

Ninth International Symposium on Subsurface Microbiology

October 5-10, 2014 • Pacific Grove, California USA

Thursday, October 9, 2014

ORAL PRESENTATION ABSTRACTS

ORAL PRESENTATION SCHEDULE

8:30 am – 10:00 am	Plenary Session #3	Merrill Hall
10:20 am – 12:00 pm	Session #7	
	A7. Chemistry	Merrill Hall
	B7. Biomes	Fred Farr Forum
	C7. Fate	Nautilus Room
2:00 pm – 3:20 pm	Session #8	
	A8. Chemistry	Merrill Hall
	B8. Contaminants	Fred Farr Forum
	C8. Fate	Nautilus Room
3:40 pm – 5:20 pm	Session #9	
	A9. Ecology	Merrill Hall
	B9. Contaminants	Fred Farr Forum
	C9. Chemistry	Nautilus Room

8:30 AM – 10:00 AM
MERRILL HALL



PLENARY SESSION

Moderated by **Joseph M. Suflita, Ph.D.**, Professor, Department of Microbiology and Plant Biology, University of Oklahoma, USA



Hot-Wired: The Nature and Extent of Extracellular Electron Transfer in Hydrothermal and Hydrocarbon Systems

Presented by Peter R. Girguis, Ph.D., Professor of Organismic and Evolutionary Biology, Harvard University, USA

Co-Authors: P.R. Girguis, M. Nielson, A. Bose, and J. Cohen

Recent years have been a watershed for research on extracellular electron transfer (EET). The broad relevance of EET in nature remains poorly constrained, and ongoing research in several laboratories is aimed at better understanding the role that EET plays in microbial ecology, evolution and biogeochemistry. Here we present data from several of our studies in which we examine the degree to which EET, both net electron flux into and out of the cell, influences biogeochemical cycles across a range of marine environments with varying physical and geochemical features. Moreover, using laboratory studies of cultivated genetically tractable strains, we have and will continue to examine the systems and mechanisms of electron transfer in microbes from both terrestrial and marine habitats. Collectively, these data reveal that EET is far more widespread than previously considered, is of great consequence to carbon and other elemental cycles in numerous environments, and has evolved among numerous bacterial and archaeal lineages. Most strikingly is the realized diversity of cellular and biochemical systems associated with EET, which underscores the relevance of this physiological capacity to life in and around anaerobic environments.



Ecological Connectivity between Surface and Groundwater Systems

Presented by Aaron I. Packman, Ph.D., Associate Professor, Department of Civil and Environmental Engineering, Northwestern University, USA

Co-Authors: A.I. Packman, J. Drummond, A. Aubeneau, and R. Davies-Colley

Historically, surface waters and groundwaters have been considered to represent distinct ecosystems with little hydrologic connectivity. However, diverse investigations have shown that surface and subsurface waters are highly connected over a wide range of scales. Rivers are connected to underlying and surrounding groundwater through the benthic and hyporheic ecotones, which represent the interfacial and subsurface domains that are strongly influenced by surface water flow. Here I will review current understanding of the river-benthic-hyporheic continuum, focusing on hydrologic connectivity and biogeochemical structure that mediate the migration, persistence, and growth of microorganisms in surface and groundwater systems. I will also present experimental results that demonstrate that hyporheic exchange processes are important to both waterborne disease transmission and the microbial metabolism of stream-borne materials, and describe model frameworks for assessing hyporheic processes and predicting their outcomes at large scales

10:20 AM – 12:00 PM

MERRILL HALL

SESSION A7 CHEMISTRY: BIOGEOCHEMISTRY OF THE SUBSURFACE

Moderated by:

- **Alexandria B. Boehm, Ph.D.**, Associate Professor, Stanford University, USA
- **Aaron I. Packman, Ph.D.**, Associate Professor, Department of Civil and Environmental Engineering, Northwestern University, USA



Influence of Viruses on Terrestrial Subsurface Biogeochemical Cycling and Heavy Metal Transport

Presented by Karrie A. Weber, Ph.D., Assistant Professor, University of Nebraska, USA

Co-Authors: K.A. Weber, D. Pan, Z.H. Tan, D. Wang, K.H. Williams, M. Robbins, D.D. Snow, N. Kananizadeh, and Y. Li

The role viruses play in biogeochemical cycles and metal transport is poorly understood. Viruses are abundant in terrestrial subsurface systems often outnumbering other microorganisms. Here, we investigated production of viruses in relationship to carbon biogeochemistry in the terrestrial subsurface and the potential for heavy metal transport. The generation of viruses was demonstrated in a series of laboratory and field experiments. Biostimulation of subsurface sediment slurries with nitrate and ^{13}C -organic carbon (OC, acetate) resulted in viral production concurrent with acetate oxidation, $^{13}\text{CO}_2$ production and nitrate reduction. Interestingly, change in viral abundance was positively correlated to OC consumption and $^{13}\text{CO}_2$ production whereas change in cell abundance was not implicating viral lysis of metabolically active cells. In situ biostimulation (O_2 injection) in a shallow aquifer also resulted in an increase in the virus to cell ratio in association with groundwater Eh and OC, thus demonstrating the stimulation of viral production in these environments. These viruses have the potential to contribute to virus mediated contaminant transport. Metal adsorption experiments conducted using a model bacteriophage, Escherichia coli phage T4, demonstrated Zn^{2+} adsorption fitting a Langmuir isotherm. Zeta potential analysis further demonstrated the surface of phage T4 is naturally electronegative capable of adsorbing positively charged ions (cations). Interestingly, the presence of Zn^{2+} significantly increased infectivity relative to unamended controls. The increase in infectivity has implications to microbial ecology and biogeochemistry contributing to an increase in cell lysis. As such, viruses should be considered as biological agents influencing microbial ecology, biogeochemical cycling and metal transport in the subsurface

Hydrodynamic Niche Differentiation beneath an Acid Mine Drainage-Contaminated Stream

Presented by William D. Burgos, Ph.D., Professor of Environmental Engineering, Pennsylvania State University, USA

Co-Authors: L.N. Larson, C. Grettenberger, J.L. Macalady, K.J. Bibby, and W.D. Burgos

In this study we used near-surface electrical resistivity imaging (ERI) and in-situ pore-water samplers to visualize the spatial and temporal extent of hyporheic exchange beneath a stream emanating from a

large abandoned deep clay mine in Cambria County, Pennsylvania, USA. Surface and subsurface water velocities were found to be faster beneath the stream center as compared to the stream banks. Distinctly different vertical pore-water profiles were observed beneath the stream center and the stream banks. The greatest extent of hyporheic exchange was beneath the center of the stream, where a shallower (< 10 cm) Fe(II)-oxidizing zone was observed. Meanwhile, less hyporheic exchange was observed near the channel banks, concurrent with a more pronounced, deeper (> 70 cm) Fe(II)-oxidizing zone. Hydrogeochemical niches were classified based on relatively “fast” versus “slow” rates of hyporheic exchange and oxic versus anoxic conditions. Sediment cores were then collected from the different niches to characterize mineralogy and microbial community structure. Microbial communities were characterized using 16S rDNA cloning and high-throughput sequencing performed on an Illumina MiSeq and utilizing a modified QIIME pipeline. Analyses included the taxonomic structure of the specific niches, as well as a beta-diversity analysis (microbial community structure relatedness) between replicates and niches. Microbial community data was also compared with existing environmentally-derived datasets to determine similarities in community composition and structure with previously characterized environmental niches. Microbial communities collected from near-surface niches were found to be much less diverse than subsurface microbial communities.

Aquifer Sediment Microbial Diversity in a Co-Mingled Contaminant Plume at the Hanford Site
Presented by Brady D. Lee, Senior Research Scientist, Pacific Northwest National Laboratory, USA
Co-Authors: B.D. Lee, M.H. Lee, E. Cordova, and J.T. Ellis

Disposal of waste related to uranium and plutonium recovery processes at the U Plant, which operated at the Hanford site from the 1940s through 1990s, has resulted in sediment and groundwater contaminated with carbon tetrachloride, uranium, technetium-99, iodine-129, nitrate and tritium. During drilling of two uranium extraction wells at the 200-UP-1 Operable Unit in the 200 West Area, split-spoon core samples were taken at three depths (10, 30 and 50 ft.) below the water table in each well. Sediments and water samples were analyzed for a variety of biogeochemical parameters, including physical properties (bulk density, particle density, and particle size), chemical properties (specific conductance, alkalinity, pH, metals, anions, uranium and other radionuclides). Phylogenetic diversity of the bacterial community in the sediments was determined using clone libraries. In addition, aerobic and anaerobic enrichments using a variety of electron donors and acceptors were performed in an effort to determine cultivable bacteria in the samples. Functional analysis of bacteria present was determined using quantitative real-time polymerase chain reaction, based on primers for total Eubacteria and Archaea, as well as, denitrifying, metal reducing and sulfate reducing bacteria. Results for these analyses will be reported and discussed. Data generated will help in understanding biogeochemical drivers for fate and transport of contaminants in 200 Area groundwater, as well as provide insight into options for remediation of some contaminants in place.

A Novel GUST Microcosm System to Assess Biostabilization of Sediments and Microbial Community Structure within Finite Sediment Layers

Presented by Thomas Reid, Ph.D. Candidate, Great Lakes Institute for Environmental Research, University of Windsor, Canada
Co-Authors: T. Reid, C. Weisener, and I. Droppo

The sediment-water interface of lakes, rivers and wetlands can control the diffusion of gas and porewater release in both contaminated and natural environments. Static microcosms have been used to model the biogeochemical processes at pond sediment-water interfaces. This static approach has provided excellent results to define gas and chemical flux controls associated with the microbial

populations for sediments. Although these studies provide a clear indication of undisturbed sediment-water interface, further research is required to simulate a more natural dynamic system. To investigate dynamic environments a modified GUST microcosm was developed which applies calibrated shear stress to mimic disturbance impacts and erodibility (e.g. wave action, storm events etc) at the interface. Recent work looking into the biostabilization of natural lake sediments has garnered serious interest in the field of contaminant transport. This research investigates the role of biofilm on the stabilization of contaminated sediments. Specifically, the change in microbial community structure as a function of pre- and post-disturbance was evaluated using next-generation sequencing (Ion Torrent™). Experiments were performed and repeated after 21 days, to examine reconsolidation of the bed and re-establishment of the biofilm as a function of contaminant sediment type. Initial static microcosm results will be presented, alongside this novel dynamic study, discussing variation between ponds in terms of biostabilization of sediment and microbial community structure within biotic systems.

Nitrogen Enrichment of Autochthonous Dissolved Organic Matter in a Surface-Influenced Karst Aquifer

Presented by Terri Brown, Ph.D. Candidate, Department of Earth and Planetary Sciences, University of Tennessee at Knoxville, USA

Co-Authors: T. Brown and A.S. Engel

Seasonal observations of dissolved organic matter (DOM) dynamics in an oligotrophic, surface-influenced cave stream and karst aquifer highlight interesting trends in DOM composition and water chemistry. FTIR spectra of DOM isolated on DEAE cellulose indicate significant contributions of autochthonous organic matter along the flowpath, in the form of carboxyl groups and secondary and tertiary amide macromolecules. Autochthonous DOM in freshwater lakes and rivers has higher nitrogen content than terrestrial DOM, and in our karst aquifer system (at low base low conditions), total nitrogen concentrations increase along the flowpath, in some cases up to 80% from the main point of surface recharge to the spring resurgence 2-km downstream. Dissolved organic carbon concentrations decrease along the flow path (up to 68% from the recharge point) under moderate to low-flow conditions. Photo-degradation can stimulate the flux of dissolved nitrogen from humic substances, but post-recharge, is negligible in the cave and karst aquifer. Comparison between upstream, surface water to downstream cave water samples reveal that changes to the carbon-to-nitrogen ratio are greatest in the spring and summer months. Increases in DOM nitrogen content could also be attributed to the partial mineralization of DOM and to a rapid loss of phytoplankton as the losing stream recharges the aquifer. Sources of organic nitrogen inputs in the water column include bacterial response and cell lysis due to temperature gradient changes, release of extracellular enzymes and metabolic waste products, nutrient cycling, and tree root leachates, which can be tracked using diagnostic FTIR spectra, and protein and lipid assays.

10:20 AM – 12:00 PM
FRED FARR FORUM



SESSION B7 BIOMES: ASTROBIOLOGY AND EXTREME ENVIRONMENTS

Moderated by:

- **Tori M. Hoehler, Ph.D.**, Research Scientist, Exobiology Branch, NASA Ames Research Center, USA
- **Karsten Pedersen, Ph.D.**, Professor of Geomicrobiology, Chalmers University of Technology, Sweden



The Subsurface Biosphere at the Sanford Underground Research Facility

Presented by Jan P. Amend, Ph.D., Professor, Department of Earth Sciences and Department of Biological Sciences, University of Southern California, USA

Co-Authors: J.P. Amend, M.R. Osburn, L.M. Momper, B.K. Reese, G.P. Wanger, and R. Bhartia

The Sanford Underground Research Facility (SURF) in South Dakota (USA) offers access to water, rock, and biofilm to a depth of 4850 feet (~1380 m) in an iron-rich paleoproterozoic metasedimentary sequence. Members of the USC-based NASA Astrobiology Institute team are investigating the microbiology and geochemistry there to inform on the extent and diversity of the continental subsurface biosphere. Preliminary analyses show that borehole fluids reflect variable water-rock interaction with high levels of ferrous iron, sulfate, calcium, and bicarbonate in the oldest fluids. Dissolved methane (≤ 400 nM), hydrogen (≤ 1.6 nM), and helium (≤ 28 nM) have been detected. Presuming that the helium is generated via α -decay, the corresponding but relatively depleted hydrogen concentrations suggest active microbial consumption. 16S rRNA gene sequencing of cells in several borehole fluids reveal variable and diverse microbial communities, with abundant bacteria and archaea from uncultivated clades and candidate phyla. Several sequences identified closely with previously described continental deep subsurface bacteria, including *Sulfurihydrogenibium subterraneum* (from Japan) and *Desulforudis audaxviator* (from South Africa). Notably, sequences similar to known thermophiles were detected, despite the relatively cool (~ 16 °C) water temperature at SURF, and sequences related to the acid-loving *Acidithiobacillus* were documented in circumneutral waters at several locations. The presence of presumably active Bacteria and Archaea in borehole water and biofilm was assessed from intact polar lipid compositions, which were uncharacteristically variable. Drilling at the 4850 foot-level in April 2014 provided fresh whole-round rock cores for DNA and RNA analysis, microbial culturing, mineralogic analysis, and deep UV fluorescence scanning of organic compounds.

Microbial Community Structure and Functionality in the Deep Biosphere Down to 2,300 M of Fennoscandian Terrestrial Crystalline Bedrock

Presented by Lotta Purkamo, Ph.D. Candidate, VTT Technical Research Centre of Finland, Finland

Co-Authors: L. Purkamo, M. Bomberg, M. Nyysönen, M. Nuppenen-Puputti, H. Salavirta, R. Kietäväinen, I. Kukkonen, and M. Itävaara

Indigenous microbial communities of six fracture zones and mixed communities from five depths of the water column of Outokumpu Scientific Drill Hole in Finnish bedrock were studied with pyrosequencing. In addition to 16S rRNA gene based characterization of bacterial and archaeal

community structures, functional marker genes for sulphate reduction and methanogenesis were used to characterize these functional groups. Further estimation of the functionality of the metagenome was conducted using PICRUSt, a computational tool exploiting 16S rRNA marker gene data produced by amplicon sequencing and a reference genome database to give insights to the functionality of the microbial community. Diverse bacterial communities differed between the intrinsic fracture zones, β -proteobacteria dominating in the upper depths (180-500 m) and Firmicutes in the deeper fracture zones. Archaeal communities in the fracture zones were more similar with each other than bacterial communities and were dominated mostly by methanogens (family Methanobacteriacea). Preliminary results of the predicted functions revealed only small differences between the biotopes in functional categories. In the archaeal dataset, genes involved in methane metabolism had the highest relative abundance. *Desulfovibrio* and *Desulfotomaculum* dominated the sulphate-reducing communities, *Methanobacterium* and *Methanosarcinales* the methanogenic communities.

NGEE Arctic: Biogeochemical Controls on Microbial CO₂ and CH₄ Production in Polygonal Soils from the Barrow Environmental Observatory

Presented by David E. Graham, Ph.D., Group Leader, Microbial Ecology and Physiology, Oak Ridge National Laboratory, USA

Co-Authors: D.E. Graham, T.R. Chowdhury, E. Herndon, M. Ladd, D.A. Elias, T.J. Phelps, B. Gu, L. Liang, and S.D. Wulfschelger

Organic matter buried in Arctic soils and permafrost will become accessible to increased microbial degradation as the ground warms due to climate change. The rates of organic matter degradation and the proportion of CH₄ and CO₂ greenhouse gasses released in a potential warming feedback cycle depend on the microbial response to warming, organic carbon structure and availability, the pore-water quantity and geochemistry, and available electron acceptors. Significant amounts of iron(II) ions in organic and mineral soils of the active layer in low-centered ice wedge polygons indicate anoxic conditions in most soil horizons. To adapt and improve the representation of these Arctic subsurface processes in terrestrial ecosystem models for the NGEE Arctic project, we examined soil organic matter transformations from elevated and subsided areas of low- and high-centered polygons from interstitial tundra on the Barrow Environmental Observatory (Barrow, AK). Using microcosm incubations at fixed temperatures and controlled thawing systems for frozen soil cores, we investigated the microbiological processes and rates of soil organic matter degradation and greenhouse gas production under anoxic conditions, at ecologically relevant temperatures of -2, +4 or +8 °C. In contrast to the low-centered polygon incubations representing in situ water-saturated conditions, microcosms with unsaturated high-centered polygon samples displayed lower carbon mineralization as either CH₄ or CO₂. Substantial differences in CH₄ and CO₂ response curves from different microtopographic samples separate the thermodynamic controls on biological activity from the kinetic controls of microbial growth and migration that together determine the temperature response for greenhouse gas emissions in a warming Arctic.

Molecular Analysis of Microbial Communities in Thawing Permafrost Environments

Presented by Mark P. Waldrop, Ph.D., Soil Microbiologist and Biogeochemist, U.S. Geological Survey, USA

Co-Authors: S.J. Blazewicz, J.K. Jansson, and M.P. Waldrop

Over 20% of Earth's terrestrial surface is underlain by permafrost that represents one of the largest terrestrial carbon pools, with an estimated ~1700 Pg of carbon (C) contained in the upper 3 m of permafrost. Models estimate that C release from thawing permafrost might represent the largest new

transfer of C from the biosphere to the atmosphere. This process is largely dependent on microbial responses, but we know little about microbial activity in intact permafrost, let alone in recently thawed permafrost soils. We investigated microbial community phylogeny, genetic functional potential, and growth activity in a recently thawed thermokarst bog using a combination of molecular and process measurement approaches including total soil community metagenomics (MG) in addition to examining the active portion of the community in frozen soil using heavy water DNA stable isotope probing (SIP). Highlights from these analyses reveal energy yielding microbial processes and potential strategies for microbial survival in permafrost soils, and linkages between biogeochemical process rates and –omics measurements. The results provide new knowledge about microbial life and activity potential in permafrost, the potential importance of freeze/thaw stimulated activity on C degradation, and the importance of methanogenesis following thaw. The targeted-omics strategy demonstrated here enables better mechanistic understanding of the ecological strategies utilized by soil microbial communities in response to climate change.

The Deep Mud-Volcano Biosphere in the Nankai Trough Accretionary Complex

Presented by Fumio Inagaki, Ph.D., Group Leader, Geomicrobiology Group, Kochi Institute for Core Sample Research, Japan Agency for Marine-Earth Science and Technology (JAMSTEC) (Japan)
Co-Authors: F. Inagaki, A. Ijiri, T. Hoshino, and Y. Morono

Submarine mud-volcanoes are globally distributed in the plate convergent margins. The muddy sediment typically contains high concentration of hydrocarbons and other elements dissolved in the porewater, which seepages support chemosynthetic benthic life, including microbes that mediate anaerobic oxidation of methane. However, the nature of deep mud-volcano subsurface biosphere has still remained largely unknown. In 2009 and 2012, during the JAMSTEC Expeditions 903 and 906 using the drilling vessel Chikyu, we drilled an active mud-volcano in the Kumano forearc basin of the Nankai Trough down to 200 meters from the summit. Cell count and molecular analyses of 16S rRNA genes indicate that relatively small but phylogenetically diverse microbial populations are present in the cored sediment samples. The carbon isotopic compositions of bicarbonate, methane, DIC and acetate suggest the occurrence of homo-acetogenesis and other microbial respirations, which is consistent with activity measurements using radioactive tracers as well as thermodynamic calculations. Our data microbiological, biogeochemical and geological data suggest that the deep mud-volcano biosphere is present, which is systematically associated with dehydration and faulting processes in the Nankai accretionary wedge.

10:20 AM – 12:00 PM
NAUTILUS ROOM

SESSION C7 FATE: BANK FILTRATION

Moderated by:

- **Chittaranjan Ray, Ph.D., P.E.**, Director, Nebraska Water Center, University of Nebraska-Lincoln, USA
- **Prof. Dr. Jack F. Schijven**, Senior Scientist, National Institute of Public Health and the Environment (RIVM), The Netherlands, and Professor, Environmental Hydrogeology Group, Department of Earth Sciences, University of Utrecht, The Netherlands



Removal of Microorganisms during Natural (Non-Engineered) Bank Filtration Occurring at the Bottom of a Glacial-Kettle Pond, Cape Cod, Massachusetts

Presented by David W. Metge, Microbiologist, National Research Program, U.S. Geological Survey, USA

Co-Authors: D.W. Metge, R.W. Harvey, G.R. Aiken, D.R. LeBlanc, T.D. McCobb, and J. Underwood

Although engineered bank filtration has long been a cost-effective means of removing pathogens from surface water, limited information exists about the efficacy of naturally occurring (non-engineered) bank filtration. Our study focused on removal of microorganisms by bottom sediments within Ashumet pond, a groundwater-flow-through, mesotrophic (2.2-2.5 mg C L⁻¹ dissolved organic carbon, DOC), low-salinity (118-123 μ S cm⁻¹ specific conductance) glacial-kettle pond on Cape Cod, Massachusetts. Injection-and-recovery tracer studies were performed at a near-shore site where pond water continuously recharges the adjacent drinking-water aquifer. Using a bag-and-barrel seepage meter (Lee type) operating under natural hydraulic-gradient conditions (groundwater-flow rates of 2-3 m/day), we introduced a conservative tracer (bromide) and variably-sized colloids [Synechococcus sp. IU625 (2.6 \pm 0.2 μ m diameter), Prochlorococcus sp. (1.6 \pm 0.1 μ m), carboxylate-modified polystyrene microspheres (1.7, 2.9, and 4.6- μ m size classes)] to the bottom sediments. These constituents were tracked as they moved across the surficial layer and through underlying aquifer sediments past push-point samplers placed at \sim 30 cm intervals along a 1.2-m long, diagonally (\sim 45 $^\circ$) downward flow path. The top 25 cm of near-surface bottom sediments removed \sim 44% of the pond DOC, and were more effective (by $>$ 9 log units) at removing colloids than underlying sediments, underscoring the importance of the surficial layer. Prochlorococcus and Synechococcus spp., two common picocyanobacteria ($<$ 3 μ m in diameter), became more readily transported once they moved past the surficial layer, suggesting they may be useful indicators of groundwater under the direct influence of surface water (GWUDI) for wells located downgradient of surface water bodies.

The Effect of Organic Carbon Composition and Concentration on Organic Micropollutant (OMP) Removal and Microbial Diversity in Riverbank Filtration Systems

Presented by Cheryl Bertelkamp, Ph.D. Candidate, Department of Water Management, Delft University of Technology, The Netherlands

Co-Authors: C. Bertelkamp, K. Schoutteten, L. Vanhaecke, J. Vanden Bussche, L. Hulpiau, N. Singhal, J.P. van der Hoek, and A.R.D. Verliefde

River bank filtration (RBF) in The Netherlands was originally designed for the removal of water quality parameters such as viruses, bacteria, and natural organic matter. Since the discovery of organic micropollutants (OMPs) in Dutch surface waters, questions have been raised about the capability of RBF systems to remove OMPs. Microbial communities present in RBF soils are responsible for OMP biodegradation. Activity and composition of these communities is influenced by the quantity and quality of organic carbon in the feed water. However, for RBF very few studies have investigated the effect of these parameters on OMP removal. This study investigated the effect of different organic carbon fractions in the feed water on the microbial community composition and accompanying OMP removal. Four organic carbon fractions (hydrophobic, hydrophilic, transphilic and the total fraction) were fed to PVC columns (L= 1m, d = 36mm) filled with soil from the RBF site of Oasen drinking water company (The Netherlands). The system was stabilized for 2 weeks on the organic carbon fractions before an OMP mixture (500 ng/L, 24 compounds) was dosed into the feeds. After 2 weeks the OMP concentration was increased to 2000 ng/L to determine the effect of the shock load on the microbial community composition. Finally, the organic carbon concentration was doubled for two

weeks to mimic decreases in river water discharge (e.g., by climate change). Process parameters such as pH, temperature, ionic composition, dissolved organic carbon, UV254, LC-OCD, OMP concentrations and microbial community composition and diversity (in the soil) were measured at the in- and outlets of the different columns.

Numerical Modelling of Transport of *Escherichia Coli* and Coliforms in a Riverbank Filtration Scheme

Presented by Chittaranjan Ray, Ph.D., P.E., Director, Nebraska Water Center, University of Nebraska-Lincoln, USA

Co-Authors: L. Sharma, I. Engelhardt, C. Ray, and H. Prommer

Riverbank filtration (RBF) systems are an important source of drinking water that help remove (or reduce) microbial pathogens through a number of sub-surface transport processes. These processes are usually modeled in groundwater assuming steady state conditions using the advection-dispersion transport equation and adding on mass-transfer processes of attachment, detachment and inactivation approximated with linear kinetic reaction expressions. Field-scale RBF pathogen transport, unlike common groundwater modeling, is compounded by the complex and dynamically changing boundary conditions, such as river level fluctuations and RBF well pumping. These induce significant spatial and temporal variations of groundwater flow velocities which in turn affect the transport characteristics rendering difficulties in modeling of microbial transport in RBF systems. To address these problems, the concepts of colloid filtration theory and the transport equations were combined to incorporate the dynamic boundary conditions into a field-scale multi-component reactive transport model using MODFLOW, MT3DMS and PHT3D. The major mass transfer processes were linked to local velocity fields and numerically computed at each time step and location. This successfully enabled the use of a single set of velocity-dependent parameters to describe the breakthrough of the pathogens in question. The numerical approach was verified on a benchmark study looking at pathogen breakthroughs in column experiments that were run at different flow velocities. It was then extended to model the transport of *Escherichia Coli* and Coliforms in an operational RBF scheme. The modeling work, currently under refinement, correctly simulated the breakthrough during high floods and the non-occurrence of it during other times.

Long-Term Trending of Missouri Riverbank Filtration

Presented by Ricky J. Mach, Water Plant Superintendent, City of Sioux City, Iowa, USA

Author: R.J. Mach

Sioux City supplies water to the approximately 120,000 persons with an average production of 13.5 MGD and peak demand of 24.1 MGD. The system obtains its water from three collector wells and seven vertical wells adjacent to the Missouri River, screened in the Missouri Alluvium and the Dakota Sandstone Aquifers. With the classification of groundwater under the direct influence of surface water, Sioux City conducted a Riverbank Filtration Study in 2001. The City's riverbank study focused on microscopic particulate analysis and particle counts. That study led to Sioux City being granted 4.0 log removal for giardia and 3.5 log removal for cryptosporidium through the riverbank and treatment plant with surrogate turbidity and disinfection requirements. In 2007, Sioux City was part of an AWWARF project evaluating methods to assess ground water under direct surface water influence and bank filtration performance. This study focused on microscopic particulate analysis of water from the river and collector wells. As part of this study, Sioux City did construct a pair of angular wells, one screened above the river bed and one screened below the river bed to track particulate infiltration from river water thru collector caisson. With LT2 regulations, Sioux City sampled both rivers and one

of our collector wells for the presence of cryptosporidium and giardia. While cryptosporidium and giardia were detected in both rivers, none was found in the collector well. Since the initial study a second plant was constructed using Membrane Technology and Pre-treatment was added to the original plant.

Toxicity Assessment of Organic Micropollutant Removal during Riverbank Filtration

Presented by Astrid Fischer, Ph.D. Candidate, Delft University of Technology, The Netherlands
Co-Authors: A. Fischer, C. Bertelkamp, A. van Wezel, A.R.D. Verliefde, and J.P. van der Hoek

Organic micro pollutant (OMP) removal during river bank filtration (RBF) has been investigated in numerous studies. However, these studies have mainly focused on the removal of parent compounds. It is known that in certain cases the transformation products can be more toxic than the parent compound. Therefore, it is important to investigate these transformation products during soil passage too. Nevertheless, it is not feasible to monitor all transformation products, as many substances have unknown degradation pathways and thus unidentified transformation products. To circumvent this problem, and provide an assessment of the efficiency of soil passage in terms of OMP removal, the variation in toxicity during soil passage could be assessed. In this study, a mixture of 50 OMPs (pharmaceuticals, pesticides, and industrial waste products) was spiked in pilot columns simulating RBF, toxicity was assessed by means of the AMES-II assay and the oxidative stress assay. AMES-II indicates the risk of genotoxicity and mutagenicity while the oxidative stress assay gives an indication of the reactivity of the OMPs initially present while also being sensitive to any reactive transformation products formed during soil passage. The unique experimental set-up simulating a real RBF site, consisted of 3 RBF pilots with different redox zones: an oxic pilot (residence time 4 days), a sub anoxic nitrate reducing pilot (residence time 20 days) and a deep anoxic iron/manganese reducing pilot (residence time 45 days). This set-up made it possible to assess if degrading OMPs and their transformation products by RBF also ensures a reduction in toxicity.

2:00 PM – 3:20 PM

MERRILL HALL

SESSION A8 CHEMISTRY: MICROBE-MINERAL INTERACTIONS (GEOCHEMISTRY FOCUS)

Moderated by:

- **William C. Ghiorse, Ph.D.**, Professor, Department of Microbiology, Cornell University, USA
- **Jonathan O. Sharp, Ph.D.**, Assistant Professor, Department of Civil and Environmental Engineering, Colorado School of Mines, USA



Structural Iron (II) of Basaltic Glass as an Energy Source for Zetaproteobacteria

Presented by Pauline Henri, Ph.D. Candidate, National Centre for Scientific Research (CNRS), Institute of Earth Physics of Paris (IPGP), France

Co-Authors: P. Henri, C. Rommevaux-Jestin, B. Menez, D. Emerson, and A. Mumford

Young basaltic glass, although profusely colonized by microorganisms, have been reported as not significantly altered, and glassy rim of basalt defined more as an habitat than an energy source.

Nonetheless, microorganisms can enhance silicate glasses dissolution, making bio-available the nutrients and metals it contains. To explore the capability of basalt be an energy source for chemosynthetic microorganisms, both in situ and in vitro colonization experiments were performed. Microbial incubators containing either reduced or oxidized synthetic basaltic glasses were deployed 9 months in an abyssal plain (35° N; 29°W). The microbial colonization was analyzed by pyrosequencing of the 16S rRNA coding gene and the basalt alterations were characterized by SEM, XANES and Raman spectroscopy. Besides, reduced basalt were incubated 2 weeks with a pure culture of *Mariprofundus ferrooxydans* DIS-1, without addition of any nutrient or minerals. The microbial diversity of the reduced glass from the abyssal plain is largely (39%) dominated by ironoxidizing Zetaproteobacteria. This sample exhibits a 1µm thick iron-oxides alteration layer. Reversely, the oxidized glass shows neither iron-oxides nor iron-oxidizers. In addition, CLSM observations of the in vitro experiment show that the glass surface is covered by twisted-stalk iron-oxides associated to an important cell density, confirming that Fe (II) from the basalt can be the only energy source for *M.ferrooxydans*. The correlation between presence of Zetaproteobacteria with Fe (II) enrichment in the basalt tends to indicate that the basalt was the energy source for the Zetaproteobacteria. Then, the alteration surface layer observed could be partly due to their action.

Dissimilatory Iron Reduction and the Redox Cycling of Green Rust

Presented by Edward J. O'Loughlin, Ph.D., Biogeochemist, Argonne National Laboratory, USA
Co-Authors: E.J. O'Loughlin, M.I. Boyanov, C.A. Gorski, M.L. McCormick, M.M. Scherer, and K.M. Kemner

Green rusts (mixed Fe(II)/Fe(III) layered double hydroxides) have been identified in Fe(III)/Fe(II) transition zones in a variety of natural and engineered environments. Many of these environments are characterized by periodic or seasonal cycling of redox conditions that create the opportunity for cycling of Fe between oxidized and reduced forms. This study examined the effects of phosphate on dissimilatory iron reduction and the redox cycling of green rust. In our experiment, the oxidation of biogenic green rust (produced during the bioreduction of lepidocrocite) by oxygen to ferric green rust occurred over a period of 24 h without the formation of other Fe(III) oxide phases. The biogenic green rust was formed in the presence of phosphate and sorption of phosphate or silicate by green rust has been shown to promote oxidation green rust to ferric green rust by suppressing green rust dissolution. After re-inoculation, total Fe(II) concentrations rebounded to pre-oxidation concentrations and Ferric green rust was reduced to green rust.

Sulfur-Mediated Electron Shuttling during Bacterial Iron Reduction

Presented by Theodore M. Flynn, Ph.D., Research Scientist, Computation Institute, University of Chicago, USA
Co-Authors: T.M. Flynn, E.J. O'Loughlin, B. Mishra, T.J. DiChristina, and K.M. Kemner

Fe(III) minerals are critical electron acceptors in many subsurface environments, but the extent to which they can provide energy for the growth microorganisms via enzymatic reduction depends greatly on the thermodynamic energy that is available from the environment. In alkaline, oligotrophic aquifers, for example, the amount of energy available to dissimilatory metal-reducing bacteria (DMRB) via the reduction of Fe(III) minerals decreases dramatically with increasing pH. Many DMRB, however, can also respire by reducing elemental sulfur [S(0)] to sulfide, a process which provides more energy under alkaline conditions than acidic ones. In a series of bioreactor experiments, we have shown that at pH 9, the DMRB *Shewanella oneidensis* MR-1 can respire S(0) but not goethite. The sulfide produced subsequently reduced goethite abiotically. In experiments conducted

with a mutant strain of *S. oneidensis* (PSRA1), which is able to reduce Fe(III) but not S(0), no ferrous iron was produced. The reduction of S(0) to sulfide and the formation of mackinawite (FeS) was confirmed by sulfur K-edge x-ray absorption near-edge spectroscopy (XANES). Because the abiotic reaction of sulfide with Fe(III) produces S(0), in the absence of geologic deposits of S(0), DMRB in alkaline aquifers may require active respiration by sulfate-reducing bacteria (SRB) in order to respire. Under these conditions, Fe(III) reduction will proceed via S(0)-mediated electron shuttling pathways that requires a mutualistic partnership between DMRB and SRB rather than direct enzymatic reduction of Fe(III) minerals by DMRB alone.

Dynamic Interplay of Microbially Mediated Oxidation-Reduction Reactions Controlling Chromium Cycling in Soils and Sediments

Presented by Debra M. Hausladen, Ph.D. Candidate, Department of Environmental Earth System Science (EESS), Stanford University, USA

Co-Authors: D.M. Hausladen, S.C. Ying, and S. Fendorf

A suite of anthropogenic activities together with natural processes, including weathering of ultramafic rocks, has resulted in hazardous chromium(VI) concentrations in groundwater across the globe. Under oxic conditions, the more mobile and toxic Cr(VI) species may form; under anoxic conditions, a combination of biotic-abiotic mechanisms reduce Cr(VI) commonly resulting in mixed Fe(III)-Cr(III) hydroxides. The solubility of the Cr-Fe hydroxides can vary significantly, having a dramatic effect on Cr(III) reoxidation by Mn-oxides. Here, we examine Cr reduction-oxidation dynamics in a diffusively controlled system to simulate soils and sediments. A series of mixed Cr-Fe hydroxides were added to one chamber while Mn-oxidizing bacteria (*Bacillus* sp.) were added to groundwater media containing 0.1 mM MnCl₂ in the other, with the chambers separated by a permeable membrane. Production of Cr(VI) is proportional to the degree of Fe-substitution in the Cr-Fe hydroxides. When the Fe-reducing bacterium *Shewanella putrefaciens* was added to the Fe-Cr hydroxides chamber, the Fe(II) generated induced Cr(VI) reduction and (re)formation of Cr(III)-Fe(III) precipitates. Our results illustrate the complex network of biogeochemical processes controlling chromium redox cycling within soils and sediments.

2:00 PM – 3:20 PM
FRED FARR FORUM

SESSION B8 CONTAMINANTS: OIL DEGRADATION AND HORIZONTAL WELLS

Moderated by:

- **Tamara N. Nazina, Ph.D.**, Head of Laboratory, Winogradsky Institute of Microbiology, Russian Academy of Sciences, Russia
- **Joseph M. Suflita, Ph.D.**, Professor, Department of Microbiology and Plant Biology, University of Oklahoma, USA



Investigating the Microbial Response to Gamma-Irradiated Oil Sands Processed Material

Presented by Danielle VanMensel, Masters Candidate, Great Lakes Institute for Environmental Research, University of Windsor, Canada

Co-Authors: D. VanMensel and C. Weisener

The open-pit mining of Alberta oil sands bitumen has generated large volumes of waste material termed fluid fine tailings (FFT). The current practice is to store the FFT in large basins (tailings ponds) for water recycling and to allow densification of solid material. These basins tend to concentrate naphthenic acids (NAs) - a group of toxic recalcitrant carboxylic acids associated with the extraction process, which are toxic to biota. Gamma irradiation (GI) is being evaluated as a possible in situ treatment to accelerate the biodegradation of these compounds. Two unknowns exist which pertain to the application of GI treatment 1) is its impact on NA toxicity and 2) how will the indigenous microorganisms respond. Specifically, will the effect of this treatment stimulate or suppress sulfate-reducing bacteria (SRB) and methanogen communities in the tailings material potentially influencing greenhouse gas emission. This research investigates the activity and community structure of indigenous microbes as a function of GI treatment. Next Generation Sequencing using an Ion Torrent platform has been optimised to provide detailed community profiles of the bacterial vs archaeal dominance within the FFT as a function of time. This research will provide better understanding of the biogeochemistry of oil sands tailings and GI impacts on microbial communities with the ultimate goal of accelerated remediation and stabilization of tailings ponds for end pit lake development.

A One-Year Microbial Metagenomics Trajectory after Hydraulic Fracturing in Marcellus Shale

Presented by Paula J. Mouser, Ph.D., Assistant Professor, Department of Civil, Environmental and Geodetic Engineering, Ohio State University, USA

Co-Authors: P.J. Mouser, R.V. Trexler, and K.C. Wrighton

Energy extraction using horizontal drilling and hydraulic fracturing technologies significantly alters biogeochemical conditions and microbial ecological function in deep hydrocarbon-bearing shale. We tracked changes in microbial community dynamics and functional potential over an 11-month period in fluids injected and produced from three wells drilled and fractured in the Marcellus shale. These systems were genomically tractable, with near complete draft genomes recovered for most of the dominant taxa identified in 16S rRNA analyses. A marked shift in microbial taxa occurred with time; community composition shifted from low-salt tolerance, mesophilic aerobic bacteria to halophilic fermentative bacteria and methanogenic archaea. Earlier time points (up to 14 days after fracturing) had an abundance of *Halolactibacillus*, members of *Vibrionales*, and *Arcobacter* spp., the latter which encoded the potential for chemoautotrophic sulfide oxidation. Relative to the earlier samples, the 11-month community was dominated by several strains of *Halanaerobium* spp. and a member of the *Halomonadaceae*. This sharp shift in the microbial community was correlated with the attenuation of injected organic carbon and a higher proportion of genes for the acquisition and degradation of aromatic compounds and the assimilation of reduced sulfur species, supporting the importance of these processes in later produced fluids when anoxic conditions prevail. *Methanohalophilus* and *Methanobolus* spp. also are enriched in later time points, both of which encode the complete pathway for producing methane from the utilization of mono-, di- and trimethylamines. These data provide insight into the trajectory of microbial dynamics after hydraulic fracturing and the microbial functional potential within methane shale.

Microbial Community Structural and Functional Differences Associated with Elevated Hydrocarbon Concentrations in Deep Gulf of Mexico Sediments Near and Far from the Deepwater Horizon Spill Site

Presented by Gary L. Andersen, Ph.D., Senior Scientist and Ecology Department Head, Lawrence Berkeley Laboratory, USA

Co-Authors: G.L. Anderson, Y.M. Piceno, P. Hu, L.M. Tom, F.C. Reid, E.A. Dubinsky, and S.E. Borglin

The blowout of the Macondo 252 (MC252) well following the explosion of the Deepwater Horizon drilling unit released approximately 4.1 million barrels of oil into the Gulf of Mexico over an 83 day time period from April to July 2010. A portion of this oil settled on the sea floor sediment near the wellhead. The Gulf of Mexico also contains numerous hydrocarbon seeps near salt domes where crude oil and methane seeps out of fissures in the ocean seabed. The goal of this study was to identify the microbial community responsible for hydrocarbon degradation in the seafloor sediment near the MC252 wellhead and compare with hydrocarbon-degrading processes around nearby hydrocarbon seeps. Sediment cores were collected in the Gulf of Mexico, ranging from less than 1 km to over 250 km from the MC252 spill site during September-October 2010 and October-November 2011 at depth from 900-1,800 m below sea level surface. Cores were sectioned at 0-1 cm and 4-5 cm and DNA was extracted for phylogenetic analysis using the Berkeley Lab PhyloChip microarray and for metagenomic analysis using Illumina HiSeq next-gen sequencing. We found that bacterial community composition in hydrocarbon seeps was similar to hydrocarbon-impacted surface sediments nearest the MC252 wellhead. The community composition was also related to the levels and composition of hydrocarbons on the surface but not in the deeper sediments. The combination of phylogenetic and functional gene information provided greater insight into the mechanisms of hydrocarbon degradation in deep-sea sediment.

Sulphur Biogeochemistry in Oil Sands Composite Tailings

Presented by Lesley A. Warren, Ph.D., Professor, School of Geography and Earth Sciences, McMaster University, Canada

Co-Authors: L.A. Warren and K. Kendra

Oil sands tailings are important, globally relevant, sulphur containing materials. However the S sulphur biogeochemistry of composite tailings (CT, a mixture of tailings, post-processed sand and gypsum) used for dry reclamation has not been investigated to date. Drill samples were collected in December of 2012 from 5 depths spanning 36 m in a CT deposit (Fort McMurray, AB, Canada), for geochemical, metagenomic and functional enrichment analyses. Results establish widespread microbial S biogeochemical cycling within the CT deposit. Porewater hydrogen sulfide was detected extensively throughout the deposit with background levels ranging from 14–23 μM and a concentrated pocket of 300 μM occurring at depth. Metagenomic characterization (454 pyrosequencing) revealed highly diverse CT microbial communities with several known iron (IRB) and sulphate (SRB) reducing bacteria. Positive growth was also observed for IRB and SRB in functional metabolic enrichments. Geochemical and metagenomic data revealed two depth dependent structurally distinct communities emerged: a shallow, depth restricted CT zone of Fe(III) reduction overlying a highly variable, but extensive, zone of sulphate reduction throughout the deeper depths. The emergence of a distinct, surficially constrained IRB zone, despite ~65% of the total bacterial community putatively having the capacity for Fe(III) reduction over the entire deposit depth, and evident and increasing Fe(III) sources down core, suggests IRB limitation by some other factor. In contrast, SRB appear limited by available sulphate in the system. These results will be presented and their implications for on-going CT reclamation discussed.

2:00 PM – 3:20 PM
NAUTILUS ROOM

SESSION C8 FATE: PATHOGEN SURVIVAL

Moderated by **Simon Toze, Ph.D.**, Research Program Leader, Urban Water Systems Engineering Research Program, CSIRO Land and Water, Australia



Role of Cold Climate and Freeze-Thaw on the Survival, Transport, and Infectivity of Selected Bacteria

Presented by Bahareh Asadishad, Ph.D. Candidate, Department of Chemical Engineering, McGill University, Canada

Co-Authors: B. Asadishad, S. Ghoshal, and N. Tufenkji

Surface and near-surface soils in cold climate regions experience low temperature and freeze-thaw (FT) conditions in the winter. Microorganisms that are of concern to groundwater quality may have the potential to survive low temperature and FT in the soil and aqueous environments. Although there is a large body of literature on the survival of pathogenic bacteria at different environmental conditions, little is known about their transport in groundwater environments in low temperatures and after FT. In this project, we studied the survival and transport of a Gram negative bacterial pathogen, *Yersinia enterocolitica*, and a Gram positive nonpathogen, *Bacillus subtilis*, when subjected to cold temperature and several FT cycles at two solution ionic strengths (IS: 10 and 100 mM). Our findings demonstrate that both bacterial strains had higher retention on sand grains after exposure to FT and starvation. Increasing the number of FT cycles increased bacterial hydrophobicity while decreased the swimming motility and viability of the bacteria. Accordingly, the transcription levels of *flhD* and *fliA*, flagellin encoding genes, and *lpxR*, lipidA 3'-O-deacylase, in *Y. enterocolitica* and *hag*, flagellin gene, and *sfp*, a gene involved in surfactin production, in *B. subtilis*, were reduced in cold temperature and after FT treatment. Both strains tend to survive for longer periods of time at low temperature in higher IS thereby posing a potential threat to drinking water supplies.

The Inactivation of *E. Coli* and *P. Aruginosa* in Anaerobic and Reduced Groundwater Targeted for Aquifer Storage and Recovery

Presented by Jennifer C. Underwood, Microbiologist, United States Geological Survey, USA

Author: J.T. Lisle

The Floridan Aquifer in the southern region of Florida has zones that are moderately saline, anaerobic and extremely reduced. These regions are permitted for the injection of treated surface water and wastewater. The type of recharge that has received the most regulatory attention in south Florida is aquifer storage and recovery (ASR). The inefficient inactivation of members of the coliform group prior to injection at some ASR facilities has been a persistent regulatory issue. To quantify the inactivation rates for *Escherichia coli* and *Pseudomonas aeruginosa*, above ground, flow through mesocosm systems were used to quantify inactivation rates when exposed to groundwater from six artesian wells in the Upper Floridan (UF) (560-1145 fbs) and Avon Park Permeable Zone (APPZ) (1300-1760 fbs). Each mesocosm contained eight membrane diffusion chambers filled with *E. coli* or *P. aeruginosa*. These mesocosms maintained native groundwater geochemical conditions, except for pressure. The *E. coli* strain followed a bi-phasic model with an inactivation rate in the UF of 0.217-0.628 hr⁻¹ and 0.540-0.684 hr⁻¹ in the APPZ. These rates decreased in the second phase ranging to

0.006-0.001 hr⁻¹ and 0.013-0.018 hr⁻¹ in the UF and APPZ, respectively. The *Ps. aeruginosa* strain also followed a bi-phasic model with an inactivation rate in the UF of 0.144-0.770 hr⁻¹ and 0.159-0.772 hr⁻¹ in the APPZ. These rates decreased in the second phase ranging from 0.003-0.008 hr⁻¹ in the UF and 0.004-0.005 hr⁻¹ in the APPZ zones. The influence on groundwater geochemistry and culture media on bacterial indicator inactivation rates will be presented.

Grazing of Heterotrophic Flagellates on (Pathogenic) Viruses Is Driven by Feeding Behavior

Presented by Dr. Christian Griebler, Institute of Groundwater Ecology, Helmholtz Zentrum München, Germany

Co-Authors: C. Griebler, L. Deng, S. Krauss, J. Feichtmayer, R. Hofmann, and H. Arndt

Pathogenic viruses once released into the aquatic environment are subject to retardation and decay by a number of abiotic processes, such as irradiation and sorption. They, however, also get involved as extrinsic participants in the microbial food web, and evidence for the significance of microbial antagonism in viral decay is increasing. We thus conducted experiments with three strains of heterotrophic groundwater flagellates characterized by different feeding behavior, mixed viral and prokaryotic communities, as well as the model bacteriophage MS2, to elucidate the importance of grazing and other antagonistic microbial activities for viral inactivation and decay. A major result is that protozoan grazing on viruses takes place, and can substantially contribute to the elimination of ‘strangers’, i.e. viruses that do not find hosts for reproduction in situ. Furthermore, we could show that the impact of heterotrophic flagellates on viral density is related to the flagellates’ feeding behavior: the model phage MS2 was actively removed by the suspension feeders *Thaumatomonas coloniensis* and *Salpingoeca* sp.; in contrast the actively raptorial grazer *Goniomonas truncate* had little impact on phage density. Both, qPCR of MS2 and the application of protein fluorescently labelled bacteriophages demonstrated that the decline of viral titre was caused by ingestion rather than by random absorption. Protists thus may have an important but yet unrecognized function in microbial food webs eliminating (pathogenic) viruses. Another outcome of our experiments was that the presence of an active prokaryotic community also contributed significantly to the decay of MS2. However, the underlying mechanisms this still await to be uncovered.

Impacts of the Charge-Regulation Effect on the Growth of Attached Bacteria and the Bioavailability of Ionizable Substrates

Presented by Derick G. Brown, Ph.D., Associate Professor, Department of Civil and Environmental Engineering, Lehigh University, USA

Co-Authors: H. Zhu, L.S. Albert, and D.G. Brown

A key aspect with bacterial surface growth is how the bacteria interact with the surface upon which they have adhered: does the surface stimulate growth and colonization or does it inhibit growth and cause the bacterium to die? Studies have shown that bacterial metabolic activity can be altered upon adhesion to a solid surface, and while these studies indicate that bacterial attachment can directly affect metabolic activity, the mechanisms for this altered activity are unclear. We are approaching this question from a system standpoint by considering how the charge-regulated bacterial cell surface affects cellular bioenergetics and substrate bioavailability. When bacteria approach a solid surface, the physiochemical charge-regulation effect alters the cell surface pH. The hypothesis we have developed is that this change in pH at the cell surface can alter two processes. First, we hypothesize that it alters the proton gradient across the cell’s cytoplasmic membrane. The energy stored in this gradient is the basis of cellular bioenergetics and we have demonstrated in prior studies how this can enhance or inhibit bacterial energy levels under non-growth conditions. Second, we hypothesize it

alters the sorption of substrates exhibiting acidic and basic functionalities and bacteria are able to make use of this process to enhance substrate bioavailability. We will present results demonstrating these effects and will discuss how they are related to the acid/base properties of the bacteria, adhering surface and sorbed growth substrate. The overall goal is to provide a framework for understanding how bacterial attachment affects cellular bioenergetics and growth.

3:40 PM – 5:20 PM
MERRILL HALL

SESSION A9 ECOLOGY: SUBSURFACE ECOSYSTEMS (AQUATIC AND OTHER)

Moderated by:

- **Rainer U. Meckenstock, Ph.D.**, Professor for Aquatic Microbiology, Biofilm Center, University of Duisburg-Essen, Germany
- **Duane P. Moser, Ph.D.**, Associate Research Professor, Division of Earth and Ecosystems Sciences, Desert Research Institute, USA



Ecophysiology of Heat-Resistant Endospores along an Estuarine Gradient Suggests Certain Thermophilic Sulfate-Reducing *Desulfotomaculum* spp. Originate in Hot Subsurface Ecosystems

Presented by Emma Bell, Ph.D. Candidate, School of Civil Engineering and Geosciences, Newcastle University, United Kingdom

Co-Authors: E. Bell, A. Sherry, N. Gray, I.M. Head, and C.R.J. Hubert

Viable anaerobic thermophilic bacteria have been found in cold marine and estuarine sediments that do not support their growth. It has been proposed that warm subsurface habitats associated with sufficient fluid flow seed the ocean with bacterial endospores. Multiple source environments may contribute to this process resulting in the dispersal of several taxa of thermophilic endospores to cold marine and estuarine sediments. To elucidate potential dispersal histories of different taxa, the diversity of thermophilic endospores of sulfate-reducing bacteria (SRB) along an estuarine gradient from freshwater (River Tyne, UK) to marine (North Sea) was investigated. Sediment heating experiments revealed a diverse population of spore-forming SRB. Microcosms incubated at 50, 60 or 70°C showed a shift in the dominant species of *Desulfotomaculum* as the temperature increased. Microcosms triple-autoclaved at 121°C prior to incubation still showed rapid and reproducible sulfate reduction and some *Desulfotomaculum* remained active after autoclaving at 130°C. Certain species were detected at every location sampled along the estuary, whereas others were only detected at the marine end, highlighting possible links to deep marine biosphere habitats such as oil reservoirs and mid-ocean ridges that harbour closely related *Desulfotomaculum* spp. These results suggest that temperature optima and heat resistance can reveal clues about warm source environments where conditions (e.g. local temperature) influence sporulation and the degree of endospore heat resistance. Temperature physiology can thus complement biogeographic patterns for teasing apart potential dispersal histories for certain species, including those displaying more cosmopolitan distributions.

Microbial Diversity and Metabolic Potential from the Coast Range Ophiolite Microbial Observatory

Presented by Katrina I. Twing, Ph.D. Candidate, Department of Microbiology and Molecular Genetics, Michigan State University, USA

Co-Authors: K.I. Twing, W.J. Brazelton, M. Crespo-Medina, T.M. Hoehler, T.M. McCollom, D. Cardace, and M.O. Schrenk

Serpentinization is a widespread geochemical process involving the water-rock reaction of ultramafic rocks, resulting in a highly reducing environment containing abundant dissolved hydrogen and potentially abiogenic organic molecules, serving as possible energy sources for microbes in the subsurface. Serpentinite environments are also characterized by extremely high pH (>10) and low availability of DIC, making them challenging to live in, despite the high energy available. Habitability models predict that these environments can sustain microbial life, however little work has been done to directly characterize the microbial communities utilizing the energy generated from this process. The Coast Range Ophiolite Microbial Observatory, a collection of 9 boreholes drilled to varying depths within the serpentinizing strata of an ophiolite in northern California, was established to allow direct access to serpentinizing fluids. Biological and geochemical samples have been collected quarterly since the wells were drilled in August 2011, allowing for inter- and intra-well comparisons of microbial communities and their environments over time. DNA samples were analyzed via tag sequencing of the V6 region of the 16S rRNA gene to assess microbial diversity, and a subset of samples were submitted for metagenomic shotgun sequencing to explore the full gene content of the community. By combining genetic and geochemical data, we hope to elucidate which environmental parameters are driving community composition in this unique environment. Preliminary results suggest there is a core community of microbes containing genes required for metabolizing the geochemical products of serpentinization and adapted to survive the harsh conditions of the serpentinite subsurface environment.

Chemolithotrophic Primary Production in a Subglacial Ecosystem

Presented by Mark Skidmore, Ph.D., Associate Professor, Department of Earth Sciences, Montana State University, USA

Co-Authors: M. Skidmore, T.L. Hamilton, J.R. Havig, E.L. Shock, and E.S. Boyd

Glacial comminution of bedrock generates fresh mineral surfaces capable of sustaining microbial communities under the dark conditions that pervade subglacial habitats. Geochemical and isotopic evidence suggests that pyrite oxidation is a dominant weathering process generating protons that drive mineral dissolution in many subglacial systems. Here, we provide evidence correlating pyrite oxidation with primary productivity and carbonate dissolution in subglacial sediments sampled from Robertson Glacier (RG), Alberta, Canada. Quantification and sequencing of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) transcripts suggests that populations closely affiliated with *Sideroxydans lithoautotrophicus*, an iron-sulfide oxidizing autotrophic bacterium, are abundant constituents of microbial communities at RG. Microcosm experiments indicate sulfate production during assimilation of radiolabeled bicarbonate. Geochemical and isotopic analysis of subglacial meltwater indicates that increases in sulfate are associated with increased calcite and dolomite dissolution. Collectively, these data suggest a role for biological pyrite oxidation in driving primary productivity and mineral dissolution in a subglacial environment and provide the first rate-estimate for bicarbonate assimilation in these ecosystems. Evidence for lithotrophic primary production in this contemporary subglacial environment provides a plausible mechanism to explain how subglacial communities could be sustained in near isolation from the atmosphere during glacial-interglacial cycles.

A Census of Deep Life to Assess Microbial Diversity in the Subsurface

Presented by Frederick Colwell, Ph.D., Professor, College of Earth, Ocean, and Atmospheric Sciences, Oregon State University, USA

Co-Authors: S. Grim, A. Boetius, B. Briggs, W. Brazelton, S. D'Hondt, K. Edwards, M. Fisk, E. Gaidos, J. Gralnick, K.-U. Hinrichs, C. Lazar, H. Lavalleur, M.A. Lever, V. Marteinson, D. Moser, B. Orcutt, K. Pedersen, R. Popa, A. Ramette, M. Schrenk, J. Sylvan, A. Smith, A. Teske, E. Walsh, M. Sogin, and F. Colwell

The initial Census of Deep Life (CoDL) surveyed microbial diversity and environmental parameters in 13 terrestrial and marine subsurface environments. Using 454 pyrosequencing of the V4-V6 hypervariable region of the 16S rRNA gene, we surveyed 148 and 56 samples for bacterial and archaeal communities, respectively. After quality filtering, 3M high-quality pyrosequences were obtained, which clustered at 97% sequence similarity into 2,254 archaeal (89 genera; 28 classes) and 55,372 bacterial (1267 genera; 75 classes) operational taxonomic units (OTUs). For each sample we recovered 35-5964 bacterial and up to 355 archaeal OTUs. Samples sequenced for archaea had on average 43% Crenarchaeota sequences, and 57% Euryarchaeota sequences. Proteobacteria was the most represented bacterial phylum (average: 33% of sequences), and Gammaproteobacteria and Betaproteobacteria the dominant classes (average: 16% and 15% of sequences, respectively). Four archaeal OTUs, attributed to Methanosarcina (Euryarchaeota); a euryarchaeal genus of the South African Gold Mine Group; ANME-1b; and a genus of Marine Group I (Crenarchaeota), were observed in a majority of environments. High-biomass communities were less likely to host Actinobacteria, Betaproteobacteria, Gammaproteobacteria, and Bacilli. Relative abundances of Hydrogenophaga OTUs increased with increasing pH. Fourteen OTUs from 13 bacterial genera occurred in samples from every CoDL site/project, with abundances of 0.1-23% of all sequences. We view ubiquitous *Ralstonia*, *Escherichia*, and *Propionibacterium* OTUs with caution. Multiple sequence variants of these OTUs with varying abundances indicate that a single organism source does not dominate these samples. High-resolution DNA sequencing with matching metadata will guide future inquiries into the diversity of deep life.

Insights into Mutualistic Interactions between Free-Living, Pleomorphic Spirochetes (FLiPS) and *Dehalococcoides Mccartyi*

Presented by Burcu Simsir, Ph.D. Candidate, Department of Civil and Environmental Engineering, University of Tennessee at Knoxville, USA

Co-Authors: B. Simsir, D. Tsementzi, K. Cusick, K. Rithalahti, K. Kontantinidis, and F.E. Löffler

Chlorinated solvents are commonly encountered toxic groundwater contaminants. Microbial anaerobic reductive dechlorination plays a major role in the transformation and detoxification of chlorinated solvents. *Dehalococcoides mccartyi* (Dhc), the only bacteria to date that capable of complete reductive dechlorination of chlorinated solvents, depend on community members to supply hydrogen and essential growth factors. Therefore, to meaningfully predict and stimulate Dhc reductive dechlorination activity, it is necessary to understand interactions between Dhc and other community members. Dhc strain FL2 isolation efforts resulted in the coincidental discovery of an unknown and mysterious bacterial group closely related to Spirochaetes. Due to their morphological characteristics, this group is designated as Free-Living, Pleomorphic Spirochetes (FLiPS). FLiPS can grow by fermentation of glucose or other sugars but exhibit no dechlorination activity. FLiPS-associated 16S rRNA sequences are typically found in a variety of contaminated or other natural environments in co-occurrence with Dhc populations. To explore nature of the interaction between FLiPS and Dhc, a combination of metagenome and metatranscriptome sequencing was performed to a Dhc-containing

enrichment culture during the course of TCE dechlorination. Preliminary results of time series gene expression revealed that FLiPS significantly contributed to the community transcriptome, being among the ten most abundant populations at transcript level during the Dhc reductive dechlorination activity. Additionally, gene expression data indicate that the most abundant FLiPS transcripts in TCE enrichment cultures related to oxidative stress response (i.e., superoxide reductase), with increasing expression levels through the dechlorination process. Current efforts to elucidate the specific functions of FLiPS in dechlorinating consortia are ongoing. Understanding the relative contribution of FLiPS activity to support Dhc growth will provide valuable information for Dhc-community interactions to improve site management and enhance bioremediation applications.

3:40 PM – 5:20 PM
FRED FARR FORUM

**SESSION B9 CONTAMINANTS: OIL AND GAS RESERVOIRS,
MICROBIOLOGICALLY INFLUENCED CORROSION (MIC)**

Moderated by:

- **Tamara N. Nazina, Ph.D.**, Head of Laboratory, Winogradsky Institute of Microbiology, Russian Academy of Sciences, Russia
- **Joseph M. Suflita, Ph.D.**, Professor, Department of Microbiology and Plant Biology, University of Oklahoma, USA



Corrosion of Carbon Steel Facilitated by Organic Acid Metabolites under High CO₂ Conditions and in the Absence of Sulfate Reducing Bacterial Activity

Presented by Mark A. Nanny, Ph.D., Professor, School of Civil Engineering and Environmental Science, University of Oklahoma, USA

Co-Authors: M.A. Nanny, J.M. Suflita, T. Lenhart, J. Chavarro, A. Westbrook, Y. Li, J. Miguel, B. Harriman, I. Davidova, and K. Duncan

Corrosion of the carbon steel infrastructure in subsurface oil production facilities is most often associated with the activity of sulfate reducing bacteria. However, a different biocorrosion mechanism was evident in the high CO₂ petroleum reservoirs of southwest Colombia, South America. That is, a sensitive 35S-sulfate reduction assay revealed that sulfide formation was not an important ecological process in these formations. The crude petroleum did not contain organic acids or neutral polar compounds. However, GC/MS analysis of oilfield production waters suggested that linear, iso-, and cyclic alkanes, alkyl monoaromatic compounds and methyl naphthalenes served as suitable parent substrates for the formation of the corresponding organic acid and neutral compounds presumably by an active subsurface microflora. Positive correlations between dissolved iron and organic acid metabolites as well as cell density (DNA and optical counting) suggest a strong relationship between microbial activity in the production waters and steel corrosion. The absence of signature aerobic intermediates (e.g. catechols) attest to the importance of anaerobic hydrocarbon bioconversions. It is proposed that such organic acid metabolites enhance CO₂ corrosion of steel by complexing oxidized iron cations and prevent them from precipitating as a protective layer of siderite (FeCO₃) onto the steel surface. Organic acids might also dissolve the protective siderite layer, thereby exposing the underlying steel surface to corrosion processes. Additionally, organic acids can increase dissolution rates at Mn inclusion sites in the steel thereby increasing pitting formation.

Factors Governing the Onset of Oil Reservoir Souring by Thermophilic Sulfate-Reducing Microorganisms in the Deep Hot Biosphere

Presented by Angela Sherry, Ph.D., Research Fellow in Geomicrobiology, School of Civil Engineering and Geosciences, Newcastle University, United Kingdom

Co-Authors: A. Sherry, E. Bell, G. Pilloni, and C.R.J. Hubert

Reservoir souring is caused by the activity of sulfate-reducing microorganisms (SRM) in subsurface oil reservoirs, and is often induced by water injection during secondary oil recovery. Souring can potentially contribute to corrosion of infrastructure, thus increasing the safety and health hazards associated with producing a resource. It can also reduce the value of the petroleum resource and causes annual losses in the billions of dollars. Endospore-forming SRM, such as *Desulfotomaculum* spp., are often suspected culprits in reservoir souring. Endospores can survive unfavourable conditions for long periods, yet remain poised to germinate and become active if conditions change for the better. Factors governing endospore germination are poorly understood, but are thought to include availability of nutrients (possibly metabolic byproducts of other anaerobic bioprocesses) as well as appropriate temperature. For *Desulfotomaculum* spores in deep hot oil reservoirs, cold seawater introduction during secondary oil recovery may create thermal viability zones for sulfate reduction near the injection wellbore. To evaluate these processes, sulfate-containing microcosms were prepared with different marine sediments as a source of spores, and amended with organic substrates. Incubation at 80°C for six days was followed by a down-shift in temperature to 60°C to mimic cold seawater injection. Souring did not occur at 80°C, but commenced within hours at 60°C. Microcosms were monitored by tracking sulfate reduction and organic acid concentrations in combination with molecular microbial community analysis of 16S rRNA genes using different methods including next generation sequencing platforms. This approach yields new insights into potential biogeochemical responses in dynamic thermal viability zones in the deep biosphere of water-injected oil reservoirs.

Metagenomic Analysis of Mostly Cold Natural Oil Sands Outcrops Samples Revealed the Presence of Thermophilic Microbial Communities with the Metabolic Potentials Driving Methanogenic Hydrocarbon Biodegradation at Fort McMurray, Alberta

Presented by Dongshan An, Ph.D., Department of Biological Sciences, University of Calgary, Canada

Co-Authors: D. An, M.-L. Wong, S. Caffrey, J. Soh, X. Dong, C. Sensen, T. Oldenburg, S. Larter, and G. Voordouw

Oil sands in the Athabasca deposit are located near the surface. These oil sands are naturally present in the environment and can be found in the river valleys carved through them. Since the presence of a network of rivers cut through the deposit, aerobic hydrocarbon degradation is likely to be the fate of bitumen hydrocarbon at outcrop sites. The wide range of ambient temperatures (-40 to +60°C) could also affect bitumen biodegradation, given that bitumen shows high viscosity at low temperatures and low viscosity at high temperatures. The latter facilitates microbial degradation of bitumen.

Pyrosequencing of 16S rRNA genes from 23 outcrops samples, collected at the surface and center parts of In-Water and Cliff sites from Fort McMurray, Alberta, in 2007 and 2011, revealed that In-Water and Cliff samples showed different microbial community structures. The microbial community of the In-Water samples were dominantly aerobic or facultative anaerobic hydrocarbon degraders, such as Comamonadaceae, Rhodocyclaceae and Chitinophagaceae, while the microbial community of Cliff samples were dominantly aerobic methanotrophs, such as Beijerinckiaceae and Methylobacteriaceae, and aerobic acetic acid bacteria Acetobacteraceae. Interestingly, the Cliff samples showed the presence of anaerobic microbial communities involved in the methanogenic

hydrocarbon degradation in the three of Cliff-center samples, which formed a subcluster within the Cliff samples. Most of these anaerobic microbial community members were recovered in the anaerobic highest ambient temperature (60°C) incubation, indicating that outcrop Cliff samples harbor thermophilic microbial communities at center part and may hold metabolic potentials to drive methanogenic hydrocarbon degradation in the Fort McMurray.

Role of Spore-Forming Firmicutes in the Degradation of Hydrocarbons in Subsurface Petroleum Reservoirs

Presented by Ana Suárez-Suárez, Ph.D., Research Associate, School of Civil Engineering and Geosciences, Newcastle University, United Kingdom

Co-Authors: A. Suárez-Suárez, C.M. Aitken, M. Jones, A. Sherry, N. Gray, C.R.J. Hubert, S. Larter, and I.M. Head

Arctic sediments contain thermophilic Firmicutes closely related to sulphate-reducing bacteria inhabiting subsurface petroleum reservoirs. Their unexpected abundance and constant input over geological time scales raises questions about their origin, and highlights the possibility of a flow of microorganisms from the deep biosphere into anoxic surface sediments. Might advective flow of fluid from warm deep environments (e.g. leaking petroleum reservoirs and hydrothermal vent systems) be transporting anaerobic hydrocarbon-degraders into the cold ocean? Firmicutes is one of the most frequently encountered bacterial phyla in subsurface petroleum reservoirs. However, little is known about their involvement in the anaerobic biodegradation of hydrocarbons observed in most World's petroleum deposits. We have employed a combination of microcosm experiments, biogeochemical analysis and next generation molecular biology techniques to demonstrate that spore-forming sulphate-reducing Firmicutes capable of hydrocarbon degradation occur in surface sediments. Pasteurized estuarine sediments incubated at 25°C in the presence of crude oil showed a significant degradation of n-alkanes coupled to the consumption of sulphate and the production of methylalkylsuccinates, key metabolic intermediates in the anaerobic degradation of n-alkanes via fumarate addition. Remarkably, diversity analysis based on 16S rRNA gene Ion Torrent sequencing pointed to a modest enrichment of known sulphate-reducers hydrocarbon-degraders such as *Desulfotomaculum*. Instead, members from of the Clostridiaceae, Planococcaceae and Peptostreptococcaceae dominated the pasteurized microcosms. Their physiology and possible connection with deep petroleum reservoirs are currently being explored.

Metabolomic and Metagenomic Analyses of Crude Oil Production Pipelines Experiencing Differential Rates of Corrosion

Presented by Boris Wawrik, Ph.D., Assistant Professor, Department of Microbiology and Plant Biology, University of Oklahoma, USA

Co-Authors: B. Wawrik, A.V. Callaghan, V. Bonifay, E.C. West, E. Aydin, J. Sunner, K.E. Duncan, A. Oldham, and I.B. Beech

Microbially influenced corrosion (MIC) is a process whereby corrosion of metals is initiated, enhanced and/or facilitated via microbiological activity. The specific processes of MIC are not well elucidated. For this work, early and late pigging materials from two pipelines experiencing dramatically different MIC rates were analyzed via 16S rRNA gene surveys, metagenomic analysis, and metabolomics. The 16S rRNA gene analysis indicated that the high corrosion pipeline (HC) was dominated by Thermotogae, Deltaproteobacteria, Firmicutes and Methanococci, whereas the low corrosion pipeline (LC) was dominated by Gammaproteobacteria most closely related to *Pseudomonas*. Reconstruction of genome scaffolds from LC metagenomic data indicated the presence

of a complete denitrification pathway, a high frequency of genes associated with aerobic hydrocarbon degradation, and very low frequencies of genes associated with anaerobic hydrocarbon pathways. In contrast, the dominant HC scaffolds were similar to the genomes of *Desulfonatronospira thiodismutans* and *Thermococcus kodakarensis*, which are known to dismutate $S_2O_3^{2-}$ and reduce S_0 , respectively. Neither genome scaffold contained sulfate reduction (e.g. *dsrA/B*) genes, and COG/BlastN analyses of all metagenomic data suggested that *dsrA/B* genes are not adequate proxies for corrosion in these systems. These observations were further supported by HPLC/Q-ToFMS analyses. Several metabolites indicative of aerobic hydrocarbon degradation and anaerobic hydrocarbon activation were detected at the LC and HC sites, respectively. In summary, the two pipelines appear to be dominated by strictly anaerobic vs. denitrifying microbes. Given the presence of nitrate in both systems, it is hypothesized that differential amounts of oxygen may govern community ecology in these pipelines.

3:40 PM – 5:20 PM
NAUTILUS ROOM

SESSION C9 CHEMISTRY: ELECTROMICROBIOLOGY

Moderated by:

- **Mohamed Y. El-Naggar, Ph.D.**, Assistant Professor of Physics, University of Southern California, USA
- **Roseanne M. Ford**, Professor and Chair, Department of Chemical Engineering, University of Virginia, USA



Subsurface Microbiology at the Microbe-Electrode Interface

Presented by Jeffrey A. Gralnick, Ph.D., Associate Professor, University of Minnesota, USA

Author: J.A. Gralnick

Microbes can influence the geochemical cycling of metals in subsurface and sediment environments by using reduced metals as a source of electrons or oxidized metals as a sink for electrons. Many dissimilatory metal reducing bacteria found in sediment and subsurface environments can respire insoluble minerals as their terminal electron acceptor for anaerobic growth. A number of these isolates have also been shown to use poised graphite or gold electrodes in the lab. Recently, it has been shown that metal oxidizing bacteria can use electrodes as an electron donor for growth and metabolism. I will present results from our lab that explore the molecular mechanism of electrode respiration in the model organism *Shewanella oneidensis* and discuss new model organisms that mediate iron redox chemistry and can interact with poised electrodes isolated from the Soudan Iron Mine - a portal into a 2.7 billion year old Banded Iron Formation located in northern Minnesota.

Integrated Genomic and Proteomic Analysis of a Stable, Robust Marine Biocathode Biofilm

Presented by Sarah M. Strycharz-Glaven, Ph.D., Research Biologist, Naval Research Laboratory, USA

Co-Authors: S.M. Strycharz-Glaven, Z. Wang, D. Leary, B. Eddie, B. Lin, A. Malanoski, J. Harvey, G. Vora, and L.M. Tender

Cell-electrode electron transfer (ET) is thought to occur through mechanisms similar or analogous to metal-reducing or metal-oxidizing bacteria. Electrodes have been suggested to serve as a proxy to study ET pathways of such organisms. *Geobacter* and *Shewanella* are two of the most intensely studied organisms in electromicrobiology. However, as the field broadens to include applications of biocathodes there is movement towards discovering more robust and diverse microbial electrode catalysts, including microbial consortia. Here, we present a cultivation-independent approach to study a stable, autotrophic multispecies biocathode biofilm consuming electricity from an electrode (+310 mV SHE) and fixing CO₂ from seawater. Metagenomic analyses by sequence composition and sequence similarity based methods are consistent with a community divided between Alpha- and Gammaproteobacteria. Metatranscriptomic and metaproteomic analyses indicate an unknown member of the order Chromatiales and a *Marinobacter* sp. are most abundant and relevant for electron transfer and CO₂ fixation, accounting for over 70% of observed proteins in these functional categories and correlating with gene expression patterns. Proteins for key components of a putative microbial iron oxidation pathway were identified in *Marinobacter* and Chromatiales and targeted for mutagenesis. Electrochemically active biofilm isolates including *Marinobacter*, *Alcanivorax*, and *Labrenzia* spp., were not able to sustain electrical activity without introducing organic carbon indicating the biofilm community is required. By providing an electrode as the electron donor for autotrophic growth we can simulate conditions for analogous subsurface communities that are difficult or impossible to maintain in culture, whose syntrophic interactions, if understood, may be exploited for development of synthetic microbial communities.

Eating Rocks as Opposed to Breathing Rocks: Enrichment and Isolation of Microbes Using Electrodes as an Electron Source

Presented by Annette Rowe, Ph.D., Postdoctoral Fellow, Center for Dark Energy Biosphere Investigations (C-DEBI), University of Southern California, USA
Co-Authors: A. Rowe, P. Chellamutha, B. Lam, and K. Nealson

Little is known about the importance of mineral oxidation processes in sediment, and part of this stems from the difficulties associated with culturing microbes with this capacity. We demonstrate that electrochemical techniques, using an electrode poised at a given redox potential, is a feasible approach to enrich and facilitate isolation of mineral oxidizing microbes. To this end we constructed marine sediment microcosms and incubated electrodes at varied and controlled redox potentials. The negative current production observed increased as the redox potential decreased. The enriched biomass from these incubations was further cultivated under sediment free conditions. Electrode oxidizing microbes isolated from these enrichments belong to the genera *Halomonas*, *Idiomarina*, *Thalassospira*, and *Pseudomonas*; organisms commonly detected in marine sediments but not generally associated with mineral oxidation. Electrochemical analysis of the isolates has demonstrated that, though these organisms have similar physiologies, they employ different biochemical mechanisms demonstrated by the variability in dominant electron transfer modes (i.e., biofilm, planktonic or mediator facilitated interactions) and the range of midpoint potentials observed for dominant redox active cellular components (ranging from -293 to +50 mV vs. Ag/AgCl). Notably, organisms isolated on sulfur tended to have higher midpoint potentials than iron-oxidizing microbes. The insight gained from these experiments is not limited to the physiology of the organisms isolated but also relevant to their potential ecologic importance. Given the abundance of reduced minerals in sediments and the Earth's subsurface this approach holds vast potential for increasing our understanding of the biogeochemical importance and abundance of mineral oxidation reactions.

Microbial Long Distance Electron Transfer in Contaminated Aquifers

Presented by Hubert Müller, Ph.D. Candidate, Institute of Groundwater Ecology, Helmholtz Zentrum München, Germany

Co-Authors: H. Müller, J. Bosch, T. Lüders, L.R. Damgaard, L.P. Nielsen, and R.U. Meckenstock

In marine sediments, filamentous Desulfobulbaceae have been shown to bridge sulfide oxidation and oxygen reduction over centimeter distances. The spatially separated redox half reactions lead to an alkaline pH where protons are consumed by oxygen reduction and to an acidic pH where sulfide is oxidized to sulfate. To analyze if long distance electron transfer occurs in contaminated groundwater we set up batch experiments with natural sediments amended with FeS as electron donor. After 2-3 months, the pore water showed pH, oxygen, and sulfide gradients characteristic of long distance electron transfer of 4-20 mm. Spatially resolved analysis of the microbial communities by tRFLP and 454 pyrosequencing indicated a relative abundance of Desulfobulbaceae of up to 45% within the suboxic zone. Fluorescence in situ hybridization revealed that the sequences belonged to filamentous Desulfobulbaceae. We then took intact sediment cores from the capillary fringe of a tar oil contaminated aquifer at 7 meters depth and analyzed the microbial communities at cm resolution. The filamentous Desulfobulbaceae were not only present in situ but accounted for up to 27% Desulfobulbaceae of the total bacterial numbers. An estimate of the total abundance showed about 3-4 m filamentous Desulfobulbaceae per ml sediment. These results suggest long distance electron transfer naturally occurs in contaminated aquifers and might be an important process in the recycling of the electron acceptor sulfate. Thus, the newly discovered process might be important in increasing degradation rates close to the capillary fringe.

Electrochemical, AFM, and Single-Cell Resolution Raman Analysis of Geobacter sulfurreducens Biofilms Early in Growth

Presented by Leonard M. Tender, Ph.D., Research Chemist, Naval Research Laboratory, USA

Co-Authors: N. Lebedev, S.M. Strycharz-Glaven, and L.M. Tender

Here we report on two studies involving early growth anode-grown *Geobacter sulfurreducens* biofilms. In the first AFM and single-cell resolution confocal resonance Raman microscopy (CRRM) were used to study transition of from lag phase (initial period of low current) to exponential phase (subsequent period of rapidly increasing current). The results indicate lag phase biofilms consist of lone cells and tightly packed single-cell thick clusters crisscrossed with extracellular filament where abundance of c-type cytochromes (c-Cyts) is similar for both cell types. By early exponential phase, cell clusters have expanded laterally and second layer of closely packed cells begins to form on top of the first where c-Cyts abundance is 4 to 5-fold greater in 2-cell thick regions. The results indicate the transition from lag phase to exponential phase involves at least two key transformations: 1) from lone cells to 2-dimensionally associated cells during lag phase accompanied by formation of extracellular filaments, where current remains low; 2) from 2- to 3-dimensionally associated cells during early exponential phase, accompanied by increased abundance of c-Cyts, where current begins to increase. In the second study, *Geobacter sulfurreducens* biofilms were grown to early exponential phase on interdigitated microelectrode arrays (IDAs). Microscopy revealed sparse cell clusters surrounded by extracellular polymeric substances (EPS). Continuous domains of EPS, but not of cell clusters, were observed bridging gaps between adjacent electrodes. Electrochemical gate measurements indicate electrical continuity between adjacent IDA electrodes. The dependency on gate potential of conducted current is peak shaped suggesting that the EPS is a redox conductor.