containing nitrite and carbonate, growth was enhanced by 40%. Strikingly, lactate was not able to support growth on nitrite in the absence of carbonate. These data suggest that *Nitrobacter hamburgensis* possesses more heterotrophic potential than previously known, yet, energy conserved during nitrite oxidation may require carbon dioxide as a carbon source despite the energetic advantage of using organic carbon.

Non-Destructive 3D Imaging of Biofilm Architecture with X-Ray Tomography

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

This effort is aimed at characterization of microbial communities as they form biofilms in porous media using Computed Microtomography (CMT). The three-dimensional architecture of biofilms in the subsurface has been the subject of limited experimental studies, particularly in terms of how this architecture evolves in response to changes in hydrodynamics and other growth-limiting or -enhancing conditions. Conversely, the influence of the evolving biomass on hydrodynamics is an important factor when designing and managing bioremediation in the field.

Past efforts to quantitatively describe biomass growth and spatial and temporal behavior have been limited to either two-dimensional (micromodel) systems (e.g. Thullner et al., 2002), to nano-scale observations of the biomass only (minus porous medium) (e.g. Thieme et al. 2003), or to destructive methods such as thin-sectioning. We

will use synchrotron-based microtomography to image the three-dimensional arrangement of biomass during growth. Hydrodynamic effects of the biogrowth will be evaluated concurrently. The main hurdle is that biomass looks similar to water to the penetrating x-rays, so contrast agents are needed for us to be able to distinguish the biomass from the water. Thus, a significant part of this research effort is focused on determining adequate dopants/tracers that can be used to establish x-ray absorption contrast among the various phases (water, solids, biomass). Selection of bacteria that are able to survive the radiation level used (such as for instance *Deinococcus radiodurans*) is another challenge that will be addressed. If successful, we will be able to characterize biomass as it evolves both spatially and temporally in a porous medium in response to various environmental changes, particularly those relating to optimization of subsurface bioremediation strategies.

Biofilms in Porous Media: An Overview of Progress on Theory and Characterization in 3-Dimensions

Brian Wood, School of Chemical, Biological and Environmental Engineering, Oregon State University

Although we often think of ‘bioremediation’ when discussing biological processes in porous media, there are, in actuality, a large number of important natural and engineered biological processes that occur in such systems. Specific examples include wastewater treatment (e.g., trickling filters or anaerobic packed bed reactors), water filtration, immobilized cell bioreactors, biofilters for removing air contaminants, and even scaffolds for growing tissues. Tremendous advances have been made in the recent past in biology in general, and this has made a significant impact in terms of research on biological processes in porous media.

In this talk, the essential features of upscaling in porous media / biological systems will be discussed. This will be an introductory level discussion about what upscaling is, how it can be applied to systems with a biological component, and the motivations for examining porous media / biological system in the context of upscaling. The concepts of multiscale systems, scaling and scaling laws, and reduction of the ‘degrees of freedom’ in a complex biological system will be introduced. Several examples where upscaling in porous media / biological systems have provided useful insight will be presented.

Comparison of Attached vs. Suspended Growth for Anaerobic Reductive Dechlorination of High TCE Concentrations

Andy Sabalowsky, Oregon State University IGERT Student

The chlorinated solvent, trichloroethene (TCE), and its lesser-chlorinated transformation products are ubiquitous groundwater contaminants known for their toxicity and/or carcinogenicity. Because of TCE’s density, source zone contamination frequently occurs with non-aqueous free product dissolving to saturation limitations in the immediate vicinity. The anaerobic bacterium, *Dehalococcoides ethenogenes*, is known to reductively dechlorinate TCE and other chlorinated ethenes completely to ethene, and obtain energy for growth from the process. However, biological dechlorination has not been documented at concentrations like those in a TCE source

zone (~10 mM). The vast majority of dechlorination experiments using *Dehalococcoides* strains have been with suspended growth in modest concentrations. Experimentation in our laboratory suggests TCE and its dechlorination product, cis-1,2-dichloroethene (cDCE) are toxic at concentrations below 10mM. It is known, however, that biofilms often confer resistance to adverse conditions. To determine if biofilms can alleviate high-TCE toxicity and ultimately treat source-zone TCE contamination, two systems were compared: a recirculating packed column for biofilm growth and a chemostat for suspended growth. Both systems were inoculated with the Evanite mixed anaerobic culture (known to contain at least one strain of *Dehalococcoides*) and fed TCE-saturated medium (~10 mM). The biofilm system yielded more stable performance, and was able to sustain TCE dechlorination to cDCE much longer than the chemostat. Both systems ultimately failed after months of operation and continuous exposure to approximately 10 mM cDCE. Suspended growth batch experiments failed to sustain TCE dechlorination past ten days at cDCE concentrations above 4 mM, suggesting simple cell retention is not the only mechanism for maintaining dechlorination activity in the attached growth system. While TCE could be dechlorinated almost completely to cDCE in the chemostat with a 16-day retention time for 50 days before failure, the column maintained complete dechlorination to cDCE for 100 days before failure with a 12-day retention time. Data suggests attached growth did alleviate high solvent concentration toxicity and may play a critical role in site remediation.

Anaerobic Oxidation of Methane and GPT: Highlights and Lessons Learned

Sara L. Caldwell¹, James R. Laidler¹, Elizabeth A. Brewer², Jed O. Eberly², Sean C. Sandborgh², and Frederick S. Colwell²

¹Portland State University

²Oregon State University

[The presentation covers GPT as a learning experience; this abstract covers the topic itself.] Microbially mediated anaerobic oxidation of methane (AOM) plays a critical role in the balance of atmospheric greenhouse gases by consuming methane produced in marine sediments, anoxic lakes and marine waters, natural wetlands, coastal plains, agricultural areas, and possibly within a wider range of environments than currently recognized. However, the contribution of AOM to the global methane cycle is often underestimated in global carbon budget estimations, which may strongly influence models that predict long-term climate change. AOM coupled to sulfate reduction is the most studied pathway, although more energetically favorable strategies, such as AOM coupled to denitrification, have also been recognized. Phylogenetic trees based on 16S rRNA clone libraries from anaerobic lake and marine sediments contain diverse methanotrophic archaea (ANME) and sulfate-reducing bacteria (SRB). Nucleic acid probes also show that these organisms form a close, physical association, or consortium. Single-species groups of methanotrophic archaea have also been found, but there are currently no candidates for single cell AOM. Furthermore, the 1:1 molar ratio of methane consumption and sulfide production reported from these consortia suggests that electrons must be transferred between species to couple these reactions, but the mechanism of interspecies electron transfer has not yet been determined. Culture-dependent and independent techniques have provided clues to how these communities function in the

environment, but they are currently limited, and many questions remain regarding the diversity of AOM-related organisms and the environmental factors that regulate their metabolisms. Future research should expand the range of recognized environments for AOM, include laboratory growth of individual AOM archaea with and without their bacterial counterparts, and use non-traditional molecular techniques to better understand the nature of microbially mediated methane oxidation.

Life and Times in the Deep Subsurface

Tommy Phelps, Oak Ridge National Lab, Oak Ridge, Tennessee

Convincing evidence has emerged that the biosphere extends to depths exceeding 3 km, raising questions regarding the limits of the biosphere, the age of the microbes in these deep ecosystems, their evolution, their activity, their sources of energy for metabolism as well as their potential applications to benefit society. The most uncompromising limit of the deep biosphere is likely temperature, followed by the availability of liquid water and energy. The most obvious pathways for water in the deep subsurface are geological fractures. Water-filled fractures form habitable spaces and provide conduits for the transport and reinoculation of microbes into sterilized zones. An important limitation common to all deep subsurface environments is space. Porosity is typically less than 1% and permeability is very low. Consequently, deep groundwater moves slowly and microbes may depend on diffusion-limited nutrient flux. The deep terrestrial subsurface depends on energy and nutrients from ancient organic matter, inorganic sources associated with the host rocks and fluids, abiotic sources such as H₂ from radiolysis, or gases from crustal sources. Hydrogen may be produced by geothermal mechanisms and accumulate beneath the biosphere. Transported via fracture systems it intercepts the biosphere, but even then the rate of consumption by microorganisms may be slow due to the numerous limitations imposed on the deep biosphere. Microbial populations are typically characterized as being sparse, isolated in tiny pores, slow growing/respiring and resource-limited. Any requirement for syntrophic interactions at very low microbial densities further limits metabolism as energy flux resources would be shared.

This relatively recent discovery of a deep subsurface biosphere has opened a new scientific frontier where earth sciences, chemistry, physics and biology can merge to provide insights into how life on this planet and even extraterrestrial life, may have originated and evolved over billions of years. The geological isolation of these deep subsurface microbial communities offers the potential to address questions related to potentials and or extent of life on Mars and other planetary bodies. Advances in our understanding of the extent, diversity, distribution and functioning of microorganisms in deep, often extreme, subsurface environments will rapidly expand our knowledge of geomicrobiological and biogeochemical processes on Earth and beyond. The discovery of novel microorganisms from deep the subsurface also provides ample opportunities for us to discover new pharmaceuticals, processes for biochemical and chiral-specific synthesis, environmental remediation and energy production, novel materials or novel biotechnology products.

Biosedimentology of Thermal Features of the Uzon Caldera, Kamchatka, Russia

Jessica Goin, Portland State University IGERT Student

Modern hot springs provide a habitat for microorganisms that are similar in metabolism, morphology, and cellular structure to the organisms that existed relatively early in Earth's history. These organisms provide architectural framework for the deposition of silica, and thus leave their trace in the form of microfossils and sinter fabric. These traces of the original microbial community are known as biosignatures and the understanding of the formation of these modern biosignatures increases our ability to interpret their ancient analogs. Analysis of the role of microorganisms in the formation of sinter fabric is essential to our understanding of the evidence for early life provided by stromatolites. While stromatolites have long been considered important biosignatures, recent research indicates that many features of stromatolites may form in the absence of biology. Further elucidation of the role that microorganisms play in the development of modern sinter fabric is essential for us to distinguish biotic and abiotic stromatolites in the rock record.

The Uzon Caldera, on the Kamchatka peninsula of far eastern Russia is home to a wide variety of hot springs of disparate temperature, pH, geochemistry, and microbiology. Analysis of biosedimentology of hot springs in the Uzon Caldera with distinct geochemistries allows for an examination of the role played by microbiology, detrital sedimentation, and authigenic mineral precipitation in the formation and potential preservation of biofabrics. The potential for the preservation of biofabrics is compared in four of these thermal features which differ in their level of detrital sedimentation, mineral precipitation, and biofilm development. Examination of biosedimentation in these thermal features involved SEM analysis of the relationship between microorganisms and minerals, thin section analysis of sinter fabric, XRD determination of mineralogy, and optical microscopy of living organisms.

Trace Metal Cycling in Continental Margin Sediments - Insights from Molybdenum Isotopes

Rebecca Poulson, Oregon State University IGERT Student

When organic matter is buried in marine sediments, microbes oxidize the organic carbon in a familiar series of diagenetic reactions referred to as the "redox ladder." Microbial degradation of organic matter proceeds through a predictable series of electron acceptors. The unique geochemistry of molybdenum leads to sediment Mo enrichments under both oxygenated and reducing conditions, each with distinct Mo isotopic signatures. It is this feature of Mo geochemical behavior that we hope to exploit as a record of past geochemical conditions. In well-oxygenated environments, Mo sorbs to Mn-oxides, leading to sediment Mo enrichments. This Mo is subsequently released when reduction of Mn-oxides is a dominant diagenetic process. In these settings, sediment Mo isotope compositions are the most negative measured to date, suggesting that Mn-controlled Mo cycling produces a unique (negative) isotopic signature. Under more reducing conditions, sulfate reduction becomes a dominant mechanism for organic carbon degradation. In the presence of sulfide, Mo is less soluble and precipitates as solid phase Mo-sulfides, also resulting in sediment Mo enrichments. These reducing sediments have a Mo isotopic signature distinct from that of more oxygenated deposits. We have

investigated Mo concentrations and isotopic compositions of modern marine sediments from the California, Mexico, and Peru continental margins. These sites represent a variety of geochemical depositional environments as reflected in the sediment Mo isotopic signatures observed. At some sites, down-core profiles reveal changes in Mo isotopic values with depth, and we speculate that this Mo isotope variability reflects changes in sediment chemistry over time. If true, this hypothesis implies that under the right conditions sediment Mo isotope values will record evolving or transient chemical conditions.

Bug Juice: Microbial Fuel Cells in Marine Sediments

Mark Nielsen and Clare Reimers, Oregon State University

Modest amounts of electrical current can be generated by bridging the seafloor's redox gradient with electrodes. Benthic microbial fuel cells (BMFCs) have been proposed as devices to harvest this electrical current to power environmental sensors which require 10 mW to 5W of power. Many factors affect the amount of power that can be produced by a fuel cell including energy losses due to biological metabolism, charge transfer limitations at the electrodes, internal circuit resistances and mass transfer limitations. As an added complication, these factors vary with time, are interdependent and respond dynamically to the operating parameters of the BMFC. We will provide an overview of BMFC performance based on an equivalent circuit model with examples from recently deployed BMFCs.

Specific factors that affected previous prototypes were the slow diffusion of reactants through the sediment to a buried anode and electrochemical passivation of the anode. We designed, built and deployed a new benthic chamber-based BMFC design that incorporates a suspended, high surface area and semi-enclosed anode to improve performance. In Yaquina Bay, Oregon, two BMFCs generated current continuously for over 200 days. One BMFC was pumped which resulted in power densities more than an order of magnitude greater than those achieved by previous BMFCs with graphite plate anodes. On average these power densities with advection were $233 \text{ mWm}^{-2}_{\text{seafloor}}$; peak values were $380 \text{ mWm}^{-2}_{\text{seafloor}}$ and performance improved over the time of the deployments. Another BMFC was deployed at a cold seep in Monterey Canyon, CA, to test the chamber design in an environment with natural advection. Power density increased five fold (140 mWm^{-2} vs. 28 mWm^{-2}) when check valves were installed to allow flow through the chamber.

Poster Abstracts

Subsurface Biosphere Initiative and IGERT Workshop Newport, Oregon June 17-19, 2007

In Alphabetical Order by Presenter

Trichloroethene Dechlorinators and Dissimilatory Fe(III) Reducers Competition for Lactate and Acetate as the Electron Donor in a Continuous Flow Column Study

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

Continuous-flow anaerobic bioreactor column experiments were conducted to evaluate the reductive dechlorination of trichloroethene (TCE) in the presence of aquifer material from the Hanford DOE site. The Hanford aquifer material contained approximately 6 wt % total Fe and about 0.15% available Fe. The column was pre-treated with 5 mM Na₂S solution to reduce Fe(III) to Fe(II) and bioaugmented with the Point Mugu (PM) culture that is capable of transforming TCE to ethene. Upon bioaugmentation and the addition of lactate as a fermenting substrate TCE, sulfate, and iron were reduced. Increasing lactate concentrations from 0.67 mM to 1.0 mM, increased TCE dechlorination to VC and ETH with almost 98% ETH after 300 days of column run. Acetate and propionate were the lactate fermentation products in the column. The degradation of acetate is strongly influenced by the availability of electron acceptors, such as sulfate or ferric iron. The dissimilatory iron-reducing organism can use acetate as an electron donor to reduce Fe(III) in anoxic environment. The presence of sulfate or ferric iron, acetate oxidizes to CO₂, while it converts to CH₄ and CO₂ in the absence of these electron acceptors (i.e., methanogenic phase). During the entire column operation, methane production was never detected and the hydrogen concentration was below detection limit (< 10 nM). Redox capacity measurements showed iron reducing conditions persisted in the column. Mass balances on the lactate oxidation showed that an estimated of 27% were associated with iron reduction (based on the sequential reducible iron oxide extraction and previous column experiments with the same aquifer material), 8.5% with sulfate reduction, and only 6.1 % were associated with dehalogenation reactions. Therefore, acetate the fermentation product of lactate and propionate was used as electron donor to reduce Fe(III) to Fe(II) in the column. The results indicate that in the subsurface environment, addition of lactate as electron donor for the dehalogenating microorganisms can stimulate iron reducers for the available acetate from the lactate fermentation products.

Woody Plant Encroachment into Grassland Alters the Composition of Soil Microbial Communities

Brewer, E.A.¹, T.W. Boutton,² and D.D. Myrold,¹

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Changes in the functional composition of plant communities and key ecosystem processes may have significant impacts on the structure and diversity of soil microbial communities. Woody plant encroachment into grasslands alters the quantity and quality of litter and root inputs to soil, and often modifies the storage and dynamics of soil organic matter. Our objective was to examine changes in soil microbial communities in the subtropical grasslands of southern Texas that have been encroached by trees and shrubs. Soils from two depths (0-15 and 15-30 cm) were collected at four positions along a transect extending from the center of shrub/tree clusters out into the surrounding open grassland. Phospholipid ester-linked fatty acid (PLFA) analysis was used to characterize the composition of the soil microbial communities. Bacteria communities were analyzed using the 16S rRNA gene for terminal restriction fragment length polymorphisms and fungal communities using the ITS region of the ribosomal gene in length heterogeneity polymerase chain reaction. All analyses revealed significant differences in communities among the two depths. Composition of the microbial communities did not differ along transect locations at the 0-15 cm or the 15-30 cm depth. Fungal communities under the woody clusters and the open grassland were different at the 0-15 cm depth. Bacterial communities did not differ across transect locations but did differ among tree clusters. Both fungal and bacterial communities correlated with soil $\delta^{13}\text{C}$ and root biomass. At the 15-30 cm depth, bacterial and fungal communities did not differ among locations but there were differences among tree clusters. These results indicate that soil microbial communities, particularly fungi, are altered following woody plant encroachment into this subtropical grassland ecosystem. Furthermore, correlations of the microbial communities with soil, root and vegetation variables suggest interdependence of microbial communities and C cycling.

Characterizing Biofilm Formation in *Acidiphilium cryptum* JF-5

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Acidiphilium cryptum JF-5 is an Fe-respiring acidophile of potential use as a metal remediation agent at low pH. Biofilm formation in this organism is not well characterized, so the goal of our research was to determine if *Acidiphilium cryptum* JF-5 forms a biofilm, and if so, what are the structures of the biofilm and composition of the biofilm matrix. **Methods:** *A. cryptum* was grown in a minimum salts media at a pH of 3. Anaerobic growth media consisted of sparged minimum salts supplemented with 15mM ferric sulfate. Initial characterization took place by filtering rafts of cells on to a polycarbonate filter. The rafts were stained with DAPI, Live/Dead, ConA, and Wheat Germ lectin, Alcian Blue or SYPRO orange. In addition, a new fluorescent Fe(II) probe was used to detect Fe(II) produced by actively growing cells. Biofilm growth was then determined in an open cell, flat plate reactor at a flow rate of 1ml/min. The same media for aerobic growth as above was used. Iron (3N5) plates were used as coupons in the flow

cell. After 24 hr coupons were removed from the flow cell, rinsed with sterile water, and stained with DAPI. Stained bacteria were visualized on an epifluorescent microscope. **Results:** Under anaerobic and aerobic growth *A. cryptum* will form large rafts of cells, as visualized with DAPI, Live/Dead, and SYPRO orange stains. Both ConA and wheat germ lectins bound to the rafts of cells. Alcian blue only stained aerobically grown cells. The Fe(II) probe detected Fe(II) only in the anaerobically grown cells, confirming Fe-reduction. Flow cell studies on iron coupons resulted in microcolonies. **Conclusion:** This study shows that *A. cryptum* is capable of forming biofilms. Lectin stains show that under aerobic and anaerobic conditions N-acetylglucosamine, N-acetylneuraminic acid, α -mannopyranosyl, and α -glucopyranosyl sugar residues are present. *A. cryptum* produces acid mucopolysaccharides only when grown anaerobically. To support physiologic studies, genome annotation was performed to find biofilm-related genes. Various polysaccharide synthesis and export genes have been identified from the genome. Taken together, our findings show both genomic and physiologic potential for biofilm formation in this organism.

Methodology of Low Volume Sampling for Monitoring Oxygen and Reduced Species in Anaerobic Cultures

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Being one of the most significant chemical species in the environment, O₂ is still not easily detected at concentrations below 1 mg/L. Many of the existing analytical methods rely on sample volumes >200 mL for minimizing oxygen contamination during sampling and detection of dissolved oxygen (DO) in suboxic or anoxic samples. In this study, we introduce a new methodology and a portable flow sampler for low volume (1 mL) sampling from a closed-anoxic laboratory scale system. The sampler includes a miniaturized preset volume (50 μ L) micropump which is controlled via a home made circuit interfaced to the laptop computer with a microcontroller module (Basic Stamp1). Oxygen contamination coming from the atmosphere and the materials in contact with the sample (i.e., tubing, containers, etc.) was minimized by reducing the number of connectors and double containment of sample bottles. Unique removable septum caps were developed to fit onto commercially available microcosm bottles and to provide double containment. These caps ensure minimum exposure to oxygen during sampling.

Anaerobic cultures under Fe(III)-reducing, sulfate-reducing and methanogenic conditions were sampled with the micropump sampler and a manual gas-tight syringe. Samples were tested for low level DO by measuring the increase in absorbance of reduced indigo carmine in an anoxic double-septum vial that serve as a cell for portable spectrometers. None of the sulfate-reducing and methanogenic cultures had detectable DO concentrations (above 0.04 mg/L). Interference of Fe(III) was observed in Fe(III)-reducing cultures due to excess amount of iron hydroxide colloids.

Presented methodology and devices are highly applicable to other sensors and spectrophotometric detection methods for low level redox-active species. The low volume sampler coupled with double septum cap protection, can provide nondestructive and accurate analysis of DO below 1mg/L in anaerobic cultures and groundwater.

“*Thiominerva volcanii*” gen. nov., sp. nov., a new genus within the Aquificales from a terrestrial hot spring, Costa Rica

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A novel thermophilic bacterium, designated strain CR11, was isolated from a filamentous sample collected from a terrestrial hot spring (~pH 7.0; 93°C) on the southwestern foothills of the Rincón Volcano in Costa Rica. Enrichment cultures were incubated at 80°C under microaerophilic conditions, using sulfur as the electron donor, and isolated to purity by consecutive serial dilutions. The new isolate is most closely related (94.5% similarity between 16S rRNA sequences) to *Thermocrinis ruber*. Its gram-negative cells are motile rods with polar flagella. Approximately 5% of cells observed under transmission electron microscopy (TEM) also exhibit intra-cytoplasmic, membrane-like structures of unknown function. Strain CR11 grows chemolithotrophically with elemental sulfur, thiosulfate, or H₂ as electron donors and with O₂ (up to 16% v/v) as the electron acceptor. However, the organism appears to grow better with elemental sulfur and thiosulfate than with H₂. Growth only occurs at NaCl concentrations below 0.4% (v/w). The isolate also grows heterotrophically on mannose, glucose, maltose, succinate, peptone, casamino acid, starch, citrate, and yeast extract in the presence of oxygen (4%) and S⁰. Strain CR11 grows between 63°C-88°C (optimum at 75°C); no growth is observed at 60°C or at 90°C. The pH range for growth of the isolate is 4.8 to 8.3 (optimum between 5.9 and 6.5). The G+C content of this strain, determined by thermal denaturation, is 40.3. Based on detailed phylogenetic analysis of its 16S rRNA gene and ITS (Intergenic Transcribed Spacer) sequences and its G+C content, a distinguishing trait for this organism, strain CR11 is proposed to represent a novel genus within the *Aquificales*. The name proposed for this isolate is “*Thiominerva volcanii*.”

Thermophilic Hydrogenases: Properties and Applications in Biological Hydrogen Production

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A comprehensive review of known thermophilic hydrogenases has revealed several with remarkable properties that make them well suited to biological hydrogen production. In addition to retaining their activity at temperatures as high as 80°C, some of these enzymes are remarkably oxygen tolerant and remain stable when purified. To date, virtually no research has addressed the application of thermophilic hydrogenases in biological hydrogen production. We have identified the hynSL hydrogenase from *Thiocapsa roseopersicina* as the ideal choice for development of a photobiological system for hydrogen production based on enzyme activity, thermostability, and oxygen tolerance. We are currently working to express this hydrogenase in the thermophilic cyanobacterium *Thermosynechococcus elongatus* in order to be able to take advantage of

the thermophilic capabilities of its photosystems. This modified organism will serve as a model for biomimetic design of an organic based bioreactor for hydrogen production.

Ecology of Thermoacidophilic Microorganisms Inhabiting Deep-Sea Hydrothermal Vent Chimneys

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The heterotrophic sulfur- and iron-reducing microorganism “*Aciduliprofundum boonei*” is the first thermoacidophilic microorganism isolated from a deep-sea hydrothermal vent. “*A. boonei*” belongs to the ubiquitous archaeal DHVE2 (deep-sea hydrothermal vent euryarchaeotic 2) lineage that had previously escaped cultivation efforts. In this project, I propose to investigate thermoacidophily and the ecology of the DHVE2 lineage by using “*A. boonei*” as a model organism. Due to the ubiquitous distribution of DHVE2 16S rRNA gene sequences found at vent sites from around the globe, the predicted pH gradient within chimney structures and the physiology of “*A. boonei*,” it is hypothesized that thermoacidophiles are found in mature sulfide chimneys and play significant roles in the biogeochemical cycling of sulfur and iron in the deep oceans. The draft genome of “*A. boonei*” has recently been sequenced and analysis is underway.

Modeling Seasonal Silica Deposition

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The deposition of silica sinter in modern hot springs provides an analogue to preservation of early life on Earth. The extreme environment excludes higher organisms, and the silicifying environment generates microfossils and stromatolite fabrics through interaction with microbial mats. Stromatolites were long accepted by the scientific community as evidence for early life on Earth, but continuing research has provided evidence that many stromatolite-like objects may form in the absence of biologic inputs. The study of the role that modern microbial mats and biofilms play in determining sinter fabric during deposition is useful in interpreting stromatolites in the rock record for biogenicity.

Sinter deposition in the outflow channel of K4 Well, a thermal feature in the Uzon Caldera, Kamchatka, Russia, has been analyzed to determine the role that the biofilm plays in determining fabric. Laminar sinter forming under a cyanobacterial biofilm was analyzed by SEM, and this analysis was used for comparison to model predictions. The evaporative concentration of silica was determined using seasonal evaporation and precipitation information. The concentration of silica is then used to predict monomeric and colloidal silica deposition. The model predicts greater deposition of colloidal silica in the summer and monomeric silica in the winter. The model predictions support seasonal deposition of the layers, as colloidal silica layers contain many cyanobacterial microfossils, while blocky silica layers have few microfossils.

Dispersion through Highly Heterogeneous Porous Media

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This project focuses on how solute and microbial transport, specifically the dispersion process, is affected by heterogeneities in porous media. In order for media to be termed ‘highly heterogeneous,’ there needs to be a high variance in the log-conductivities of the different media within the system. Solute transport behavior through highly heterogeneous media is easy to distinguish due to its non-Fickian behavior. This is observed experimentally as tailing in the spatial concentration field or the resultant breakthrough curve. For non-Fickian behavior, mathematically the conventional dispersive flux term in the conservation of mass equation does not capture all of the transport behavior. It is hoped through this research to develop a mathematical model for a binary spatial conductivity field. The binary field will be represented as a high-conductivity matrix material (the η -phase) with low-conductivity inclusions (the ω -phase). A 100 liter flow cell will be filled with coarse silica sand representing the η -phase, with sintered spherical inclusions of much finer silica sand representing the ω -phase. The η - and ω -phases will have particle diameters of ~ 0.24 mm and ~ 71 μ m respectively. The inclusions will be placed ‘randomly’ within the flow cell in order to create a highly heterogeneous binary system. The data collected from this system will be used to correlate the experimental and the mathematical models developed through upscaling and volume averaging techniques. 3-D computer models will also be developed to simulate the processes occurring within the experimental system and ensure correlations between experimental and mathematical results. Preliminary experiments have been conducted in a small cylindrical column with three spherical inclusions for 1-D analyses. The experimental protocol and results will be discussed.

Spatial Distribution of Biofilms in Porous Media

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Current understanding of subsurface microbial processes and their impact on fluid hydrodynamics is limited by our ability to observe microbial colonies in their natural spatial arrangement. Biomass in porous media has only been observed in two dimensions, at the nanoscale, or at limited resolution in three dimensions; scientists are therefore lacking significant information about biofilm form. Three dimensional film arrangements can significantly alter pore flow velocities and overall mass transfer between the aqueous and biological phases. Further complicating this issue is the potential for differences in biofilm density, arrangement, and distribution based on the geometry of porous media and fluid velocities. We are focusing efforts on resolving images using synchrotron-based x-ray microtomography, we plan to image biofilms without disturbing their natural spatial arrangement. Imaging of the biomass will be accomplished by adding particles to the aqueous phase; preliminary experiments indicate that silver nanoparticles are the most effective at providing contrast. Current research efforts are focused on testing the size of particles that penetrate into the biomass and how they distribute as a function of biofilm thickness and flow rate in a two dimensional flow cell. Results from our two dimensional experiments combined with calculations using Stoke’s Law will aid in the determination of particles to be used in x-ray tomography imaging. Data collected will provide

information about physical structures of biomass and how they alter the physical properties of soil, e.g. porosity and hydraulic conductivity. Ultimately, we will gain a greater understanding of how changes in physical parameters may impact the rate microbes degrade contaminants and therefore alter the effectiveness of bioremediation.

Microfluidics in the Subsurface: Towards In-Situ Analysis of Microliter Volumes in a Miniaturized Package

Koch, Corey, Vincent T. Remcho, and James D. Ingle, Department of Chemistry, Oregon State University

Microfluidics is a state-of-the-art tool that is allowing fields such as analytical chemistry, chemical engineering, and industrial engineering to scale operations down by several orders of magnitude. By fabricating micrometer sized channels with integrated circuit technology, analyses can be performed on nanoliter volumes. Microfluidics therefore requires less sample/reagent, analyses can be conducted more rapidly, and instruments can be miniaturized like never before. For environmental analysis this presents us with the potential to create completely automated and in-situ devices that can fit in the palm of your hand. With this new technology field monitoring can become more remote and automated and less expensive devices are more amenable to vast monitoring networks.

We are currently developing a micro-total-analysis platform to implement common colorimetric methods of water quality monitoring. The device will be in-situ (immersed in the sample), able to acquire a sample, mix small volumes of sample and a colorimetric reagent, perform optical detection, and have on-board electronics/communication for data acquisition and instrument control. Currently proof-of-concept for the device is being demonstrated by analyzing river water for iron via the 1,10-orthophenanthroline method. The ultimate goal is to deploy the device in a ground water monitoring well and also show its usefulness with other analytes such as sulfide. It is our hope that not only will an in-situ device be valuable for monitoring purposes but that it will also provide knowledge about contamination in current well sampling methods.

To date, an integrated micro-mixer and 1-cm pathlength absorbance flow cell have been characterized with iron and the colorimetric reagent thiocyanate. The performance characteristics of the microfluidic analysis are comparable to those of the analysis performed manually and measured with a benchtop spectrophotometer. A microfabricated filter has also been developed to eliminate clogging of the microchannels and was tested using Willamette river water. The filter was able to continuously filter 40 mL of Willamette river water over 13.5 hr. This accounts for approximately 2500 analyses (~15 μL /analysis) without clogging of the microchannel or degradation of flow rate.

Preliminary work on design of micropumps and valves will also be presented along with strategies to integrate the device and provide for wireless communication. An integrated filter-mixer-flow cell will be tested in a laboratory river simulator and a field test will then be performed on the completely integrated device (including pumps, valves, and electronics).

Viral Mineralization in a High-Silicate Environment

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The discovery of the first microbial fossils by Walcott in 1914 and subsequent finds in later decades have had a tremendous impact on both biology and paleontology. Although controversy continues to surround some of these fossils, the existence of microbial life as far back as the Archaean period is gaining wider acceptance.

One of the significant contributions of the early microbial fossils was to provide researchers with an idea of what microbial fossils might look like. This undoubtedly led to further discoveries of microbial fossils.

The current lack of viral fossils can be attributed to two factors: [1] few, if any, people are looking for them and [2] there is no information on what viral fossils might look like. Many people looked at the same sort of carbonaceous deposits in the Gunflint Chert formation before Tyler and Barghoorn realized they were cyanobacteria. Likewise, paleontologists and geologists may have repeatedly looked at electron microscope views of viral fossils and not recognized them for what they are.

The purpose of this project is to determine if viruses can be mineralized and to give some idea what fossilized viruses might look like. Since microbial mineralization has been observed in contemporary hot springs as well as in ancient hot spring relics, I will attempt to mineralize viruses under conditions similar to a silica-rich hot spring.

The project will be carried out in three phases. The first phase, which has already yielded positive results, will be to mineralize a range of viruses in the laboratory and examine them under the electron microscope. Once the mineralized viruses have been characterized, the second phase will be to look for the same characteristics in the water and sinter from silica-rich hot springs. If mineralized viruses can be unambiguously identified in contemporary hot spring silica deposits, the third phase will be to examine cherts that have fossilized microbes for the presence of fossilized viruses.

Effect of the CSTR Hydraulic Retention Time on Tetrachloroethene Dehalogenation and the Lactate Fermentation Pathway

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This study is being performed to evaluate the effect of cell hydraulic retention time on the anaerobic dehalogenation of tetrachloroethene (PCE). The changes in the microbial community that occur will also be evaluated along with the fermentation pathways using lactate as an electron donor. For this research a continuous stirred tank reactor (CSTR), with a total volume of 5.6 L is being operated anaerobically without headspace and fed with media (see Yang and McCarty, 1998) containing 1 mM PCE (saturation concentration in water) as electron acceptor and lactate (2 mM: required electron equivalents for complete PCE transformation to ethene) based on produced H₂ through the fermentation as electron donor. The feasibility of dehalogenating high concentrations of PCE to ethene at various PCE loading rates is being determined, as well as the optimal electron donor/acceptor ratio. The effect of high concentration of PCE dehalogenation on the dehalogenation of less chlorinated ethenes will also be evaluated

using activity based kinetic tests and molecular based methods. Additionally, model analysis will be performed where H_2 production from organic substrate fermentation will be linked with dehalogenated based hydrogen utilization. Results are presented here of the initial stages of the CSTR operation that indicate the transformation of PCE to cis-dichloroethene (cis-DCE) is maintained with the gradual decrease in the HRT. In batch experiments using lactate and H_2 as electron donors, the complete PCE dehalogenation to ethene was obtained.

Numerical Simulation of the Anaerobic Transformation of Tetrachloroethene (PCE) to Ethene in a Continuous Flow Aquifer Column

Mustafa, Nizar , M. Azizian, M. Dolan and Lewis Semprini, School of Chemical, Biological and Environmental Engineering, Oregon State University

Enhanced desorption resulting from anaerobic reductive dechlorination of Tetrachloroethene (PCE) in continuous flow column experiments was modeled and compared with experimental observations. The column studies were performed with Evanite (EV), enrichment culture and Hanford aquifer solids. The experiments showed that cis-DCE concentrations in the column effluent exceeded the influent PCE concentration. This increase in cis-dichloroethene (c-DCE) is due to enhanced PCE desorption and transformation. Moreover, column studies show dynamic changes both spatially and temporally in chlorinated aliphatic hydrocarbon (CAH) distributions, and provide a reasonable means of mimicking processes that would be encountered in the field. The results of these experiments provide a unique data set for modeling analysis. A 1-D reactive transport model was developed, accounting for dispersion, advection, rate-limited sorption and desorption, reductive dechlorination kinetics and microbial growth. The model was tested and validated by mass balances, comparisons with analytical solutions and a batch kinetic model. The 1-D model was then applied to evaluate the response of CAHs to biostimulation of the laboratory column. Initially, kinetics for the EV culture used in the model simulations were those determined by Yu and Semprini (2004). Sorption sediment:water distribution coefficients (K_d) for PCE and its anaerobic transformation products were determined in batch laboratory studies. The system of model equations were solved numerically using (COMSOL 3.3) which used 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. Trichloroethene (TCE) was shown not to accumulate in the column effluent. The model predicted steady state effluent concentrations of ethene and VC that did not match the column observations. This likely results from a limitation of using a single dehalogenating population model.

Dispersal and Degradative Mechanisms of Selenium, Arsenic and Organic Compounds from Sediments of the Colorado River Delta in the Upper Gulf of California by Physico-Chemical processes

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The hostile conditions of the Sonoran desert and the Gulf of California have shaped the evolution of unique pristine habitats along the shores of Colorado River Delta (CRD) and Upper Gulf of California (UGC). The unique biodiversity of the region led to the establishment of the Reserva de la Biosfera Alto Golfo de California y Delta del Rio Colorado as a protected natural area (PNA) in 1993. The UGC is a shallow (<30 m), evaporative marine environment characterized by gravity currents and reversible, seasonal surface current gyres.¹ The CRD was accreted during the past 5 Ma and consists of fine-grained sandstone, siltstone and volcanic detritus materials of Mesozoic marine sedimentary and volcanic rocks from the upper and lower Colorado drainage basins.² As a result of large water reclamation and retention projects in the upper Colorado River, (Hoover and Glenn Canyon Dams, 1935 and 1952); the annual freshwater and sediment discharge that maintained the CRD and UGC ceased, transforming the mouth of the Colorado River into a negative estuary.³ These conditions and the hydrodynamics of the UGC result in the erosion of the CRD via resuspension, off-shore and long-shore transport of deltaic sediments throughout the region.⁴ A review of current research in this region revealed a gap between the geochemistry of the sediments, their transport, and potential effects on local biota. Given the physico-chemical dynamics of the region, we hypothesize that tidal resuspension of CRD sediments has specific impacts on the geochemistry of those sediments, and that these are transported and deposited in nearby tidal lagoons and marshes with potential bioaccumulation in local biota. This study will focus on four of those effects: 1) Solubilization of selenium, arsenic and other redox-sensitive species from the sediments by oxidative chemical processes ensuing from deeper penetration of the dissolved oxygen profile into the interstitial pore space and /or resuspension of the sediment matrix; 2) Mobilization of the organic matter OM and chronological important biomarker indicators from, and with the sediment by advective processes; and 3) The role and effects of microbially mediated decomposition of OM and biotracers, prior and post advection; and 4) The transportation mechanism of the redox-sensitive chemicals and the OM. Herein it's proposed to elucidate on the dispersal and degradative mechanisms of these species and substrates in the UGC region.

References:1 (Lavin et. al; 1998).2 (Muffler and Doe, 1968).3 (Shumilin et. al. 2002). 4 (Carriquiry and Sanchez, 1999).

Resistance is Inevitable: Seeking the Relationship Between Permeability and Formation Factor

Nielson, Mark, Hatfield Marine Science Center and IGERT Student and Clare Reimers, College of Oceanic and Atmospheric Sciences, Oregon State University

There is growing appreciation that advective transport through permeable sediments may play a role in biogeochemical cycles. Conventional wisdom used to be that the small standing stocks of organic carbon and other reactants resulted in low rates of remineralization and inconsequential interaction with the overlying water. Recent studies, however, show a positive correlation between organic C degradability (measured as a first-order decay constant) and grain size. This indicates that the observed low

concentrations of organic carbon in permeable sediments could be due to high rates of remineralization rather than low accumulation. In fact, about 30% of the oceanic primary production occurs in continental margin sites (~10% of the total ocean area) which are predominantly underlain by permeable sediments. Approximately 50 percent of the organic material produced through primary production in the coastal water column sinks to the seafloor.

Measuring exchange between permeable sediments and overlying water is a technical challenge. The combination of permeable sediments and flow over sedimentary bedforms result in advective transport in to (and out of) the uppermost layer of sediments. The key parameter to modeling this transport process is permeability of the sediment. Measuring this parameter is complicated by the fact that in situ permeability is difficult to measure (requires multiple boreholes) and permeability of core samples is not always a reliable indicator of in situ conditions. Therefore, it would be advantageous to relate an easily measured field parameter such as electrical resistivity (expressed as formation factor) to permeability. This would allow a more comprehensive investigation of spatial and temporal scales of permeability in coastal sediments.

Our hypothesis is that there is a significant correlation between permeability and formation factor. We propose to test this hypothesis by performing a series of permeability measurements and electrical resistivity measurements on carefully selected and characterized cores. Once the relationship between the parameters is demonstrated in the laboratory, field resistivity measurements can be made using a benthic profiler thus allowing us to assess in situ permeability. This relationship can be used broadly by researchers interested in the role of permeable sediments in diagenetic processes.

Zinc Inhibition of *Nitrosomonas europaea* in a Continuous Culture

Radniecki, Tyler S., Mark E. Dolan and Lew Semprini, School of Chemical, Biological and Environmental Engineering, Oregon State University

Nitrosomonas europaea plays a critical role in the removal of toxic ammonia (NH_3) from wastewater treatment plants (WWTPs) by oxidizing NH_3 to nitrite (NO_2^-). *N. europaea* and is widely considered the most sensitive organism in the removal of NH_3 from WWTPs being inhibited by many compounds found in WWTP influent including aromatic hydrocarbons. *N. europaea* is capable of co-metabolizing benzene into phenol and toluene into benzyl alcohol and benzaldehyde at the cost of inhibiting its own ammonia oxidation rate. The daughter products of toluene oxidation, benzyl alcohol and benzaldehyde, were found not to inhibit *N. europaea*, indicating that toluene directly inhibits the ammonia oxidation rate. However, the daughter product of benzene oxidation, phenol, does inhibit *N. europaea*. The accumulation of phenol throughout the 3-hour batch experiment appears to be the source of inhibition, not the presence of benzene, and is responsible for the curvature of the nitrite production curve. Surprisingly, there was no significant up- or down-regulation of genes in *N. europaea* cells exposed to 20 μM toluene, enough to cause 50% inhibition after 60 minutes of exposure. However, exposure of *N. europaea* to 40 μM benzene resulted in the up-regulation of an operon that appears to be involved with fatty-acid metabolism and membrane protein synthesis and include the genes NE 1545 and NE 1546. NE 1545 and NE 1546 appear to be specific for benzene and phenol inhibition as they show significant up-regulation in the presence of benzene or phenol but are not up-regulated in the

presence of toluene. Both genes are also up-regulated within 30 minutes of exposure to benzene and stay highly up-regulated for at least 3 hours making them a sensitive and robust signal of benzene/phenol inhibition.

Microbial Adhesion and Transport

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Understanding the phenomenon governing the interactions between bacteria and solid surfaces is essential to the success of many biological systems, such as bioremediation scheme, deep-bed filtration etc. These interactions also govern the transport of bacteria in natural and engineered systems. Microbial adhesion and transport is inherently a “multi-scale” phenomenon and includes interfacial scale (nm to μm) at the smallest level to the field scale (10 's of cm to 100 's of m). This disparity of length scales lead to difficulties in application of experimental and theoretical results to natural and engineered biological systems. Upscaling offers a solution to this problem by preserving only the important features of applicable processes from the smallest to the largest scale of interest. The heterogeneity of scales is addressed through a series of upscaling efforts to move between adjacent length scales. The current research is aimed at exploring the phenomena of microbial adhesion and transport through different length scales, using both experimentation and theoretical constructs. Specifically two levels of upscaling, (1) Interfacial to pore scale (Level I to II), and (2) Pore scale to Darcy-Scale (Level II to III) would be investigated and the theoretical developments would be verified by lab experimentation. Several representative strains of bacteria (including both Gram-positive and Gram-negative) would be used for experimental work. The proposed research is expected to result in (1) the development of an upscaled conservation equation for describing the microbial transport in the presence of a solid surface (i.e. at interfacial scale), and (2) a filtration model for bacteria for continuum (REV) scale.

Effect of Chlorobenzene on *Nitrosomonas europaea*: Physiology, Proteomics and Genetic Expression

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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. 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 $2\ \mu\text{M}$ inhibited nitrite production in batch cultures of *N. europaea* by approximately 50%. Based on oxygen uptake, chlorobenzene was found to inhibit AMO activity, while not significantly inhibiting the downstream process of hydroxylamine oxidation to nitrite. Inhibition of nitrification increased with increased exposure time and coincided with the cessation of

chlorobenzene transformation. 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 upregulated 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, and the results are herein presented.

Single Well Push-Pull Tests for Determining In-Situ Rates of Anaerobic Naphthalene Biodegradation

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A single-well, push-pull method was implemented at the McCormick and Baxter Creosoting Company Superfund Site in Portland, Oregon—a site known to have extensive creosote contamination. Polycyclic aromatic hydrocarbons (PAHs) are the major constituents of creosote (Mueller, 1989). The purpose of this study was to determine a rate of in-situ anaerobic biodegradation of naphthalene, a low molecular weight PAH, that can be quantified and used for predictions of PAH containment, bioavailability, and remedial options. The rate of dilution in the well must be slower than the rate of biodegradation; therefore tracer tests were conducted on 20 wells based on existing PAH concentrations. Wells 62i and 45s (see map) were selected based on dilution rate and the presence of putative metabolites formed during the anaerobic degradation of naphthalene. Water amended with deuterated naphthalene and a conservative tracer was injected into the two wells. Decline in deuterated naphthalene and the tracer concentrations were monitored over time. In addition, the production of deuterated and non-deuterated naphthalene metabolites and several geochemical parameters were measured throughout the push-pull tests.

A Method for Biofilm Growth of *Nitrosomonas europaea*

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Changes in gene expression are being studied in *Nitrosomonas europaea*, an ammonia oxidizer in waste water treatment, when it is exposed to toxic chemicals such as benzene and heavy metals. The intent of the research is to study the gene expression in biofilm grown cells of *N. europaea* as well as suspended (planktonic) cultures. This is because organisms in waste water can exist as biofilm flocs and may exhibit different responses than planktonic cells. The design of a reactor system in which to grow a monoculture of *N. europaea* as a biofilm is presented here. The design involves a packed column reactor with a liquid volume of 25 mL with glass beads as packing material. Dissolved oxygen (DO) was found to limit ammonia utilization in the reactor even after sparging the media with pure oxygen gas for a DO concentration of 20 mg/L. This was observed at residence times of 55, 76 and 100 minutes. Nitrite production rates were found to be correlated with the amount of dissolved oxygen fed to the columns and increased with shorter residence times. Both columns operated with the same influent

conditions and showed very similar responses. A biofilm was observed to be formed at the column influent. Currently FISH methods are being developed in order to visualize the biofilm using a confocal microscope. Future plans for design and operation of the columns involve determining an ideal hydraulic residence time for oxygen delivery and addition of filters to prevent influent colonization. Ultimately, this model will be used to test the response of *N. europaea* biofilms to toxic compounds.

Utilization of Fluoroethene as a Surrogate for Aerobic Vinyl Chloride Degradation

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Vinyl chloride (VC) contamination in ground water systems is often the result of incomplete reductive dechlorination of the industrial solvents perchloroethene (PCE) and trichloroethene (TCE). Reduction of VC to ethylene (Eth) during anaerobic reductive dechlorination is frequently cometabolic and the rate-limiting step, which leads to VC accumulation. VC is more water-soluble and has a lower octane/water partitioning coefficient (K_{ow}) than either PCE or TCE and sorbs less strongly to aquifer materials. VC has the potential to move out of the reductive zone and into the aquifer where the presence of soluble oxygen allows aerobic processes to occur.

It is difficult to estimate rates of aerobic VC degradation in situ because complete mineralization of VC yields CO_2 and Cl^- ; neither of which can be tied solely to VC degradation. Utilizing a surrogate for VC that would yield a discrete analytical response when aerobically metabolized would allow estimation of VC transformation rates in contaminated aquifers. FE is a stable molecule in aqueous solution and its aerobic degradation yields F^- , which is a unique signature in most aquifers. The objective of this work was to evaluate if FE is a suitable surrogate for monitoring aerobic VC utilization or cometabolic transformation. Experiments were carried out with various oxygenase containing aerobic bacteria that either cometabolically or catabolically metabolize VC, to evaluate (i) if rates of FE transformation are similar to those of VC transformation, (ii) if VC and FE have similar affinities for the monooxygenase that mediates the initial transformation, (iii) if a competitive inhibition kinetic model accurately simulates concurrent FE and VC degradation, and (iv) if the rate of F^- accumulation can be correlated with that of VC utilization.

Experiments were carried out with three VC-utilizing isolates, *Mycobacterium* strain EE13a, *Mycobacterium* strain JS60 and *Nocardioides* strain JS614, that cometabolize VC and FE, catabolize VC and cometabolize FE, or catabolize VC and FE respectively. There were no significant differences between the K_s or k_{max} values of each of the isolates for FE and VC, and there was little difference between the isolates in their rates of transformation or affinity for the halogenated substrates. Competitive inhibition successfully modeled the temporal responses of FE and VC transformation during batch reactor experiments when both substrates were present. Mass balances of the VC transformed and Cl^- released were nearly stoichiometric regardless of whether transformation was direct or cometabolic, but transformation of FE and release of F^- was only stoichiometric during growth-linked metabolism by JS614. Also, during

cotransformation, the initial rates of halide release matched that of substrate transformed for each VC-degrading isolate. This allowed competitive inhibition to model Cl^- and F^- release along with VC and FE transformation.

Both the rates of FE transformation and rates of F^- accumulation (two unique signals and two different analytical methods) could be correlated with the rate of aerobic degradation of VC, and showed promise for estimating rates in situ.