

Ninth International Symposium on Subsurface Microbiology

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

Tuesday, October 7, 2014

ORAL PRESENTATION ABSTRACTS

ORAL PRESENTATION SCHEDULE

8:30 am – 10:00 am	Plenary Session #2	Merrill Hall
10:20 am – 12:00 pm	Session #4	
	A4. Chemistry	Merrill Hall
	B4. Methods	Fred Farr Forum
	C4. Ecology	Nautilus Room
2:00 pm – 3:20 pm	Session #5	
	A5. Chemistry	Merrill Hall
	B5. Methods	Fred Farr Forum
	C5. Contaminants	Nautilus Room
3:40 pm – 5:20 pm	Session #6	
	A6. Chemistry	Merrill Hall
	B6. Methods	Fred Farr Forum
	C6. Biomes	Nautilus Room

8:30 AM – 10:00 AM
MERRILL HALL



PLENARY SESSION

Moderated by **Rainer U. Meckenstock, Ph.D.**, Professor for Aquatic Microbiology, Biofilm Center, University of Duisburg-Essen, Germany



Part I: QMRACatch - A Tool for Microbial Quality Simulations of Freshwater, Including Risk Assessment

Part II: Empirical Formulas to Predict Virus Attachment as a Function of Geochemical Conditions

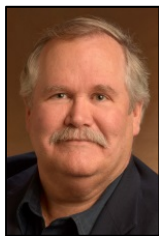
Presented by Prof. Dr. Jack F. Schijven, Senior Scientist, National Institute of Public Health and the Environment (RIVM), The Netherlands, and Professor, Environmental

Hydrogeology Group, Department of Earth Sciences, University of Utrecht, The Netherlands

Co-Authors (Part I): J.F. Schijven, A.H. Farnleitner, J. Derx, and A.P. Blaschke

Co-Authors (Part II): J.F. Schijven, G. Sadeghi, A. Waade, and S.M. Hassanizadeh

A computational interactive tool named QMRACatch was developed to simulate concentrations in fresh water of waterborne pathogenic microorganisms (enterovirus, norovirus, Campylobacter, Cryptosporidium), microbial fecal indicators and genetic faecal markers (E. coli and Bacteroidetes marker). The aqueous domain encompasses a river in which microorganisms enter by means of raw and treated wastewater discharges, runoff from agricultural land and a floodplain and from direct fecal input. Exposure to these microorganisms occurs from swallowing of water during bathing or from consumption of drinking water that was produced from treating the river water by riverbank filtration. The processes that determine microorganism concentrations in the river are dilution and temperature-dependent inactivation or die-off. Travel times in the river are calculated on the basis of the Manning-Gauckler-Strickler formula for gravelly or sandy river beds. On the basis of precipitation and amounts of manure and fecal droppings, pathogen concentrations in suspended feces on land are calculated. Part of the suspended pathogens infiltrates into the groundwater and part runs off as described by a runoff coefficient. Infection risks are calculated for exposure via drinking water consumption and via bathing in the river. The tool also calculates required removal by riverbank filtration to not exceed a certain health based target. The tool provides concentration values and parameter values of Gamma distributed concentrations that can be taken as input parameters for source water concentration in QMRASpot or in riverbank filtration models to include a more detailed analysis of drinking water treatment efficiency into the risk assessment. In addition empirical formulas are being developed that predict virus attachment to soil as a function of physico-chemical conditions at field scale. The data that are the basis for these formulas come from four field studies under a variety of redox conditions. Depending on what conditions are known a different formula applies. These formulas can be used to calculate adequate setback distances to adequately protect groundwater wells from virus contamination.



Microbial Community Structure Predicts Groundwater Geochemistry

Presented by Terry C. Hazen, Ph.D., UT/ORNL Governor's Chair Professor, University of Tennessee and Oak Ridge National Laboratory, USA

Co-Authors: A.M. Rocha, M. Smith, C. Smillie, T.L. Mehlhorn, J.E. Earles, K.A. Lowe, J. Phillips, D.B. Watson, C. Paradis, K. Bailey, D. Joyner, J.L. Fortney, S.

Pfiffner, J.J. Zhou, J.D. Van Nostrand, L. Wu, P. Zhang, Z. He, D. Curtis, D. Xu, D.

Elias, M. Adams, F. Poole, R. Chakraborty, A.P. Arkin, E. Alm, and T.C. Hazen

At the Department of Energy's Oak Ridge field site, over 20 years of historical and published data for more than 800 groundwater wells is available in a computer queryable database. In this study, we conducted a survey of 99 groundwater well clusters in order to (1) characterize key microbial populations at geochemically distinct locations, and (2) identify associations between environmental gradients and microbial communities. To optimize geochemical diversity, wells were selected using k-medians clustering to group 818 wells into 100 clusters by 14 geochemically similar measurements. At each well, in situ groundwater measurements were recorded and unfiltered and filtered groundwater samples were collected for both geochemical measurements and analysis of microbial communities. Nucleic acids were collected by filtering water through a 10.0µm pre-filter and 0.2µm-membrane filter and then extracted using a Modified Miller method. Evaluation of divergence of microbial communities across all the wells indicates the microbial communities are fairly distinct. Comparison of microbial communities within each well shows taxa are not as divergent compared to across all wells. Metadata correlations of all the wells show many of the geochemical parameters are independent of each other. To evaluate potential microbial-geochemical associations, a random forest classification system was used and trained on the OTU abundances to predict continuous values for each geochemical parameter. Results indicate that with careful design and a large dataset, the groundwater microbial community structure can be used to accurately predict the water geochemistry.

10:20 AM – 12:00 PM
MERRILL HALL

SESSION A4 CHEMISTRY: MICROBIAL ENERGETICS

Moderated by:

- **Timothy D. Scheibe, Ph.D.**, Staff Scientist, Pacific Northwest National Laboratory, USA
- **Ean Warren**, Hydrologist, U.S. Geological Survey, USA



Seasonal Temperature and Unsaturated Zone Gas Changes Due to Microbial Activity at a Crude Oil-Contaminated Site

Presented by Ean Warren, Hydrologist, U.S. Geological Survey, USA

Co-Authors: E. Warren, J. Trost, N. Sihota, and B. Bekins

Crude oil at a spill site near Bemidji, Minnesota has been undergoing aerobic and anaerobic biodegradation for over 30 years, creating a 150-200 meter (m) dissolved contaminant plume. Continuous temperature measurements at multiple locations in the vadose and saturated zones revealed a thermal plume of 2 to 6 degrees above background emanating from near the residual oil

body at the water table. The thermal plume is associated with heat produced by aerobic methane oxidation in the unsaturated zone overlying the oil body. Temperatures and concentrations of methane, oxygen, and carbon dioxide in the unsaturated zone were measured throughout the year concurrently with surficial measurements of carbon dioxide efflux. Oxygen concentrations decreased from 21% at the land surface to approximately 0% in the methane oxidation zone (about 3 m depth), while methane concentrations peaked at about 8% immediately above the oil body and decreased to 0% in the methane oxidation zone. Carbon dioxide and methane concentrations above the oil body varied seasonally (~7 to 15% and 2.6 to 7.4%, respectively), suggesting seasonal variations in microbial activity. In the methane oxidation zone, temperatures increased to 4-6 degrees above background and also showed seasonal variations. This talk will discuss the drivers of observed seasonal variations at the site.

Quantifying Energy Yields for Methanogens in Serpentinizing Systems

Presented by Sanjoy Som, Ph.D., Research Scientist, Blue Marble Space Institute of Science, NASA Ames Research Center, USA

Co-Authors: S. Som, M. Alperin, and T. Hoehler

Geological settings dominated by water-to-rock reactions are natural targets for geobiological investigations decoupled from surface processes. The geochemical diversity that characterizes such sites provides abundant energy to support subsurface microbial life. Hydrogen oxidizers are of particular interest because H₂-based metabolisms are widespread and deeply rooted throughout the phylogenetic tree of life, implying they may have emerged extremely early in the evolution, and possibly even the origin of life on Earth and potentially any other rocky body bearing liquid water. Initial work on lithogenic H₂ production has focused on ultramafic serpentinization, as it is occurring on Earth, is known to have occurred on Mars, and is likely occurring on icy satellites such as Europa. Serpentinization is modeled using the “Geochemist’s Workbench” (GWB) whereby ultramafic harzburgite rocks are reacted with oxygen and sulfate depleted seawater. Reaction kinetics are not explicitly considered, but comparable effects of partial reaction are approximated by assuming post-reaction dilution of equilibrated fluids. The output of GWB serves as the input to a single-cell bioenergetic model, which calculates energy yields based on spherically-symmetrical diffusion of substrates to a cell followed by reaction at the diffusion-limited rate. Membrane selectivity for substrate transport is explicitly considered. Methanogenesis is the metabolism of focus.

Microbial Hydrogen Oxidation and Carbon Cycling in the Deep Subsurface

Presented by Rizlan Bernier-Latmani, Ph.D., Associate Professor, École Polytechnique Fédérale de Lausanne (EPFL), Switzerland

Co-Authors: A. Bagnoud, B. Schwyn, O. Leupin, I. de Bruijn, A. Andersson, and R. Bernier-Latmani

Autotrophic growth is a metabolic strategy that is well described but for which there are few studies, particularly in the deep subsurface. The ability of microorganisms to carry out this type of metabolism is of importance due to their independence from sunlight-driven biogeochemical cycling. We performed an in situ experiment in which we amended a 25m long borehole, 300 meters deep in a Clay Rock formation in Western Switzerland with hydrogen that has been running for over a year. The goal of the experiment was to characterize the response of the microbial community to this amendment and to document the succession or parallel metabolisms that ensue. Within a month of the start of the amendment, Fe(II) and sulfide appeared in the porewater, suggesting microbial Fe(III) reduction and sulfate reduction. Community changes were monitored using 16S rRNA

pyrosequencing as well as metagenomic approaches. Binning of the metagenomic data was carried out using tetranucleotide frequency and contig coverage patterns. Approximately fifty clusters were identified and about 19 corresponded to pure and close to complete genomes. Using the bins, the Joint Genome Institute itag sequencing and the geochemical data collected over the course of the experiments, we identified autotrophic sulfate-reducing bacteria, fermenting bacteria and heterotrophic sulfate-reducing bacteria, suggesting light-independent carbon-cycling in the deep biosphere.

Genetic Evidence that *Geobacter Sulfurreducens* Uses Specific Pathways of Electron Transfer for Metals of Different Redox Potential

Presented by Caleb E. Levar, Graduate Assistant and Ph.D. Candidate, Gortner Laboratory of Biochemistry, University of Minnesota, USA

Co-Authors: C.E. Levar, C.H. Chan, M.G. Mehta-Kolte, C.L. Hoffman, B.M. Toner, and D.R. Bond

It remains an open question how *Geobacter sulfurreducens* transfers electrons from the inner membrane to external electron acceptors, and if the over 70 expressed multiheme c-type cytochromes are used for reduction of different electron acceptors. Deletion of *imcH*, coding for a previously unstudied inner membrane c-type cytochrome containing 3 transmembrane helices, a region of NrfH homology, and 7 heme binding motifs, yielded a *G. sulfurreducens* strain unable to reduce soluble Fe(III)-citrate, insoluble Mn(IV)-oxides, or electrodes poised at a potential of +0.24 V vs. SHE. However, this $\Delta imcH$ mutant retained the ability to reduce synthetic ferric oxyhydroxide, suggesting the presence of alternative electron transfer pathways used to reduce specific substrates. We hypothesized that the utilization of these pathways was dependent upon the redox potential of the terminal electron acceptor. To test this, electrodes were poised at lower redox potentials, which was found to be permissive for the respiration and growth of the $\Delta imcH$ mutant. By synthesizing additional Fe(III)-oxides with potentials predicted to be either permissive or non-permissive for the growth of the $\Delta imcH$ mutant, we show that *G. sulfurreducens* alters its respiratory pathway in response to the redox potential of the terminal electron acceptor provided. The ability to express respiratory pathways in response to the redox potential of the minerals encountered is consistent with the organism shifting to conditions of lower energy availability. Identification of these redox tuned respiratory pathways has implications in monitoring metal reduction in subsurface environments and provides a hypothesis for the diversity of cytochromes in *Geobacter*.

Microbial Fuel Cells under Extreme Salinity: Analysis and Optimization of Microbial Community

Presented by Yu Yang, Ph.D., Postdoctoral Researcher, Rice University, USA

Co-Authors: Y. Yang, O. Monzon, and P.J.J. Alvarez

Many industries generate hypersaline wastewaters with high organic strength, which represent a major challenge for pollution control and resource recovery. This study assesses the potential for microbial fuel cells (MFCs) to treat such wastewaters and generate electricity under extreme salinity. A power density of up to 71 mW/m² (318 mW/m³) with a Coulombic efficiency of 42% was obtained with 100 g/L NaCl, and the capability of MFCs to generate electricity in the presence of up to 250 g/L NaCl was demonstrated for the first time. Pyrosequencing analysis demonstrated that the bacterial communities colonizing the anode and dissociating in the broth were very similar, but significantly different from the original bacterial community in sea sediments (Gulf of Mexico). Anode-associated biofilm showed the predominance of a single genus in phylum Firmicutes, *Halanaerobium* (85.7%), commonly detected in late flowback fluids and widely distributed in shale formations and oil

reservoirs, while Proteobacteria and Bacteroidetes (68.6% and 18.3% respectively) were the most abundant in the sediment sample. Surprisingly, archaeal population was absent on the anode or in the broth of MFC; in the sediment sample, 50.3% of the archaeal community belong to Fervidicocaceae spp., and hydrogenotrophic methanogens (21.2% as Methanobacteriales, 14.9% as Methanomicrobiales and 8.0% as Methanococcales) and mixotrophic methanogens (4.6% as Methanosarcinales) account for the other half of archaeal population. Overall, this work encourages further research to assess the feasibility of MFCs to treat hypersaline wastewaters generated by the oil and gas industry.

10:20 AM – 12:00 PM
FRED FARR FORUM

SESSION B4 METHODS: MOLECULAR STUDIES

Moderated by:

- **Prof. Dr. Kirsten Küsel**, Chair of the Aquatic Geomicrobiology Department, Institute of Ecology, Friedrich Schiller University Jena, Germany
- **David W. Metge**, Microbiologist, National Research Program, U.S. Geological Survey, USA



Metagenomic Analysis in Deep-Biosphere Sediments of the El'gygytyn Crater Lake: How Has the Microbial Community Changed over the Last 3.6-Million Years?

Presented by Janine Görsch, Ph.D. Candidate, German Research Centre for Geosciences (GFZ), Helmholtz Zentrum Potsdam, Germany
Co-Authors: J. Görsch, M. Alawi, and D. Wagner

The El'gygytyn Crater Lake (Far East Russian Arctic) was formed by a meteorite impact 3.6 million years ago and is one of the best-preserved, large impact structures on Earth. The sediment layers, which have been formed since the impact, contain information on the earlier climate history, and the evolution of life since the meteorite impact. To investigate this climate archive, the sediments were drilled in 2009 within the scope of the ICDP project Scientific Drilling in El'gygytyn Crater Lake. In initial investigation, we revealed the existence of a diverse archaeal community in up to 470 ka old sediments. Additionally, we found a strong correlation between the biogenic silica and the diversity and abundance of bacteria and archaea. This indirect microbial response can be explained by the improved nutrient supply during an algal bloom within warmer periods. Using up to 3.6 million year old sediment deposits, we separated extracellular DNA (potential ancient community) and intracellular DNA (recent community), carried out fingerprint techniques and quantitative PCR to find and investigate areas of high microbial activity. On this basis, we performed metagenomic analysis for sediments of different depths. A higher bacterial than archaeal abundance was revealed over the entire sediment core. Moreover, both extractable DNA pools are indicating high differences in their population structure. Our results are showing the successful application of our combined methodical approach to obtain the first microbiological dataset of such an old lake ecosystem.

Viromes in Cryptic, Isolated, and Enduring Ecosystems

Presented by Renee J. Smith, Ph.D., Postdoctoral Research Fellow, School of Biological Sciences, Flinders University, Australia

Co-Authors: R.J. Smith, S.S. Tobe, T.C. Jeffries, B. Roudnew, J.R. Seymour, A.J. Fitch, K.L. Simons, P.G. Speck, K. Newton, M.H. Brown, and J.G. Mitchell

Pore water microbial communities change as they move below the surface. The continuing isolation and evolution of a groundwater community may continue for hundreds of thousands of years. There is little known regarding the viral diversity and abundance of deep groundwater communities. Recent work in 1500 year old, 80 m deep, confined-aquifer groundwater showed unexpected viral groups to be abundant. Specifically, genomes of the large animal virus family Circoviridae were diverse and readily identifiable. These viruses have been found in human faeces, but whether they are human pathogens is still unknown. However, they are known to cause disease in most major livestock groups. Geminiviridae, Nanoviridae and Microviridae were also present. These results demonstrate that viruses can spread and endure in groundwater as common members of the microbial community. The extent of the spread and endurance, as well as the extent to which the porous matrices of aquifers act to concentrate viruses is the focus on ongoing work. The results also raise the question of the extent to which relic viruses of some groundwater communities are a record of venerable if not quite ancient surface fauna and flora.

Novel Phylotypes of Nitrite-Dependent Anaerobic Methane-Oxidizing Bacteria in Surface and Subsurface Sediments of the South China Sea

Presented by Jing Chen, Ph.D. Candidate, School of Biological Sciences, University of Hong Kong, China

Co-Authors: J. Chen, Z.-C. Zhou, and J.-D. Gu

Nitrite-dependent anaerobic methane oxidation (n-damo) process is unique in linking the microbial carbon and nitrogen cycles, but the presence of n-damo bacteria in marine ecosystem and the associated environmental factors are still poorly understood. In the present study, detection of n-damo bacteria using 16S rRNA and pmoA genes-based PCR primers was successfully employed to reveal their diversity and distribution in the surface and subsurface sediments of the South China Sea (SCS). The widespread occurrence of n-damo bacteria in both the surface and subsurface sediments with high diversity has been confirmed in this study. The pmoA gene-amplified sequences clustered within three newly erected subclusters, namely SCS-1, SCS-2 and SCS-3, suggesting the unique niche specificity of n-damo bacteria in the marine ecosystem. Results indicated the presence of n-damo bacteria in the west Pacific Ocean with a wide distribution from the continental shelf (E201S) to the deep abyss (E407S and E407B). Community structures of n-damo bacteria in SCS were clearly different from the current known non-marine ones. It is also found that NO_x- and NH₄⁺ affected the community structures and distribution of n-damo bacteria in the SCS sediments differently. Salinity is another important factor identified shaping the n-damo communities in marine environments. The community based on both pmoA gene-amplified sequences, and community richness and diversity based on 16S rRNA gene-amplified sequences correlated with temperature.

Microbial Community Structure of Subsurface Oil Sands Samples

Presented by Christina M. Ridley, Ph.D. Candidate, Petroleum Microbiology Research Group, University of Calgary, Canada

Co-Authors: C.M. Ridley, D. An, and G. Voordouw

The subsurface microbial community of the Alberta oil sands is not widely understood. Although this environment was assumed to be anaerobic, previous analysis has indicated the presence of aerobic bacteria in similar samples. By gaining a better understanding of the microbes residing in this unique

niche, it may be possible to selectively stimulate certain indigenous populations to aid in microbially enhanced oil recovery (MEOR). Microbial processes such as gas, acid or surfactant production, or biodegradation may help lower the viscosity of the bitumen found in the oil sands. Due to the high viscosity associated with this bitumen, extraction from the subsurface is completed using a method called steam-assisted gravity drainage (SAGD). SAGD is extremely energy intensive and it is, therefore, associated with a large carbon footprint. Samples have been collected from two exploratory SAGD wells in the Athabasca oil sands at 75 meters apart. This presents a unique opportunity to study two subsurface microbial environments in close proximity to each other. The community structures data, thus far, demonstrates a high percentage of aerobic bacteria and very few methanogens. Anaerobic and aerobic microcosms have been set up at 60°C and 6°C to study microbial activity at thermophilic and reservoir temperatures, respectively. This will help determine which microbes can be stimulated, and possibly be used for MEOR purposes. By studying subsurface microbial communities of the oil sands, MEOR strategies may be developed to lower viscosity, thus lowering the energy demands and greenhouse gas emissions associated with oil sands operations.

Phylogenetic and Functional Gene Analysis of Microbial Biofilms from Within the Basaltic Crust

Presented by Andrew R. Gross, Ph.D. Candidate, University of Southern California, USA

Co-Authors: A.R. Gross, J.B. Sylvan, K. Becker, and K.J. Edwards

Subsurface fluids flowing through fractures and pore spaces within ocean crust provide an expansive and global environment for microbial growth. These environments have the potential to exert a significant influence on global chemical fluxes. They also constitute the best modern analogue for possible early earth or terrestrial ecosystems. Previous studies have demonstrated that subsurface communities can carry out processes such as carbon fixation, methanotrophy, and sulfur oxidation which impact the composition of the crust and climate over geologic time scales. This study examines the metabolic potential of communities collected from a borehole penetrating 500 m into 8 Ma crust below North Pond in the northern Atlantic ocean, on the western flank of the Mid Atlantic ridge. In order to identify possible metabolic functions employed by biofilm communities within basalt, DNA was extracted from a cable incubated in ODP Hole 395A, which had been sealed from the overlying water column for 14 years. Phylogeny was described using full length 16S rRNA clone libraries from three depths within the borehole. Ribulose Bisphosphate Carboxylase genes were amplified and a metagenomic library was produced from the shallowest depth. The 16S rRNA clone libraries were comprised primarily of uncultured Alphaproteobacteria and Bacteroidetes which were closely related to uncultured organisms extracted from the surfaces of sea-floor exposed basalts in previous studies. Form I and form II Ribulose Bisphosphate Carboxylase genes were detected by PCR. These genes were also detected within the metagenome, along with sulfur oxidation genes *soxB* and *dsrAB*. This study provides further evidence that subsurface communities -- particularly within the crust -- are comprised of specialized microbes suited to sustaining complex ecologies.

10:20 AM – 12:00 PM
NAUTILUS ROOM

**SESSION C4 ECOLOGY: SUBSURFACE ECOSYSTEMS (TERRESTRIAL
HARDROCK AND GROUNDWATER)**



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

Analysis of Microbial Ecology and Metabolism in Marcellus Shale Produced Water Eighteen Months Post Completion

Presented by Daniel Lipus, Ph.D. Candidate, University of Pittsburgh, USA

Co-Authors: D. Lipus, K. Bibby, and A. Vikram

Hydrocarbon reservoirs following hydraulic fracturing represent an extreme environment for microorganisms characterized by unique geochemical conditions including high salt concentrations. Despite the extreme conditions and the attempts of well operators to minimize microbial contamination through the extensive application of biocides, several studies have suggested that microbial diversity in the wastewater from hydraulic fracturing is considerable, suggesting the potential for microbial activity in the reservoir. The presence of microbes in hydraulic fracturing wells is an issue of concern for the fracking industry and the general public, leading to biofilm formation, corrosion issues and clogging of shale fractures. While several projects have characterized microbial diversity in flowback water from newly completed wells, very little is known about the long term presence of microbial communities in hydraulic fracturing wells. In this study we use 16S rRNA sequencing and shotgun metagenomics to analyze the microbial ecology of produced water from an 18 month old well. Analysis of metagenomic data allowed identification of microbial taxonomy, common gene categories and metabolic pathways, suggesting methane metabolism, sulfur metabolism and osmotic stress response to play an important role in the hydraulic fracturing downhole environment. This study is a first attempt at understanding and reconstructing the possible microbial ecology in hydraulic fracturing produced water, with the long term goal to minimize the excess use of chemicals and biocides in hydraulic fracturing operations and encourage produced water recycling.

Microbes: A Tracer of Groundwater in Volcanic Mountains

Presented by Prof. Dr. Kenji Kato, Department of Geosciences, Graduate School of Science, Shizuoka University, Japan

Co-Authors: T. Furuta, K. Nagaosa, and K. Kato

Microbiological study of spring water in volcanic mountain is still limited. We conducted DNA analysis of prokaryotes in spring water flushed out from the boundaries between larva with nearly 200m thick and ancient geological setting in Mt. Fuji, a tallest dormant volcanic mountain located in the central part of main island in Japan. We retrieved over 200 bacterial clones which belonged to 8

bacterial phyla and two archaeal phyla though the absolute abundance was as low as 10×10^3 cells /ml in the examined 16 springs, which covered all phyla previously reported from terrestrial subsurface environments. In addition, we retrieved several anaerobic and thermophilic clones from various sites examined. This suggests possible route of groundwater as temperature of spring water ranges from 10 to 15°C being almost saturated with dissolved oxygen. Similar findings were obtained from the study in the foot of Mt. Aso, an active volcano located in Kyushu Island. Microbial characteristics were similar to those of Mt. Fuji. Findings of microbial DNA particularly thermophilic clones and sulfur reducing clones support the hydrological understanding on the route of groundwater in Mt. Aso (Kagabu et al. 2011) in particular about the possibility of mixing of the groundwater which is consisted of two sources of groundwater; one is originated from near the hydrothermal chamber and another one is originated from relatively shallow aquifer which is less influenced by volcanic activity. Microbes could ascertain the route of groundwater particularly in complex hydrological system of volcanic environment as a possible indicator.

Metagenomic Analysis of Microbial Communities of the Underground Thermal Waters of Western Siberia

Presented by Vitaly V. Kadnikov, Ph.D., Centre for Bioengineering, Russian Academy of Sciences (RAS), Russia

Co-Authors: V.V. Kadnikov, A.V. Beletsky, A.V. Mardanov, O.V. Karnachuk, and N.V. Ravin

The deep subsurface ecosystems are characterized by anaerobic conditions, extreme pressure and high temperature. We analyzed extremophilic microbial communities of the underground thermal waters springing out of two different oil-exploration boreholes in Tomsk region, Western Siberia, Russia. The outcoming water at the first site, Parabel, is anoxic, neutral-to-slightly alkaline with a temperature of 46-51°C. Two approaches were used to characterize microbial communities: identification of microorganisms based on amplification and pyrosequencing of 16S rRNA genes fragments, and sequencing of the total community DNA. The data obtained for Parabel sample indicated the dominance of bacteria in the clone library, while hydrogenotrophic methanogenic archaea of the genus *Methanothermobacter* accounted for the rest. The most abundant lineage, Firmicutes, was represented by bacteria of the genera *Desulfoviregula* and *Desulfotomaculum*, as well as by organisms related to *Thermacetogenium* and uncultured groups. The results of metagenomic analysis suggest the following ecological relationships. Sulfate-reducing *Desulfoviregula* and *Desulfotomaculum* can anaerobically oxidize low-molecular organic compounds and intermediate metabolites (lactate, butyrate, propionate) to acetate, and then to carbon dioxide. Bacteria of genus *Thermacetogenium* and other minor groups can hydrolyze organic polymeric compounds. The second sample, isolated at Bely Yar site (40-44°, pH 8.0), as revealed by sequencing of the total community DNA, contained different community comprising representative of *Chloroflexi*, *Nitrospira*, *Deltaproteobacteria* and Firmicutes. Particularly, contigs highly similar to *Desulforudis audaxviator*, previously found in a single-species ecosystem of South Africa gold mine, were identified.

Biodiversity of Bacteria and Archaea in Nitrate- and Radionuclides-Contaminated Groundwater

Presented by Tamara N. Nazina, Ph.D., Head of Laboratory, Winogradsky Institute of Microbiology, Russian Academy of Sciences, Russia

Co-Authors: T.N. Nazina, T.L. Babich, N.K. Kostryukova, D.Sh. Sokolova, R.R. Abdullin, E.V. Zakharova, S.N. Kalmykov, I.G. Tananaev, A.B. Poltarus, K. Nagaosa, and K. Kato

Distribution of microorganisms and nanoparticles in groundwater in the area of the “industrial reservoir” – lake Karachai (“PO “Mayak”) was investigated by multidisciplinary approaches. The groundwater had been contaminating with radionuclides, acetate- and nitrate-ions. Cultivable aerobic organotrophic, and anaerobic fermentative, Fe(III)-reducing and denitrifying bacteria were obtained from the groundwater. Microbes responsible for sulfate-reduction and methanogenesis were not detected. Among the cloned sequences of 16S rRNA genes of Bacteria, sequences of representatives of the genera Polynucleobacter, Pseudomonas, Methylophilum, Candidatus "Pelagibacter ubique", and of OD1 and OP11 groups were retrieved. For Archaea, sequencing of 16S rRNA genes showed that aerobic ammonium-oxidizing Thaumarchaeota are dominating. In total 40 strains of bacteria of various physiological groups were isolated from groundwater. Pure cultures of ultramicrobacteria of the genera Chryseobacterium, Microbacterium and Salinibacterium were isolated also. Quantitative PCR targeting nir S/K genes suggested denitrification occurred in the studied groundwater, which might change the in situ environment by lowering redox potential. This may alter the precipitation behavior of radionuclides. High activity in adsorption of microbes was found for ²⁴¹Am(III), ²³⁷Np(V) and ²³³U(VI) through the laboratory experiment using isolates from the groundwater, while adsorption by microbes was low for ⁹⁰Sr. Colloidal particles in groundwater near the lake were represented by Fe-oxides, while particles in groundwater obtained far from the lake were represented by clay. Actinides were mainly associated with ferrihydrite. Those findings provide a better understanding of the function of microorganisms and nanoparticles in migration of radionuclides in contaminated groundwater. This work was supported by RFBR (13-04-92105) and JSPS (13037711-000001).

Natural and Manmade Windows into Deep Microbial Ecosystems of the Death Valley Regional Flow System, USA

Presented by Duane P. Moser, Ph.D., Associate Research Professor, Division of Earth and Ecosystems Sciences, Desert Research Institute, USA

Co-Authors: D.P. Moser, S.D. Hamilton-Brehm, G. Zhang, J.C. Fisher, J.C. Bruckner, B. Kruger, J. Thomas, A. Wheatley, L. Stewart, and T.C. Onstott

The Death Valley Regional Flow System (DVRFS) is a highly-fractured, mostly- carbonate rock aquifer covering thousands of square km and extending from inferred recharge zones associated with Central Nevada volcanic uplands and dolomite mountain blocks to large-discharge springs in Amargosa- and Death Valleys (Nevada/California). Opportunities for sampling deep microbial ecosystems include monitoring wells on and near the Nevada National Security Site (NNSS) and exploration/production wells and fracture-controlled springs in and near Death Valley. To date, fluids from about sixty-five sites have been sampled and analyzed for physical and chemical parameters and microbial community structure using 454 pyrotag, Illumina, and PCR-amplified 16s rRNA gene libraries. Results revealed dissolved oxygen to unexpectedly great depths (up to 1300 m) along the full ~140-km flow path. Oxygenated zones contain mostly proteobacteria; whereas, archaeal populations are dominated by Thaumarchaeota, especially relatives of Nitrososphaera spp., Nitrosopumilus spp., and SAGMA-A. Archaeal populations correspond strongly between adjacent wells and springs, suggesting a shared origin of produced fluids and utility of microbial populations for tracking the connectivity of groundwaters. Several suboxic/anoxic wells appear to sample a deeper, hotter end member, hosting distinct microbial communities dominated by Nitrospirae, predicted sulfate-reducing Firmicutes, and methanogens (Eurychaeota). Sequences from “dark matter” phyla (e.g. OP3) and nearly identical to Candidatus Desulfurudis audaviator are common (up to 6 – 10% of bacteria) at some sites. A culture collection, obtained from anoxic and suboxic wells,

features sulfate-reducing and peptide-utilizing Firmicutes related to *Desulfotomaculum*, *Thermotalea*, or *Pelotomaculum* spp. and *C. D. audaxviator* (up to 91% identity).

2:00 PM – 3:20 PM
MERRILL HALL

SESSION A5 CHEMISTRY: BIOGEOCHEMISTRY OF THE SUBSURFACE

Moderated by:

- **Alexandria B. Boehm, Ph.D.**, Associate Professor, Stanford University, USA
- **Derick G. Brown, Ph.D.**, Associate Professor, Department of Civil and Environmental Engineering, Lehigh University, USA



Effects of CO₂ on Subsurface Microbial Communities for a Saline Storage Site, EOR Site, and a Freshwater Aquifer

Presented by Djuna Gulliver, Ph.D., Environmental Engineer, National Energy Technology Laboratory-U.S. Department of Energy (NETL-DOE), Carnegie Mellon University, USA

Co-Authors: D. Gulliver, K. Gregory, and G. Lowry

Effective geological carbon sequestration will depend on biogeochemical processes that occur in subsurface reservoirs after CO₂ injection. A critical need exists to understand the evolution of CO₂ exposed microbial communities that influence these biogeochemical processes within these reservoirs. The evolution of microbial ecology along a CO₂ concentration gradient was investigated using fluid-slurry samples obtained from three subsurface reservoirs: a deep saline aquifer (1220 m depth, 14 MPa, 40 °C, Wellington, KS), an EOR site (490 m depth, 3.4 MPa, 40 °C, Zabata, TX), and a overlying fresh water aquifer (55 m depth, 0.5 MPa, 22 °C, Escatawpa, MS). Batch vessel experiments were conducted with samples from each site, varying pCO₂ of 0% to 100% under reservoir temperature and pressure for 56 days. The microbial communities of each batch reactor were analyzed with 16S rRNA clone libraries and qPCR. In both the saline aquifer and the EOR site, DNA concentration and diversity decreased with increasing CO₂ exposure. In contrast, DNA concentration and diversity of the freshwater aquifer did not display a clear trend with CO₂ exposure. Findings provide insight into a dynamic biogeochemical system that will alter with CO₂ exposure. Adapted microbial populations will eventually give rise to the community that will impact vital bioprocesses and may lead to pore plugging, metal mobility, and sour gas production. Knowledge of the surviving microbial populations lead to better strategies for ensuring the security of carbon storage.

Carbon Cycling in the Basaltic Oceanic Crust Deep Biosphere

Presented by Beth Orcutt, Ph.D., Senior Research Scientist, Bigelow Laboratory for Ocean Sciences, USA

Author: B. Orcutt

One of the major discoveries of the scientific ocean drilling program over the past three decades is the confirmation of active and abundant life "buried alive" in the marine "deep biosphere," the largest potential habitat for life on Earth. This biosphere consists of deeply buried sediments and igneous oceanic crust; yet, the existence and extent of a deep biosphere hosted in igneous oceanic crust — the hard rock that resides beneath sediment or exposed near mid ocean ridge spreading centers and on

seamounts— is considerably less understood than in the sedimentary realm. Only a handful of sites have been sampled with results that document which microbes are present and their general abundance. Microbial activity and the role of microbial metabolic reactions in altering basaltic crust have yet to be addressed. Here, recent studies that aim to understand the function of microbial groups in the basaltic deep biosphere, focusing on carbon cycling, will be discussed. Specifically, evidence for carbon fixation potential by basalt hosted microbial communities will be presented, along with recent single cell genomic data that reveals the function for carbon cycling in some basaltic systems. In the context of other datasets, the impact of microbial activity in stimulating or inhibiting carbon sources and sinks in the igneous crustal deep biosphere will be discussed.

Methanogenic Oil Degradation in the Dagang (China) Oil Field

Presented by N ria Jim nez, Ph.D., Postdoctoral Researcher, Federal Institute for Geosciences and Natural Resources (BGR), Germany

Co-Authors: N. Jim nez, M. Cai, N. Straaten, J. Yao, H.R. Richnow, and M. Kr ger

Anaerobic biodegradation, particularly under methanogenic conditions, is one of the main in situ oil transformation processes in subsurface oil reservoirs. Methane may enhance flow characteristics of oil but it can also be used as energy source. The present study aimed to provide reliable information on in the in situ biotransformation of oil under methanogenic conditions in a thermophilic water-flooded oil reservoir in Dagang (China). Geochemical and isotopic data from reservoir fluids were consistent with in situ biogenic methane production linked to aliphatic and aromatic hydrocarbon degradation: GC-MS profiles of petroleum samples confirmed that Dagang oil is mostly highly weathered. Carbon and hydrogen isotopic signatures of methane were consistent with previously reported values for methane formation during hydrocarbon degradation. Furthermore, laboratory degradation experiments carried out under methanogenic conditions, revealed that autochthonous microbiota are capable of significantly degrade oil within several months, with biodegradation patterns resembling those observed in situ, and of producing heavy methane from ¹³C-labelled n-hexadecane or 2-methylnaphthalene. These results suggest that in situ methanogenesis may occur from the aliphatic and polyaromatic fractions of Dagang reservoir fluids. In addition, methane-producing Archaea (hydrogenotrophic, methylotrophic and acetoclastic) and hydrocarbon-degrading Bacteria were abundant in produced oil-water samples. Syntrophic Bacteria and methanogenic Archaea were predominant in oil and 2-methylnaphthalene-degrading enrichment cultures, as well. In summary, the studied areas of the Dagang oilfield may have a significant potential for the in situ conversion of oil into methane as a possible way to increase total hydrocarbon recovery.

Carbonate-Hosted Microbial Community Ecology and Dynamics at the Hydrate Ridge Methane Seep, OR, USA

Presented by David Case, Ph.D. Candidate, California Institute of Technology, USA

Co-Authors: D. Case, J. Steele, B. Grupe, L. Levin, and V.J. Orphan

Microbial activity at methane seeps drives precipitation of authigenic carbonates, which in turn act as host habitats for microbial communities including, but not limited to, anaerobic methane oxidizing archaea and sulfate reducing bacteria. However, the mechanisms and dynamics by which microbial communities colonize and inhabit seep carbonates – some extending many meters below the seabed – are not well understood. Two novel 13-month in situ experiments were performed in order to explore carbonate-associated microbial community dynamics at Hydrate Ridge, OR, USA. These experiments for the first time employ deep 16S rRNA gene sequencing to understand seep carbonate-associated microbial community ecology. First, six seafloor carbonates were transplanted from active seeps to

low-activity sites (n=4) and vice versa (n=2). Results demonstrate that characteristic active-seep communities require ≤ 13 months to populate transplanted carbonates, but that characteristic low-activity-seep communities require longer time scales to populate newly transplanted carbonates from active sites. Second, lab sterilized calcite and dolomite samples (n=20) were placed on the seafloor in order to test the mineralogy- and activity-dependent microbial colonization potential in the presence of new habitat substrate. The colonizing microbial community was significantly affected by seep activity, and ANME-1 demonstrated a strong mineralogy preference. Molecular signatures recorded in authigenic carbonates may reflect the time-integrated biological history of the host methane seep, or merely a temporary community established during seep quiescence. These experiments begin to characterize the relationship between seep activity, molecular biomarkers, mineralogy, and biogeography, which will aid in future interpretation of carbon cycling within modern and ancient seep carbonates.

2:00 PM – 3:20 PM
FRED FARR FORUM

SESSION B5 METHODS: MOLECULAR STUDIES

Moderated by:

- **Prof. Dr. Kirsten Küsel**, Chair of the Aquatic Geomicrobiology Department, Institute of Ecology, Friedrich Schiller University Jena, Germany
- **David W. Metge**, Microbiologist, National Research Program, U.S. Geological Survey, USA



Microbiology of the Deep Fracture Subsurface in the Canadian Shield

Presented by Danielle Beaton, Biochemist, Atomic Energy of Canada Limited, Canada

Co-Authors: B. Stevenon, B. Stamps, H. Nunn, K. Sharp, S. Stroes-Gascoyne, I. Gurban, M. Stuart, and D. Beaton

A geoscientific characterisation of the Chalk River Laboratories site (CRL, Canada) was designed to examine its suitability to host a deep geologic nuclear waste management facility. Part of this study included analyses of the groundwater microbial community structure in relation to the hydrogeochemistry of the groundwater. The CRL site is underlain by Precambrian gneissic rocks within the structural framework of the Ottawa-Bonnechere graben and is situated near the southern limit of Quaternary Wisconsinian glaciation. Boreholes have investigated geological features and fracture conditions across a large region of the site framed between two major lineaments. Groundwater samples from intervals from four packed-off boreholes and one open borehole were analysed. Bacterial abundances and diversity were high in all intervals. Major bacterial lineages within the packed-off boreholes included Proteobacteria, Bacteroidetes, and Firmicutes, whereas those within the open borehole were distinct and dominated by Nitrospira, Acidobacteria, and Methylocystaceae. The relative abundances and diversity of archaea were low in all intervals, being comprised mainly of Methanobacteriales. Multivariate Mixing and Mass Balance (M3) modeling of three source waters (glacial melt water, saline/marine water, precipitation) suggest glacial melt and precipitation signatures dominated the groundwater compositions to depths of 500 m. Saline/marine water signatures were distinct in the shallow open borehole transecting a fault and in the groundwaters below 500 m, these deep intervals also had higher abundances of archaea. The community structures,

therefore, reflected these hydrogeochemical differences, which were created by mixing of different source waters and which, in turn, are ultimately controlled by the hydrology and fracture characteristics of the site.

Transient and In Situ Activity and Population Differences in Groundwater Aquifers

Presented by Murray Close, Principal Scientist, Institute of Environmental Science and Research, Ltd. (ESR), New Zealand

Co-Authors: L. Weaver, A. Hickson, J. Webber, P. Abraham, B. Robson, W. Williamson, E. McGill, and M. Close

The study presented compared the differences in activity and populations present in transient groundwater, in-situ sediments and gravel biofilm bags placed in the aquifer. To fully understand the functioning of an aquifer all components need to be studied (transient and in-situ populations). Due to the inherent difficulties in sampling aquifer sediments an alternative approach was investigated. Comparisons were made between the transient groundwater, gravel bags and the sediment itself. The study site (alluvial gravel aquifer in Canterbury, New Zealand) was designed to allow collection of sediment samples, collection of groundwater and placement of gravel biofilm bags at the water table. Gravel bags were left for 4 months before collection when groundwater and sediment samples were also taken. All samples were immediately returned to the laboratory and analysed for biomass (protein and heterotrophic plate counts), enzyme activity (general catabolism, carbon, nitrogen and phosphorous acquisition). DNA was extracted from all samples and sent for metagenomic sequencing using the Illumina platform. Our results show that when studying microbial activity within groundwater environments, both the transient and in-situ phases should be examined. Comparing the biofilm gravel bags with sediment samples gave comparable results and so demonstrates the potential for further studies in this difficult environment using the biofilm bag system. Groundwater samples showed lower enzyme activity and biomass than either in-situ sample types. Differences were seen between nitrogen and phosphorous activity between the gravel bags and sediment. Gravel bags placed in-situ had greater activity than naturally occurring sediment samples.

Lignin-Degrading Microbes of the Eastern Mediterranean Sediment and Seawater

Presented by Hannah L. Woo, Ph.D. Candidate, University of Tennessee, USA

Co-Authors: H.L. Woo, S.M. Techtmann, J.L. Fortney, D.C. Joyner, and T.C. Hazen

With the rising commercialization of cellulosic biofuels, the plant's lignin component is becoming a major waste stream. Microbial enzymes could convert the waste into higher-value products, but few microbes are known to modify lignin. Deep-ocean microbes are known to degrade recalcitrant aromatics in crude oil but their capacity to degrade similar organics like lignin has not been thoroughly studied. Therefore, we investigated both marine surface water and sediment communities for lignin-degrading potential. Furthermore, we hypothesized the sediment microbes would have more efficient lignin-degrading enzymes because these microbes feed on more recalcitrant carbon that sink and accumulate at the ocean floor. To obtain a simplified lignolytic microbial community, oxic Eastern Mediterranean surface water and sediment slurry were incubated separately in bottles with insoluble lignin for 2 weeks. Microcosms were monitored for carbon dioxide production. Both sample types had 10-fold higher respiration rates than un-amended controls within 1 day. The lignolytic enzyme activity was tested using the model lignin L-3,4-dihydroxyphenylalanine. The sediment microcosms had significantly higher activity than the seawater. Microbial community structure, as determined by 16S rRNA gene amplicon sequencing, showed that the higher enzymatic capabilities of the sediment may be due to the greater abundance of Acidobacteria, Crenarcheota, and Euryarchaeota.

These taxa represented 1-4% of the microbial community within the sediment enriched on lignin but less than 0.03% of the surface water on lignin. Our findings show that the sediment is home to microbes that respire well on lignin, which could provide novel enzymatic mechanisms that transform lignin waste efficiently.

Microbial Community Shifts associated with the Microbiologically Influenced Corrosion (MIC) of Carbon Steel

Presented by Giovanni Pilloni, Ph.D., Senior Researcher, ExxonMobile Research and Engineering, USA

Co-Authors: G. Pilloni, P. Mishra, M. Ruhmel, X. Chen, F. Cao, R. Smith, and D. Enning

Microbiologically Influenced Corrosion (MIC) can be involved in the deterioration of carbon steel and other structural materials in a variety of industries. The economic impacts of this phenomenon are highly relevant. In particular, within the oxygen-free engineered environments that often characterize oil and gas operations, MIC can be a dominant process in the degradation of technical equipment. Despite the broad economic implications of MIC and its long tradition as a field of academic and applied research, the mechanisms by which microbial activity leads to corrosion are still insufficiently understood. There is a particular need to better understand the interplay between complex and diverse microbial communities with an involvement in microbial corrosion. The recent emergence of comprehensive and cost-effective DNA sequencing technology offers an opportunity to elucidate multi-species microbial corrosion phenomena in this regard. We used a microbial consortium from marine soil and enriched it under a large variety of different physicochemical parameters in the presence of carbon steel. Microbially mediated changes in water chemistry were recorded under the defined test conditions and interpreted in the light of observed microbial community changes in these enrichments. Microbial community analyses revealed a dramatic drop in diversity from the natural sediment community to systems dominated by well-known deltaproteobacterial sulfate reducers along with clostridial fermenters. This was particularly obvious in test series at ambient temperature. Surprisingly, ion chromatography data indicated acetate production from CO₂ under these conditions, highlighting a fascinating and largely overlooked aspect of MIC, i.e. the role of microbial syntrophies in metal degradation.

2:00 PM – 3:20 PM
NAUTILUS ROOM

SESSION C5 CONTAMINANTS: LANDFILL AND MINE TAILINGS

Moderated by **Bradley S. Stevenson, Ph.D.**, Associate Professor, Department of Microbiology and Plant Biology, University of Oklahoma, USA



Mineral Composition as Structuring Factor of Bacterial Communities in Landfill Leachate Plumes

Presented by Wilfred F.M. Röling, Ph.D., Associate Professor of Geomicrobiology, Molecular Cell Physiology Research Group, VU University Amsterdam, The Netherlands

Co-Authors: W.F.M. Röling, E. Zaura, and S. Direito

Large heterogeneity in sediment composition and microbial community structure over short distances is observed in subsurface landfill leachate plumes. We hypothesized that the type of mineral

determines which microorganisms can grow on it, and that heterogeneity in mineral composition leads to heterogeneity in microbial community structure. Therefore, we performed an in situ incubation experiment in an iron-reducing aquifer affected by landfill leachate (Banisveld, the Netherlands). Thirteen different minerals (iron oxides, clays, feldspars, quartz, apatite, and jarosite) were individually placed in bacteria-permeable membrane pockets. Triplicates of each mineral were randomly positioned in a perforated polypropylene holder, which was inserted below groundwater level. After 9 months of incubation, sequencing of bacterial 16S rRNA gene amplicons consistently revealed that each mineral harboured an unique bacterial community. Surprisingly, species compositions were comparable, while community structures (relative abundances of individual taxa) varied: most dominant taxa (taxa contributing >1% of sequences) occurred on all minerals while a few showed a mineral (class)-specific occurrence. Especially the availability of ferric iron in minerals had a large effect on community structure, with a single Geobacteraceae taxon becoming dominant on ferric iron-rich minerals. We also observed differences between minerals in the occurrence of taxa which are not known to be iron reducers. The results indicate that mineral composition is an important structuring factor of microbial communities. Differences in soil mineral composition will contribute to differences in microbial community structure, which could influence the degradation of pollutants in landfill leachate. This heterogeneity should be taken into account in sampling design.

Landfills Select for Distinct, Diverse, and Novel Microbial Communities

Presented by Bradley S. Stevenson, Ph.D., Associate Professor, Department of Microbiology and Plant Biology, University of Oklahoma, USA

Co-Authors: B.S. Stevenson, B.W. Stamps, C.N. Lyles, J.M. Suflita, J.R. Masoner, D.W. Kolpin, and I.M. Cozzarelli

Landfills are a ubiquitous final repository for heterogeneous mixtures of residential, commercial and industrial waste. Microorganisms decompose this solid waste in a physically and chemically heterogeneous environment that quickly becomes anaerobic and limited for electron acceptors. Decomposition can have serious environmental impacts due to the solubilization of a wide array of non-volatile dissolved organic carbon (NVDOC) compounds, many of which are contaminants of emerging concern, and the production of landfill gas (mainly CO₂, H₂ and CH₄). The environmental conditions within landfills are unique, diverse and dynamic. In order to investigate the influence of this selective landscape on microbial life, microbial assemblages found in fresh leachate were characterized from 19 non-hazardous landfills across 16 U.S. states. These landfills represented a range of size, age of waste, waste composition and management strategy, and were located in multiple geographic regions and climates. Leachate chemistry was characterized as an indirect measure of microbial metabolic activity. Microbial biomass was collected in triplicate, genomic DNA was extracted, 16S rRNA gene fragments from bacteria and archaea were amplified and sequenced. A “core” landfill microbiome was evident among all sites and distinct from other environments, with members of the Epsilonproteobacteria (Arcobacter), Gammaproteobacteria (Pseudomonas), and the candidate division OP3 being most abundant. Variation in the composition of these microbiomes was correlated to the concentration of NVDOC and heavy metals. This first comprehensive molecular survey of landfill microbiology has identified a distinct, unique and novel core microbiome.

Inferences from Microbes under a Mine

Presented by John R. Spear, Ph.D., Professor, Department of Civil and Environmental Engineering, Colorado School of Mines, USA

Author: J.R. Spear

Mines and caves present approachable opportunities to explore and better understand the subsurface. A thorough geobiological investigation typically characterizes the geology, geochemistry and microbiology of a given system. From the analysis of these different kinds of datasets, much can be revealed about the ecosystem function of Earth's subsurface. For example, in a study of a geothermal mine adit, we have shown that ammonia oxidizing archaea are actively engaged in nitrogen cycling while building a soda straw speleothem that is 'their' home at the same time. Herein, I follow up on a study we conducted on the microbial communities found in a rock core taken beneath the Henderson Mine, a deep, hardrock molybdenum mine outside of Empire, Colorado. We found novel candidate phyla that have since been found in other unique locations that could indicate how microbial community type and composition, may serve as a barometer of the activities associated with a mine, or the activities of natural processes themselves. Lessons learned may better serve how we approach, work with, interpret and diagnose the ecosystem's of the subsurface—from exploitation to protection. Subsurface 'fracing' for oil and gas production, CO₂ sequestration and cave management are but a few examples. To better conduct any of these activities next generation geology, geochemical and microbiological tools must be applied.

From Surface to Subsurface: Microbiological Dynamics of the Development of Massive Acid Mine Drainage-Derived Fe(III) (Hydr)Oxide Deposits

Presented by John M. Senko, Ph.D., Assistant Professor, Department of Geosciences, The University of Akron, USA

Co-Authors: J.M. Senko, J.S. Brantner, Z.J. Haake, J.E. Burwick, C. Menge, and S.T. Hotchkiss

We studied microbiological processes associated with deposits composed almost exclusively of Fe(III) (hydr)oxides that developed upon emergence of Fe(II)-rich coalmine-derived acid mine drainage (AMD) at the terrestrial surface. The formation of these deposits (referred to as an "iron mound") was initiated approximately 20 years ago, when AMD began flowing over previously pristine soil. Since then, rapid aerobic Fe(II) oxidizing activities have led to the development of a 10-12 cm thick iron mound, indicating an iron mound "growth" rate of approximately 0.5 cm/yr. To evaluate the initial stages of iron mound formation, we incubated AMD-unimpacted soil with AMD. Exposure of soil to AMD led to increasingly rapid Fe(II) biooxidation, and increased abundances of Fe(II) oxidizing bacteria (FeOB). Nucleic acid-based profiling of microbial communities associated with these incubations revealed the development of similarities to the communities in mature iron mound sediments, notably an increased abundance of phylotypes attributable to acidophilic Fe(II) oxidizing and Fe(III) reducing Xanthomonadales. To evaluate how iron mound-associated microbial communities adapt to continuous burial within the biogenic Fe(III) (hydr)oxide matrix, we conducted depth-dependent evaluations of microbial communities using culture-dependent and nucleic acid-based approaches. Fe(II) oxidation was observed at O₂-depleted depths within the iron mound, abundances of culturable FeOB remained uniform throughout iron mound sediments, and Fe-metabolizing Xanthomonadales phylotypes were the most abundant constituents of sequence libraries regardless of depth. These results suggest that soil-associated microbial communities rapidly adapt to intrusion of AMD and develop robust Fe(II) oxidizing activities that may be maintained at O₂-depleted depths within iron mound sediments.

3:40 PM – 5:20 PM

MERRILL HALL

SESSION A6 CHEMISTRY: BIOGEOCHEMISTRY OF THE SUBSURFACE

- **Alexandria B. Boehm, Ph.D.**, Associate Professor, Stanford University, USA
- **Derick G. Brown, Ph.D.**, Associate Professor, Department of Civil and Environmental Engineering, Lehigh University, USA



Coupling Metagenomics and Geochemistry to Unveil Microbial Diversity and Functions in Hardrock Aquifers

Presented by Sarah Ben Maamar, Ph.D Candidate, Observatoire des Sciences de l'Univers de Rennes, Université de Rennes 1, France

Co-Authors: S. Ben Maamar, L. Aquilina, A. Quaiser, H. Pauwels, V. Vergnaud-Ayraud, T. Labasque, and A. Dufresne

New high-throughput sequencing offers large prospects for enhancing our understanding of the microbial biodiversity patterns in many habitats. Still, some environments such as aquifers remain poorly explored while some aquifers have been recognized as hotspots of biodiversity. Hard-rock aquifers are usually characterized by a heterogeneous and compartmented environment. They are indeed structured into two layers: a superficial weathered layer ensuring a water storage function, and a deep underlying fissured/fractured layer ensuring a water transmission function. Strong gradients of groundwater ages, hydrochemical concentrations and water circulations have been evidenced indicating a low connectivity between these two compartments. So far, no studies have considered the compartmentalization effect on groundwaters microbial diversity and processes in hard-rock aquifers. Neither has been specified in time the functional response of microbial communities to water renewal during recharge. Using mass sequencing of 16S rRNA amplicons, we analyzed patterns of microbial diversity in three hard rocks aquifers at a regional scale (Brittany, France). We also examined the recharge incidence on the microbial communities structure and functioning dynamics over several months in hard-rock aquifers with very ancient waters (> 10 000 years) through metagenomic approaches. Our results enhance the current knowledge of phylogenetic and metabolic diversity in hard-rock aquifers. They highlight the effect of groundwaters residence time and hydrological cycle as major drivers of microbial community structure and functions. This work provides new results which help untangling the complex interplay between microbial communities and the hydrogeochemical parameters that control the functioning of aquifer ecosystems.

Large Fractions of Bacteria in Deep Pristine Limestone Aquifers Are Involved in CO₂-Fixation Coupled to the Oxidation of Nitrogen and Sulfur Compounds

Presented by Prof. Dr. Kirsten Küsel, Chair of the Aquatic Geomicrobiology Department, Institute of Ecology, Friedrich Schiller University Jena, Germany

Co-Authors: K. Küsel, M. Herrmann, A. Rusznyak, C. Lazar, D.M. Akob, S. Optiz, P. Lange, and K.-U. Totsche

Despite growing evidence of an important role of lithoautotrophy in aquifer carbon flow, the microbial metabolisms linked to CO₂-fixation and their effects on aquifer biogeochemistry are still

poorly understood. We investigated groundwater samples from two superimposed limestone aquifers in the Hainich-Dün region (Thuringia, Germany) with sampling depth ranging from 12 to 88 m. The aims of this study were (i) to assess the quantitative importance of autotrophic microbial groups using gene markers for the Calvin-Benson-Bassham cycle (cbbM, cbbL), and the reverse tricarboxylic acid (rTCA) cycle (aclB), and (ii) to identify the relevance of these groups within the total microbial community by combined analysis of 16S rRNA, cbbL, cbbM, and aclB genes and transcripts. Quantitative PCR targeting RubisCO-encoding genes indicated that up to 17 % of the microbial community had the potential to fix CO₂ via the Calvin cycle. In the oxygen-rich lower aquifer, 16S rRNA reads related to nitrifying groups such as Nitrosomonas and Nitrospira accounted for up to 20 % of the total 16S rRNA reads. A suggested strong link between autotrophy and nitrification was confirmed by cbbL- and aclB-based analysis and measurements of nitrification activity. Sequence reads related to Sulfuricella denitrificans accounted for 5.6 % and 78 % of the 16S rRNA and cbbM reads, respectively, suggesting that CO₂-fixation was also driven by the oxidation of reduced sulfur compounds. Microbial communities in the oxygen-deficient upper aquifer appeared to be more influenced by surface soils as suggested by a dominance of soil-related heterotrophic genera including Pedobacter, Albidiferax, and Polaromonas.

Reactive Transport Model of Growth and Methane Production by High-Temperature Methanogens in Hydrothermal Regions of the Seafloor

Presented by James F. Holden, Ph.D., Associate Professor, Department of Microbiology, University of Massachusetts Amherst, USA

Co-Authors: J.F. Holden, L.C. Stewart, C.K. Algar, B.D. Topçuoğlu, C.S. Fortunato, B.I. Larson, G.K. Proskurowski, D.A. Butterfield, J.J. Vallino, and J.A. Huber

Methanogens are keystone high-temperature autotrophs in deep-sea hydrothermal vents and tracers of habitability and biogeochemical activity in the volcanic seafloor. At Axial Seamount, nearly all methanogens are Methanothermococcus and Methanocaldococcus species, making this site amenable to modeling through laboratory pure culture experiments coupled with field studies. Based on field microcosm incubations with 1.2 mM, 20 μ M, and no hydrogen, the growth of methanogens at 55°C and 80°C is limited primarily by temperature and hydrogen availability, with ammonium amendment showing no consistent effect on total methane output. In laboratory experiments, the Arrhenius constants for methane production by Methanocaldococcus jannaschii and Methanothermococcus thermolithotrophicus were determined in bottles. Both organisms were also grown in a 2-liter chemostat at two dilution rates; 55°C, 65°C and 82°C; and variable hydrogen concentrations, to determine the organisms' Monod constants. M. jannaschii showed higher k_s and V_{max} constants than M. thermolithotrophicus. In the field, hydrogen and methane concentrations were determined in hydrothermal end-member and low-temperature diffuse fluids, and the concentrations of methanogens that grow at 55°C and 80°C in diffuse fluids were determined using most-probable-number estimates. Methane concentration anomalies in diffuse fluids relative to end-member hydrothermal concentrations and methanogen cell concentrations are being used to constrain a 1-D reactive transport model using the growth and methane production kinetics determined in laboratory studies. By varying flow path length and seafloor cell concentrations in the model, our goal is to determine solutions for the potential depth of the seafloor biosphere coupled with the amount of methanogenic biomass it contains.

Influence of Groundwater Hydrology and Geochemistry on Nitrogen-Cycling Processes and Microbial Community Structure in the Sediments of a Groundwater Flow-Through Pond

Presented by Deborah A. Reper, Microbiologist, U.S. Geological Survey, USA

Co-Authors: D.A. Repert, D.L. Stoliker, R.L. Smith, B. Song, R.B. Thomas, C. Conaway, D.R. LeBlanc, T.D. McCobb, S.P. Hyun, D.-C. Koh, H.S. Moon, K. Ha, and D.B. Kent

Wastewater-contaminated groundwater discharges into a groundwater flow-through kettle pond on Cape Cod, MA. Hydrologic and geological controls on N-cycling processes and microbial community structure in pond sediments were compared at four sites characterized by (1) inflow of oxic pristine groundwater; (2) inflow of sub-oxic nitrate-containing groundwater; (3) stagnant, anoxic ammonium-containing pond-bottom groundwater; and (4) pond water recharging the aquifer. Seasonal vertical and horizontal biogeochemical profiles of groundwater in the shallow (<1 m) sediments below the pond showed an elevated nitrate zone (100 μM) at the nitrate site. The distributions of oxygen and nitrate concentrations in porewater under the pond-bottom resembled the distributions in the wastewater plume upgradient of the pond and shifted seasonally with pond stage. Ammonium concentrations at the ammonium site were 150-300 μM and showed considerable small-scale horizontal and vertical heterogeneity. Pond-bottom sediment-slurry incubations indicated that denitrification-potential rates were greatest in sediments from the nitrate site; N mineralization-potential rates were greatest at the ammonium site. Sediment microbial community structure (16S rDNA pyrosequencing) and N-cycling gene abundance (*nirS*, *nosZ*, and *hzo*) were assessed. The abundance of the *nirS* and *nosZ* genes, an indication of denitrifying bacteria, was greatest at the nitrate and pristine groundwater-inflow sites. The *hzo* gene abundance, an indication of anammox bacteria, was greater at the nitrate site than at the ammonium site but was not detectable at the pristine site. Pyrosequencing showed higher abundance of nitrifying bacteria in the nitrate site. Results underscore the importance of hydrologic conditions on pond-sediment N-cycling processes and microbial community structure.

Deep Coalbed Biosphere Incubations Contain Active Community with Methylophilic Metabolisms

Presented by Elizabeth Trembath-Reichert, Ph.D. Candidate, Division of Geological and Planetary Sciences, California Institute of Technology, USA

Co-Authors: E. Trembath-Reichert, Y. Morono, F. Inagaki, and V.J. Orphan

IODP Expedition 337, Deep Coalbed Biosphere off Shimokita, was the first IODP expedition to use riser drilling and was thereby able to retrieve core from a record breaking 2466 mbsf. The microbiological focus was also unique to this cruise, with a goal to observe if life is able to exist at these depths fed by deeply buried terrigenous coalbeds. Onboard scientists prepared nearly 1,700 microbiology samples shared among five different countries to study life in the deep biosphere. Samples spanned over 1 km in sampling depths and include representatives of three main lithologies (shale, sandstone, and coal). Findings from previous IODP and deep mine expeditions, suggest genetic evidence for deep life capable of methylophilicity. 231 of these 1,700 incubations were dedicated to look for this metabolism. Methylophilicity incubations were set up using a range of ^{13}C -carbon (methane, bicarbonate, methylamine, and methanol), ^{15}N -nitrogen (ammonium and nitrogen gas), and deuterated (water) substrates. Incubations were maintained at three temperature ranges to keep incubations near their respective in situ temperature. After 17 months of incubation, the headspace and porewater were sampled for a range of geochemical and microscopic analyses. These data indicate both active cell populations and metabolism of labeled substrates, across the full range of conditions and lithologies. This suggests cells from the deep coalbed biosphere off Shimokita are capable of methylophilicity and nitrogen assimilation. IODP Expedition 337, Deep Coalbed Biosphere off Shimokita, was the first IODP expedition to use riser drilling and was thereby able to retrieve core from a record breaking 2466 mbsf. Also unique to this cruise, was the microbiological focus with a

goal to observe if life was able to exist at these depths fed by the deeply buried terrigenous coalbeds. Onboard scientists prepared nearly 1,700 microbiology samples shared among five different countries to study life in the deep biosphere. These samples include the record-breaking core sampled at 2.4 km below sea floor. Based on findings from previous IODP and deep mine expeditions, there is genetic evidence for deep life capable of methylotrophy. 231 of these 1,700 incubations were dedicated to look for this metabolism. These incubations were set up using a range of ¹³C-carbon (methane, bicarbonate, methylamine, and methanol), ¹⁵N-nitrogen (ammonium and nitrogen gas), and deuterated substrates. Samples spanned over 1 km in sampling depths and include representatives of three main lithologies (shale, sandstone, and coal). Incubations were maintained at three temperature ranges to keep incubations near their respective in situ temperature. After 17 months of incubation, the headspace and porewater were sampled for a range of geochemical and microscopic analyses. These data indicate both active cell populations and metabolism of labeled substrates, across the full range of conditions and lithologies. This suggests methanotrophy, methanogenesis, and nitrogen assimilation are all metabolic capabilities of deep biosphere cells from the deep coalbed biosphere off Shimokita.

3:40 PM – 5:20 PM
FRED FARR FORUM

SESSION B6 METHODS: MOLECULAR STUDIES

Moderated by:

- **Prof. Dr. Kirsten Küsel**, Chair of the Aquatic Geomicrobiology Department, Institute of Ecology, Friedrich Schiller University Jena, Germany
- **David W. Metge**, Microbiologist, National Research Program, U.S. Geological Survey, USA



Taxonomic and Catabolic Bacterial Profiles in Contaminated Soils: A High-Resolution Analysis Shows the Same Players with Different Scripts

Presented by Ramiro Vilchez-Vargas, Ph.D., Postdoctoral Researcher, Laboratory of Microbial Ecology and Technology, Department of Biochemical and Microbial Technology, Ghent University, Belgium

Co-Authors: R. Vilchez-Vargas, A. Waliczek, F.-M. Kerckhof, B. Rodelas, D.J. Näther, R. Jauregui, and D.H. Pieper

The adaptation of microbial communities to stress factors generated by pollutants was assessed in one area where non-contaminated sites coexisted with sites contaminated with alkanes, toluene-xylene-ethylbenzene, or alkanes-toluene-xylene-ethylbenzene. Phylogenetical diversity and catabolic gene potential were analysed in parallel. Taxonomical characterizations were carried out using the Illumina deep sequencing technology obtaining 200335 OTUs, while catabolic gene potential was evaluated by a novel systematic methodology comprising four steps. Screening for catabolic potential was performed initially by using a targeted microarray containing 1426 probes for catabolic genes detection. Subsequently, a set of 56 primers was designed based on the positive signals detected. Finally, 134 clone libraries were constructed, and 1638 gene fragments related to key catabolic genes were sequenced using Sanger technology. As a result of these analyses, it was found that the relative abundance of Xanthomonadaceae was strongly correlated with the incidence of α -Proteobacteria-

related alkane hydroxylase genes at site contaminated with alkanes. Rhodocyclaceae were enriched at toluene-xylene contaminated areas, and correlated to the incidence of extradiol dioxygenases EXDOD and EXDODbt types and diiron monooxygenases evolutionarily related to α -Proteobacteria. However, when both contaminants were present, an enrichment of both taxa was observed, but microarray profiles as well as clone libraries results showed a drastic shift of the prevalent pathways, suggesting that the catabolic organization was a function of the number of pollutants present in the environment.

Single-Nucleotide Primer Extension Assays to Reveal Key Microbes and Functional Genes Taking Part in the Degradation of BTEX Compounds under Oxygen-Limited Conditions

Presented by Andras Tancsics, Ph.D., Postdoctoral Researcher, Regional University Center of Excellence in Environmental Industry, St. Istvan University, Hungary

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Aromatic hydrocarbons including benzene, toluene, ethyl-benzene, and xylenes (BTEX) are frequent contaminants of groundwater. Consequently, aerobic and anaerobic degradation pathways of BTEX-compounds are widely investigated. However, most studies deal with clear oxic or anoxic conditions, and the behavior of degradation pathways are often studied by using pure strains in highly artificial laboratory environments. In subsurface environments the availability of oxygen is commonly restricted and the hydrocarbon contamination causes oxygen-limited conditions even in shallow groundwaters. The major question in these microoxic environments is that which microbes and degradation pathways play key role in the degradation of contaminants. To assess this question single-nucleotide primer extension (SNUPE) assays were developed for monitoring presence and expression of subfamily I.2.C catechol 2,3-dioxygenase (C23O) genes. In situ monitoring revealed the relevance of these genes in oxygen-limited, BTEX-contaminated groundwater. Moreover a yet uncultured member of the genus *Rhodoferrax* and an unclassified Rhodocyclaceae bacterium were identified as possible microaerobic BTEX degraders. Enrichment culturing of iron-reducing bacteria from the investigated groundwater further verified our assumption that the detected *Rhodoferrax* related bacterium harbors subfamily I.2.C C23O gene. Consequently, the involvement of iron-reducing, facultatively anaerobic bacteria in the degradation of BTEX-compounds under microoxic conditions can be also assumed. Groundwater microcosm experiments were carried out to investigate how the decreasing availability of dissolved oxygen affects the expression of subfamily I.2.C C23O genes. Results revealed that mRNA transcripts of some of these genes were constantly detectable regardless of the availability of oxygen, assuming that oxygen does not necessarily modulate their expression.

Pyrosequencing Reveals the Dominance of Actinobacteria in Coal Core Samples from Powder River Basin Coals

Presented by Elliott P. Barnhart, Ph.D., Microbiologist, Wyoming-Montana Water Science Center, U.S. Geological Survey, USA

Co-Authors: E.P. Barnhart, A.C. Clark, W.H. Orem, D.M. Akob, A.B. Cunningham, and M.F. Fields

Microorganisms in the deep subsurface comprise a large proportion of the biomass on Earth, yet many questions about this important ecosystem remain unanswered. Coalbeds provide an excellent environment for investigating subsurface carbon utilization. Core samples obtained above, within, and below a methane-producing coal seam were analyzed with 454-pyrosequencing to relate microbial community features to the substratum lithology, and better understand the in-situ microbial community. The drilling fluid used to obtain the core samples also was analyzed to determine if the core samples had been contaminated. The results of this study indicate the drilling fluid did not contaminate the core samples. Actinobacterial SSU-rRNA sequences were more abundant in coal core

samples than in clay or sand cores. Actinobacteria have been shown to play an important role in the decomposition of organic matter such as cellulose and chitin, but this research provides novel evidence implicating their importance in coal degradation and coal bed methane (CBM) production. A better understanding of anaerobic microbial coal degradation provides insight into subsurface carbon cycling that could lead to new CBM producing technologies from deep, unminable coal.

Identifying Methanogens in Gulf of Mexico and Cape Lookout Bight Methane Seeps

Presented by Karen Lloyd, Ph.D., Assistant Professor, Department of Microbiology, University of Tennessee in Knoxville, USA

Co-Authors: K. Lloyd, S. Underwood, L. Lapham, R. Kevorkian, and A. Teske

Much biogeochemical research in marine methane seeps has centered on the role of anaerobic methanotrophic archaea (ANME) in sulfate-dependent anaerobic methane oxidation. Less effort has been made to identify the organisms responsible for methane production below the zone of sulfate depletion. We identified potential methanogens and sulfate reducers using 16S rRNA genes and cDNA, as well as genes and mRNA for dissimilatory sulfite reductase (*dsr*, present in sulfate reducers) and methyl co-enzyme M reductase (*mcr*, present in methanogens and methanotrophs) at seven depth layers up to 4.2 m beneath an active methane seep in Gulf of Mexico MC118. Sulfate was depleted at 1.3 m and 16S rRNA for sulfate reducers and *dsr* were only found at 11-13 cm and 40-44 cm. ANME-1 and Methanomicrobiales were the dominant methanogen-like archaea in methanogenic sediments, as shown by 16S rRNA genes, *mcr* genes, and *mcr* rRNA. We therefore hypothesized that ANME-1 produce methane, and are not obligate methane oxidizers dependent on methane generated by other organisms. To test this, we incubated sediments from a more accessible methane seep at Cape Lookout Bight, NC, in an anaerobic, methane-free environment for 122 days. ANME-1, ANME-2, and Methanomicrobiales all increase in CARD-FISH cell counts after sulfate is depleted and methane begins to be generated. An initial seeding of methane was not necessary for ANME-1 and ANME-2 growth in duplicate incubations. Given that no evidence exists to rule it out, we suggest that ANME produce methane along with Methanomicrobiales in methane seep sediments.

Bacterial Community Structure and Dissolved Organic Matter in Repeatedly Flooded Subsurface Karst Water Pools

Presented by Tatiana Shabarova, Ph.D., Postdoctoral Researcher, Limnological Station, University of Zurich, Switzerland

Co-Authors: T. Shabarova, J. Villiger, O. Morenkov, J. Niggemann, T. Dittmar, and J. Pernthaler

Bacterial diversity, community assembly, and the composition of the dissolved organic matter (DOM) were studied in three temporary subsurface karst pools with different flooding regimes. We tested the hypothesis that microorganisms introduced to the pools during floods faced environmental filtering towards a 'typical' karst water community, and we investigated if DOM composition was related to floodings and the residence time of water in stagnant pools. As predicted, longer water residence consistently led to a decline of bacterial diversity. The microbial assemblages in the influx water harbored more 'exotic' lineages with large distances to known genotypes, yet these initial communities already appeared to be shaped by selective processes. Betaproteobacterial operational taxonomic units (OTUs) closely related to microbes from subsurface or surface aquatic environments were mainly responsible for the clustering of samples according to water residence time in the pools. By contrast, several Cytophagaceae and Flavobacteriaceae OTUs were related to different floodings, which were also the main determinants of DOM composition. A subset of compounds distinguishable by molecular mass and O/C content were characteristic for individual floods. Moreover, there was a

transformation of DOM in stagnant pools towards smaller and more aromatic compounds, potentially also reflecting microbial utilization.

3:40 PM – 5:20 PM
NAUTILUS ROOM

SESSION C6 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



Springs and Wells and Cores: What Do These Diverse Access Points Tell Us About Microbial Processes in the Serpentinizing Subsurface?

Presented by Matthew O. Schrenk, Ph.D., Assistant Professor, Department of Geological Sciences, Michigan State University, USA

Author: M.O. Schrenk

The aqueous alteration of ultramafic rocks through serpentinization is a widespread phenomenon occurring on each of the continents and over wide areas of the seafloor. Serpentinization was even more pervasive on the early Earth and likely occurs on other planetary bodies. While serpentinization is the source of copious reduced compounds that can fuel metabolism, microorganisms near sites of active serpentinization face challenges in terms of access to oxidants, availability of nutrients, and high pH (>11). My laboratory has focused on describing microbial community compositions and physiological adaptations in a range of environments associated with active serpentinization including ultrabasic springs, wells in alkaline aquifers, and rock cores directly from the serpentinizing subsurface. Studies using next generation sequencing as well as traditional cultivation approaches have served to illuminate the identity of microbial communities in the serpentinizing subsurface and how they function under extreme environmental conditions. Betaproteobacteria related to the genus *Hydrogenophaga*, and alkaliphilic Firmicutes related to the order Clostridiales were found to be common inhabitants of serpentinites in Europe, North America, and Central America. Physiological studies have revealed the potential for hydrogen oxidation as well as fermentation of small organic molecules and hydrogen production. At the same time comparison of niches within individual serpentinites has revealed subtle microbiological variations related to subsurface hydrology, development of biofilms, and mixing between surface fluids and groundwaters. The relationship between the occurrence of specific microbial taxa, their physiological adaptations, and their habitat will be explored in the context of habitability of serpentinizing subsurface environments.

Diversity of Nanoarchaeota and Their Archaeal Hosts from Terrestrial Hot Springs in New Zealand

Presented by Kristen Brileya, Ph.D., Postdoctoral Researcher, Portland State University, USA

Co-Authors: K. Brileya, Y. Liu, M. Podar, M. Stott, and A.-L. Reysenbach

It is evident from 16S rRNA gene sequences that the Nanoarchaeota are widespread and biogeographically diverse. However, they are currently represented in culture by a single marine organism *Nanoarchaeum equitans*, in coculture with the Desulfurococcales, *Ignicoccus hospitalis*. The nature of the association remains unclear, with evidence suggesting parasitism whereby *N. equitans* derives amino acids, lipids, and energy from *I. hospitalis*. For *I. hospitalis* the relationship has been shown to prevent cell division, yet it remains a possibility that some benefit is incurred from association with *N. equitans*. Since the isolation of *N. equitans* in coculture with *I. hospitalis* in 2002, little new information has been obtained about other members of the Nanoarchaeota until the first nearly complete genome sequence of a terrestrial Nanoarchaeum, 'Nst1' from Yellowstone National Park was recently released. This sequence was obtained through single cell sorting, and a pure coculture of Nst1 with its putative Sulfolobales host was never established. This new association demonstrated that *Ignicoccus* spp. are not the only potential hosts for Nanoarchaeota, and helped direct enrichment culturing efforts toward other potential Archaeal hosts. We have now successfully enriched novel Nanoarchaeota from terrestrial hot springs in New Zealand that represent different geochemical and community compositions. Using qPCR to monitor Nanoarchaeota abundance in the enrichments, we have tracked community response to culture conditions and identified potential hosts, expanding the knowledge of this enigmatic phylum.

Carbon Transformations in High Temperature Terrestrial Geothermal Spring Communities

Presented by Matthew R. Urschel, Research Assistant and Ph.D. Candidate, Department of Microbiology, Montana State University, USA

Co-Authors: M.R. Urschel, M.K. Kubo, T.M. Hoehler, J.W. Peters, and E.S. Boyd

Microbial communities inhabiting high temperature (>73°C) and non-photosynthetic geothermal springs are commonly assumed to be supported primarily by chemolithoautotrophic metabolisms. However, recent isotopic evidence suggests that chemosynthetic microbial populations in some geothermal environments in Yellowstone National Park (YNP), Wyoming utilize both organic and inorganic carbon sources, and may be capable of metabolic switching between autotrophy and heterotrophy depending on organic substrate availability. To test this hypothesis, we used a radiotracer approach to quantify and compare rates of dissolved inorganic carbon (DIC), formate (HCOO⁻), and acetate (C₂H₃O₂⁻) assimilation and/or mineralization in 13 YNP chemosynthetic communities that span a range of geochemical conditions (pH, temperature, DIC/DOC levels, etc). These data were combined with taxonomic profiling of and geochemical measurements to identify populations and geochemical regimes that may dictate substrate preferences and transformation rates in these communities. Our results indicate that, while DIC assimilation is greater than that of formate and acetate in most YNP chemotrophic communities, formate and/or acetate assimilation and/or mineralization (heterotrophy) also occur in these systems at rates that are often comparable, and in some cases exceed, those of DIC assimilation. Moreover, substrate suppression experiments indicate that populations preferentially utilize formate over DIC as a carbon and/or energy source, suggesting the capability of metabolic switching between these carbon sources in response to changing substrate concentrations. Native DIC, HCOO⁻, and C₂H₃O₂⁻ concentrations were inversely correlated with rates of their utilization, indicating a role for biological activity in maintaining low in situ substrate concentrations.

A Multi-Extreme Thermal Environment in Idaho

Presented by Timothy S. Magnuson, Ph.D., Professor, Department of Biological Sciences, Idaho State University, USA

Co-Authors: T.S. Magnuson, A.C. Johnson, J.C. Rask, and L. Baker

The Snake River Plain and Idaho Batholith harbor numerous and unique hot spring thermal areas, due in part to heat sources beneath southern and central Idaho. We have focused our efforts on an unusual thermal area in S Idaho, Worswick Hot Springs. Worswick is composed of over 20 distinct thermal features, with temperatures ranging from 37°C to 90°C, and average pH values of 9. Radiation levels were measured and showed radiation associated with hot thermal sources and channels. Despite these conditions, there is abundant microbial activity associated with the thermal waters. Worswick thus represents a ‘multi-extreme’ environment where microbes must cope with temperature, radiation, and pH extreme. Preliminary data suggested that the radiation levels along the stream channel vary by at least a factor of two, ranging from 14.5 $\mu\text{rem/hr}$ up to 35.5 $\mu\text{rem/hr}$. Water samples were analyzed for total elemental composition using inductively-coupled plasma-mass spectrometry (ICP-MS), and this analysis revealed some very interesting properties of the hot spring waters, including potential sources of radiation. Significant amounts of metals such as Fe and As provide energy sources. X-ray fluorescence measurements show similar elements in rocks/minerals surrounding the springs. Gas analysis of thermal waters showed CO and H₂, central to extremophile metabolism. Microbiological assessment of the site has resulted in cultivation of several types of thermophilic organisms, including sulfur-oxidizing and sulfate-reducing microbes. Worswick is a microbiologically and geochemically diverse and unusual environment that supports abundant microbial growth, and will be a resource for understanding how subsurface geochemistry drives terrestrial extreme environments.

Functional Potential of Epsilonproteobacteria in Sulfidic Karst Microbial Communities

Presented by Annette S. Engel, Ph.D., Associate Professor, Department of Earth and Planetary Sciences, University of Tennessee at Knoxville, USA

Co-Authors: A.S. Engel, K. Rossmassler, T.E. Hanson, A.T. Paterson, and B.J. Campbell

Although much is known about the phylogenetic diversity of Epsilonproteobacteria in several karst systems, very little is known about their functional potential. They are known as chemolithoautotrophs that mediate sulfur cycling and carbonate dissolution. We combined geochemical and metagenomics approaches to investigate the genomic diversity and potential ecological role of Epsilonproteobacteria-dominated karst microbial communities from a sulfidic cave (Lower Kane Cave, LKC, Wyoming) and a subterranean sulfidic spring (Glenwood Hot Springs, GHS, Colorado). The two sites had contrasting chemistries, with 20 $\mu\text{mol/L}$ dissolved sulfide at LKC (pH = 7.3, 22.3 °C) and 56 $\mu\text{mol/L}$ sulfide at GHS (pH = 6.4; 50 °C). Two metagenomes were sequenced from spring orifices, 60 m apart. Epsilonproteobacteria dominated, representing ~50% of the communities upstream and 30% downstream based on rRNA profiles. The GHS metagenome contained ~80% Epsilonproteobacteria. Epsilonproteobacterial types varied in each sample, but were typically most closely related to *Sulfurovum* or *Sulfuricurvum*. However, Epsilonproteobacteria in all the metagenomes were unable to be definitively classified, indicating novel groups at these sites. The diversity of autotrophic pathways from the cave metagenomes reflected the phylogenetic diversity, with prevalence of rTCA cycle genes, and then CBB cycle genes. The GHS metagenome was dominated by genes in the rTCA cycle. Genes from other metabolic pathways were also found, such as sulfur and nitrate respiration and reduction, as well as hydrogen oxidation. Epsilonproteobacterial metagenomes from terrestrial, low-temperature habitats are underrepresented, so this research fills a gap in our understanding of their evolutionary history and ecological roles in karst.