

Ninth International Symposium on Subsurface Microbiology

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

Monday, October 6, 2014

ORAL PRESENTATION ABSTRACTS

ORAL PRESENTATION SCHEDULE

8:30 am – 10:00 am	Welcome and Plenary Session #1	Merrill Hall
10:20 am – 12:00 pm	Session #1	
	A1. Fate	Merrill Hall
	B1. Contaminants	Fred Farr Forum
	C1. Biomes	Nautilus Room
2:00 pm – 3:20 pm	Session #2	
	A2. Fate	Merrill Hall
	B2. Contaminants	Fred Farr Forum
	C2. Biomes	Nautilus Room
3:40 pm – 5:20 pm	Session #3	
	A3. Fate	Merrill Hall
	B3. Chemistry	Fred Farr Forum
	C3. Biomes	Nautilus Room

8:30 AM – 10:00 AM
MERRILL HALL



PLENARY SESSION

Moderated by **Karsten Pedersen, Ph.D.**, Professor of Geomicrobiology, Chalmers University of Technology, Sweden



Microbial Life in Oil

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

Co-Authors: R.U. Meckenstock, F. von Netzer, C. Stumpp, T. Lueders, N. Hertkorn, P. Schmitt-Kopplin, M. Harir, R. Hosein, S. Haque, and D. Schulze-Makuch

Anaerobic microbial degradation of hydrocarbons influences the quality of oil reservoirs and can lead to the generation of highly viscous bitumen. Previous work suggests such biodegradation mostly occurs at the interfaces of oil and water compartments. However, we show at the example of the world's largest asphalt lake in Trinidad & Tobago that microorganisms thrive in minuscule water droplets (1-3 μ l) entrapped in oil. Pyrotag sequencing of individual droplets revealed complex methanogenic microbial communities actively degrading the oil as shown by metabolite analysis with NMR and FTICR/MS. High salinity and water stable isotopes of the inclusions indicated a deep subsurface origin suggesting entrapment of the droplets in the oil reservoir itself. The large surface area of the water droplets and 13.5 % water content of the oil reveal a currently underestimated potential for biodegradation of oil reservoirs and a new, extreme habitat for microbial life.



Tracking Electromicrobial Activity with Electric Potential Microsensors

Presented by Lars Peter Nielsen, Ph.D., Professor, Center for Geomicrobiology and Section for Microbiology, Aarhus University, Denmark

Co-Authors: L.P. Nielsen, L.R. Damgaard, A. Revil, and N. Risgaard-Petersen

In geophysical surveys distinct selfpotential anomalies at the soil surface are often found to correlate with organic contamination of aquifers meters below. With the so-called biogeobattery model this is explained by electromicrobial activity creating electric fields when electrons from oxidation processes are passed to oxic layers via yet unidentified biogenic or minerogenic electron conductors. In marine sediments biogeobatteries have recently been identified in the form of filamentous bacteria with internal electron conductors bridging sulfide oxidation at depth with oxygen reduction near the sediment-water interface. With a novel microsensor for electric potential we have successfully measured electric fields associated with the activity of these electric cable bacteria. The microscale measurements allow both accurate location of electron sources and sinks, calculations of current densities, and instantaneous recordings of microbial metabolic responses to experimental and natural perturbations. Based on this experience and some preliminary tests we will evaluate to what extent the micro- and macroscale approaches may converge with the aim of finding and characterizing biogeobatteries in the subsurface.

10:20 AM – 12:00 PM

MERRILL HALL

SESSION A1 FATE: MICROBIAL FATE AND TRANSPORT

Moderated by:

- **Thomas Harter, Ph.D.**, Robert M. Hagan Endowed Chair, Water Management and Policy, Department of Land, Air and Water Resources, University of California, Davis, USA
- **Prof. Dr. Jack F. Schijven**, Senior Scientist, National Institute of Public Health and the Environment (RIVM), The Netherlands, and Professor, Environmental Hydrogeology Group, Department of Earth Sciences, University of Utrecht, The Netherlands



Episodic Transport of Microorganisms with Transients in Solution Chemistry and Water Saturation

Presented by Scott A. Bradford, Ph.D., Research Soil Scientist, U.S. Department of Agriculture, Agricultural Research Service, U.S. Salinity Laboratory, USA

Co-Authors: S.A. Bradford, Y. Wang, H. Kim, S. Torkzaban, and J. Simunek

Most microbial transport studies have been conducted under steady-state water flow and saturation, and solution chemistry conditions. Results typically suggest limited mobility of microorganisms in the subsurface because of retention to solid-water and air-water interfaces. Retention is well known to be sensitive to a wide variety of physical, chemical, and microbiological factors that may vary temporally in the subsurface environment. This presentation demonstrates the sensitivity of microbial transport to transient solution chemistry (ionic strength, pH, and cation type), input concentration, and water velocity and saturation conditions. The conventional first-order detachment model cannot describe this behavior. We present a mathematical modeling framework that relates microbe release under transient conditions to changes in the solid-water and air-water interfacial areas that contribute to retention. Methods to predict these interfacial areas are demonstrated. Equilibrium and/or kinetic expressions for microbe release with transients are developed and utilized to simulate microbe release and transport over a wide range of conditions. Experimental and modeling results indicate that episodic microbe transport in the subsurface is expected because of transient conditions.

The Impact of Nanoscale Charge Heterogeneity on the Fate and Transport of Viruses: Effects of Solution pH, Ionic Strength, and Phosphate

Presented by Salini Sasidharan, Ph.D. Candidate, National Centre for Groundwater Research and Training, Flinders University of South Australia and CSIRO Land and Water, Australia

Co-Authors: S. Sasidharan, S. Torkzaban, S. Bradford, and P. Cook

Chemical heterogeneity is an intrinsic property of all naturally-occurring mineral surfaces in the subsurface environment. An extensive set of column experiments was conducted to examine the effects of solution chemistry such as ionic strength (IS), pH, and anion type on attachment and inactivation of three bacteriophages (MS2, PRD1, ϕ X174), as surrogates for human pathogenic viruses, in a chemically heterogeneous (metal oxides) sand. Patchwise nanoscale chemical heterogeneity was introduced to the sand surfaces by modifying the surface chemistry of sand grains via reaction with a weak acid. The attachment efficiency of all viruses to the sand surfaces increased with increasing IS and decreasing pH. Attachment of MS2 and PRD1 was found to be more sensitive

to the changes in pH and IS than ϕ X174 because of their lower isoelectric points. The presence of phosphate (10 mM PO₄-2) in the eluting solution significantly reduced the virus attachment efficiency due to a reduction in the chemical heterogeneity as a result of complexation of phosphate ions with metal oxides. At pH 7.5 about 40% of the attached viruses were released when the columns were eluted using a beef extract solution (pH 9.5) immediately following the attachment phase. Conversely, when the viruses remained attached for about 2 days on the sand surfaces, less than 2% of viruses were recovered following elution with beef extract. This difference in release was attributed to rapid inactivation of the attached viruses.

Power-Law Size-Distributed Heterogeneity Explains Colloid Retention on Glass and Other Mineral Surfaces in the Presence of Energy Barriers

Presented by William P. Johnson, Ph.D., Professor, University of Utah, USA

Co-Authors: W.P. Johnson, E. Pazmino, J. Trauscht, and B. Dame

The mechanisms governing immobilization and release of microbes and all other colloids in the presence of colloid-surface repulsion (presence of energy barriers to attachment) are not yet incorporated into a functioning theory for prediction of transport. This presentation concerns reading the nanoscale heterogeneity thought responsible for colloid retention on surfaces in the presence of energy barriers (unfavorable attachment conditions). We back out this heterogeneity on glass and other mineral surfaces by comparing mechanistic simulations incorporating discrete heterogeneity with colloid deposition experiments performed across a comprehensive set of experimental conditions. Original data is presented for attachment to these surfaces for three colloid sizes (0.25, 1.1 and 1.95 μ m microspheres) under a variety of ionic strengths and fluid velocities in an impinging jet system. Comparison of mechanistic particle trajectory simulations incorporating discrete surface heterogeneity represented by nanoscale zones of positive charge (heterodomains), indicates that a power-law size distribution of heterodomains ranging in size from 120 to 60 nm in radius was able to explain observed retention for all conditions examined. In contrast, uniform and random placement of single-sized heterodomains failed to capture experimentally-observed colloid retention across the range of conditions examined. Notably, incorporation of discrete heterogeneity into mechanistic models also simulated the release of immobilized colloids in response to ionic strength reduction, thereby mechanistically capturing an important aspect of colloid immobilization in the presence of energy barriers to attachment.

Microbiological Indicators and Pathogen Transport in a Riverbank Filtration System

Presented by Inge H. van Driezum, Project Assistant and Ph.D. Candidate, Institute of Hydraulic Engineering and Water Resources Management, Vienna University of Technology, Austria

Co-Authors: I.H. van Driezum, D. Savio, P. Reiner, J. Derx, A.K.T. Kirschner, R. Sommer, A.H. Farnleitner, and A.P. Blaschke

Groundwater locations at alluvial backwaters and its riverbank filtration (RBF) systems are widely used as a means of obtaining public water supplies. Riverbank filtration is an effective way to remove pathogens and micropollutants from the receiving surface water. Due to the low abundance of pathogenic and indicator microorganisms in groundwater, concentration of the samples is needed. One way to concentrate the groundwater samples is by means of high volume ultrafiltration. The main study area covers a Porous GroundWater Aquifer (study site PGWA) – an urban floodplain extending on the left bank of the river Danube downstream of the City of Vienna, Austria. High volume samples (500-1000L) were taken in two different transects ranging from the river to the drinking water wells. The piezometer frequency is highest directly at the river to especially account for concentration

changes in the hyporheic zone. As fecal markers *Escherichia coli*, enterococci, spores of *Clostridium perfringens*, somatic coliphages and human genetic markers as well as chemical wastewater markers were measured. Additionally, a series of different micropollutants, including the 'natural' tracer carbamazepine, will be determined simultaneously. First results of the analyses of different micropollutants showed a gradient from the river towards the drinking water well. Under inclusion of temperature and groundwater level data, pathogen transport will be modeled in the PGWA.

Increasing the Produced Oil Using Microbes Found in the Subsurface of Oil Reservoirs in the Plains of North America

Presented by Edwin Hendrickson, Ph.D., Senior Research Microbiologist, DuPont Company, USA
Co-Authors: A.K. Luckring, M.P. Perry, R.D. Fallon, R.E. Jackson, S.J. Keeler, A.M. Manaresi, J. Norvell, and S.C. Jackson

Microbial populations in the oil reservoirs in the western plains of North America are very similar to microbes found in the oceans and deep sea thermal vents. The subsurface waters in these reservoirs have salinity equal to or greater than that of seawater (up to 3x). The presence of these waters chemistries, marine microbes and the origins of oil deposits can be explained by their connection to a large, ancient North American interior seaway. This seaway stretched from the Arctic Sea to the Gulf of Mexico; and from what is now is the Rocky Mountains to just west of the Appalachians. Using selected electron donors and acceptors, we are able to enrich for microbes that are used in a tertiary oil recovery treatment known as microbial enhanced oil recovery (MEOR). This technology increases the oil production in older oil reservoirs. Some of these microbes have been shown to reduce the permeability of high perm streaks, which can result in improved sweep efficiency. The rate of permeability modification is very reproducible, but can vary depending on the specific treatments used. The microbe and the nutrients are tailored to the conditions of each reservoir thus giving MEOR the greatest chance for success. We will show data from a unique set of slim tube experiments that simulates an oil reservoir and demonstrates the recovery of residual oil through permeability modification.

10:20 AM – 12:00 PM
FRED FARR FORUM

SESSION B1 CONTAMINANTS: BIORESTORATION

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



Impacts of Alkaliphilic Microorganisms on the Transport Characteristics of Sandstone: Implications for a Radioactive Waste Geological Disposal Facility

Presented by Sarah Smith, Ph.D. Candidate, University of Manchester, United Kingdom
Co-Authors: S. Smith, A. Rizoulis, J. West, and J. Lloyd

Radionuclide migration through the geosphere may be influenced by microbial processes. Examples include inhibition of fluid (and therefore radionuclide) transport resulting from porosity decreases due to bioclogging; microbially facilitated CaCO₃ or other mineral phase precipitation; and, alteration of hydrosphere redox chemistry. Previous studies focusing on microbial impacts on transport in rock have been carried out under circumneutral conditions; little is known about such processes under the hyper-alkaline conditions present in a geological disposal facility (GDF) for intermediate level waste

(due to extensive use of cementitious materials). This study involved flow-through column experiments using crushed sandstone and fluid (pH 12) from a hyperalkaline spring as a source of alkaliphilic/ alkali-tolerant microorganisms. Fluids pumped through the columns were amended with acetate and lactate (surrogates for cellulose degradation products) alongside control columns (no added carbon). Significant differences were observed in bacterial community composition between carbon amended (dominated by Clostridiaceae) and control columns (dominated by Comamonadaceae). Community composition differed with distance travelled, suggesting spatial variation in biogeochemical processes that were established in the columns. Decreases in flow rate were observed in “biostimulated” carbon amended columns throughout the experiment. Laser confocal micrographs demonstrated that biomass accumulation occurred at column inlets. Fermentation of lactate to propionate in carbon amended columns (producing CO₂), coupled with retention of calcium within the columns suggest microbial activity caused CaCO₃ precipitation. Further long-term experiments are currently running, investigating pressure changes caused by microbial processes in both intact and crushed sandstone, alongside detailed characterisation of the microbial communities and potential (bio)minerals formed.

Determining the Abundance and Activity of Vinyl Chloride Oxidizing Bacteria at Contaminated Sites

Presented by Timothy Mattes, Ph.D., Associate Professor, University of Iowa, USA

Co-Authors: T.E. Mattes, Y.O. Jin, X. Liu, and Y. Liang

Vinyl chloride (VC), a known human carcinogen, commonly accumulates during anaerobic reductive dechlorination of the more chlorinated ethenes in contaminated groundwater plumes. There are a variety of aerobic bacteria that can fortuitously oxidize VC in groundwater in the presence of other compounds (e.g. methanotrophs). A potentially important group of VC-oxidizers are the ethene-assimilators (i.e. “ethenotrophs”). We have developed a quantitative, real-time PCR (qPCR) method for ethenotrophs in groundwater. This qPCR method targets two functional genes ethenotrophs use to degrade VC and ethene: *etnC*, which encodes the alkene monooxygenase (AKMO) alpha subunit and *etnE*, which encodes the epoxyalkane:coenzyme M transferase (EaCoMT). The qPCR method was also extended to reverse transcribe RNA extracted from groundwater to cDNA so that *etnC* and *etnE* expression can be evaluated. Our methodology allows us to assess changes in the abundance and functionality of VC-oxidizing microbes in the subsurface. This information could be useful for site managers interested in understanding and documenting the natural attenuation of VC in dilute groundwater plumes. Initial application of the ethenotroph qPCR to DNA from three VC-contaminated sites showed that *etnC* and *etnE* abundance ranged from 10³-10⁵ copies/L of groundwater. Successful RT-qPCR experiments with groundwater from several wells at one VC-contaminated site show *etnC* and *etnE* transcript per gene ratios of 0.4 to 5. Continued application of this method to additional sites over extended time periods is expected to shed new light on the prevalence and contribution of ethenotrophs with respect to natural attenuation of VC.

Long-Term Removal of Indicator Bacteria and Viruses in Intermittently-Fed Stormwater Columns

Presented by Andrew A. Torkelson, Graduate Student, Department of Civil and Environmental Engineering, University of California at Berkeley, USA

Co-Authors: A.A. Torkelson, S.K. Mohanty, A.B. Boehm, and K.L. Nelson

Increased use of impermeable surfaces in urban environments has led to both larger stormwater flows and higher pollutant concentrations. Indicator organisms have been found to spike during storms from

sewer overflows and leaking sewer systems. Bioinfiltration basins, containing sand and compost, are used to retain and treat stormwater before discharge. However, few studies have examined indicator organism removal in these basins, and little is known about indicator virus removal or the influence of a mature biological community. We are examining these unknowns in four media types - plain sand, conventional bioinfiltration media (CBM), plain sand + zero valent iron (ZVI; 10% by weight), CBM + ZVI (10% by weight). To simulate stormwater, raw creek water is spiked with indicator bacteria (*Escherichia coli*) and viruses (MS2 coliphage). The stormwater is introduced once per week to 10 replicate columns per media type for 5 hrs to approximate a 1-year storm in Berkeley, CA; columns will be operated for at least 6 months. After 7 weeks, indicator bacteria and virus removal in plain sand + ZVI was consistently ~1.4 and 1.95 logs, respectively. No removal was observed in plain sand while indicator bacteria removal in CBM and CBM + ZVI increased with time to ~0.5 log. Removal mechanism differences between media types and ages are examined by characterizing the net surface charge (by potentiometric titration) and microbial community (by ATP measurement and flow cytometry). These results provide insight into pathogen removal mechanisms in mature bioinfiltration basins and the long-term effectiveness of media amendments.

13C Labeled Carbon Tetrachloride to Demonstrate Its Transformation to Carbon Dioxide in a Continuous Flow Column

Presented by Lewis Semprini, Ph.D., Professor, Oregon State University, USA

Co-Authors: L. Semprini and M.F. Azizian

In a continuous flow column study ¹³C labeled carbon tetrachloride (CT) was used to demonstrate its anaerobic transformation to ¹³CO₂. The column was packed with quartz sand and bioaugmented with Evanite Culture (EV) that is capable of transforming tetrachloroethene (PCE) to ethene and fermenting lactate to H₂ and acetate, but was not previously exposed to CT. The column was fed a synthetic groundwater that contained 0.1 mM PCE, unlabeled and ¹³C labeled CT (up to 0.1 mM), lactate (1.1 mM) and sulfate (1 mM). After bioaugmentation, effective sulfate reduction and PCE transformation to ethene was achieved with lactate fermenting to acetate and H₂. When unlabeled CT (0.015 mM) was added (along with PCE) it was completely transformed with a transient build-up of CF and chloromethane, which were subsequently removed below their detection limits. When the CT concentration was increased to 0.03 mM, it was completely transformed with no detectable transformation products. The unlabeled CT was then switched to labeled ¹³CT (0.03 mM). GC-MS analysis showed that 85% of ¹³CT was transformed to ¹³CO₂. The ¹³CO₂ concentration was double that present as a natural background due to the bicarbonate buffer plus the CO₂ produced from lactate fermentation. When ¹³CT concentration was increased in steps to 0.1 mM, 85% of the transformed CT continued to be observed as ¹³CO₂, with the remaining present as non-volatile organics, likely acetate. The results clearly demonstrate that high concentrations of CT can be transformed to CO₂, while maintaining effective PCE dehalogenation to ethene.

Community Interaction during Biostimulation for Technetium Biotransformation

Presented by M. Hope Lee, Ph.D., Senior Research Scientist, Pacific Northwest National Laboratory, USA

Co-Authors: M.H. Lee, D.L. Saunders, J.T. Ellis, and B.D. Lee

Phylogenetically diverse bacteria play an important role in immobilizing contaminants by directly reducing pertechnetate (⁹⁹TcO₄⁻) species to less soluble forms. Although much has been learned about the physiology and metabolic potential of individual bacterial isolates that immobilize ⁹⁹Tc, major gaps exist in our understanding of the functioning of these and other microorganisms in mixed

communities, under environmentally relevant conditions. These gaps include interaction with other microbes as well as consumption for carbon and electron sources for competing biogeochemical processes. As such, tools that integrate the chemical and biological reaction network influencing the mobility of ^{99}Tc in the subsurface need to be developed and tested. Isolates and the designed consortium consisting of pure cultures isolated from Oak Ridge FRC sediments (*Geobacter sulfurreducens* strain PCA, *Geobacter daltonii* strain FRC-32, and *Anaeromyxobacter dehalogenens* sp. strain 2CP-2), from Hanford subsurface sediments (*Cellulomonas* sp. strain ES6) and from freshwater lake sediments (*Shewanella oneidensis* MR-1) were used during testing. The $^{99}\text{TcO}_4^-$ -reducing capacity for the above isolates and consortium consisting of three and five members was determined in the presence and absence of Hanford fine sands. Genome and existing metabolic model information for the isolates was used to draft an initial community model and the implications for ^{99}Tc reduction and immobilization will be discussed. Results generated will provide important information related to ^{99}Tc reduction by mixed microbial communities. Output from the community models can then be coupled to geochemical models providing a microbial component which is often missing from these types of models.

10:20 AM – 12:00 PM
NAUTILUS ROOM

SESSION C1 BIOMES: CAVE, KARST, AND FRACTURED ROCK

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



H₂-Utilizing Sulfate-Reducing Bacteria in Hard Rock Aquifers

Presented by Karsten Pedersen, Ph.D., Professor, Microbial Analytics Sweden AB, Sweden
Co-Authors: K. Pedersen, A. Bengtsson, A. Chukharkina, L. Eriksson, J. Edlund, L. Hallbeck, J. Johansson, and L. Rabe

Desulfobacterium, *Desulfobacula*, *Desulfobulbus*, *Desulfosporosinus*, *Desulfovibrio* and *Desulfuromonas* constitute a large part of many high-throughput sequencing 16 rDNA libraries constructed with DNA extracted from groundwater and biofilms of Fennoscandian hard rock aquifers. The K_m for sulfate reduction by sulfate-reducing bacteria (SRB) using H_2 as electron donor was previously determined to $1\ \mu\text{M}$ with pure cultures of *Desulfovibrio aespoeensis* and with planktonic and biofilm communities under in situ conditions at 450 m depth in the Äspö hard rock laboratory (HRL). Analysis of >150 samples for dissolved H_2 in groundwater over depth in Olkiluoto, Finland, showed that H_2 never exceeded $1\ \mu\text{M}$ in groundwater with sulfate and SRB while the concentration can increase over depth to $100\ \mu\text{M}$ or more in sulfate-free groundwater suggesting a deep source of H_2 . A long term observation experiment at 450 m depth in the Äspö HRL that followed numbers of SRB, H_2 and groundwater chemistry including sulfide, sulfate and chloride lasted from 1999 to 2011. A draining incident of the observation site removed cultivable SRB and induced an up-coning event of saline groundwater with H_2 . When the draining incident was stopped, SRB returned and rapidly grazed down the H_2 to $<0.5\ \mu\text{M}$ with a corresponding peak in sulfide concentration. This and similar experiments suggest that the concentration of H_2 in groundwater where SRB and sulfate are present will normally be below K_m , i.e. $<1\ \mu\text{M}$. Understanding the sustainability of the deep biosphere in hard rock consequently must include understanding of the transport of H_2 in groundwater and the rock matrix.

Massive Methane-Fueled Microbial Biofilms in an Iodine-Rich Spring Cavern

Presented by Tillmann Lueders, Ph.D., Group Leader, Institute of Groundwater Ecology, Helmholtz Zentrum München, Germany

Co-Authors: C. Karwautz, M. Stöckl, and T. Lueders

Massive microbial biofilms were discovered in the cavern of a former medicinal spring in southern Germany, where iodine-rich formation water reaches the surface. The substantial biomass produced completely covers the walls and ceilings of the cave, the latter bearing snottites of over 10 cm length. Yet, the nature of these microbiota, their ecophysiology and the putative chemolithoautotrophic processes fuelling the biofilms have not been elucidated. We hypothesize that methane emerges with the formation water, and that microaerophilic methane oxidation is a major driver of biofilm formation, possibly also involving a novel coupling of methane and halide cycling. To address these questions, we have sampled biofilms from horizontal and vertical transects in the cave, for a detailed dissection of biofilm composition by 454 pyrotag sequencing. Gas emissions in the spring water itself contained methane (>50%) of fossil origin, as confirmed by stable isotope analysis (-41‰ delta 13C/12C). Sequencing revealed distinct methanotrophic and methylotrophic populations within the Alpha- and Gammaproteobacteria, and potentially even the Verrucomicrobia to dominate the biofilms. Methylotenera and Methylobacter spp. were abundant on the walls and especially in the cavern water, but surprisingly not so in ceiling biofilms. Here, ribosomal and functional gene analysis rather hinted at methylotrophic and potentially also iodine-cycling populations, supporting a postulated production and oxidation of iodomethane in this unique habitat. In conclusion, primary insights into a dominantly chemolithoautotrophic microbial ecosystem are revealed, largely independent from phototrophic carbon an energy inputs, but just a several meters under our feet.

Bacterial Diversity of Ferromanganese Crust Communities in Fort Stanton-Snowy River Cave

Presented by Diana E. Northup, Ph.D., Visiting Associate Professor and Professor Emerita, Biology Department, University of New Mexico, USA

Co-Authors: D.E. Northrup, N.A. Caimi, C. McMillan, and K.J. Hughes

The longest calcite formation in the world (~18 km in length currently), Snowy River, in Fort Stanton-Snowy River Cave National Conservation Area near Capitan, New Mexico, is lined with brown-black manganese oxide crusts on walls and ceilings above the usually dry river bed. These crusts contain varied bacterial communities that include many novel organisms. Next generation sequencing techniques revealed the presence of 32 bacterial phyla across nine samples. The most abundant phyla included Proteobacteria, Actinobacteria, SPAM (CD), NC10 (CD), Nitrospirae, Firmicutes, Bacteroidetes, Acidobacteria, Chloroflexi, OP8 (CD), GN04 (CD), Planctomycetes, and WS3 (CD). Several of these are candidate divisions (CD) of recently discovered phyla of bacteria. Genera present in the next gen sequences suggest the bacteria are involved in the cycling of iron, sulfur, and nitrogen and also include methotrophs and halophiles. Samples taken from within a few meters of each other showed much less overlap than expected. Together these results suggest a very novel community of bacteria in the Snowy River ferromanganese crusts and give us insight into subsurface communities with little human impact and long isolation. These results will help inform management decisions concerning Fort Stanton and the very fragile Snowy River, and are revealing novel and complex subsurface microbial communities.

Microbial Ecology of Groundwater along a Hydraulic Fracturing Proximity Gradient

Presented by Lee F. Stanish, Ph.D., Research Associate, University of Colorado at Boulder, USA

Co-Authors: L.F. Stanish, S. Osborn, M. Norman, and N. Pace

Microbes are critical components of biogeochemical cycling in subsurface environments such as groundwater. With the recent boom in hydraulic fracturing activities, concern over groundwater contamination has increased, and microbes may prove useful for tracking the various types of contamination of concern to environmental and human health. The goal of this study is to determine the processes driving the diversity and composition of groundwater microbes and to elucidate the relationships between microbial consortia, methane flux, and proximity to hydraulic fracturing activity. Groundwater samples were collected from 40 private wells located in three neighboring counties in Colorado. Concurrent chemical and 16s rRNA gene sequence analyses were conducted, and relationships between bacterial and archaeal community composition and water chemistry were determined. Dissolved methane increased with distance eastward, with the highest concentrations in Weld County, which is experiencing rapid shale gas development. Despite higher concentrations, stable isotope analysis suggests that the dissolved methane is derived in-situ. Furthermore, the occurrence of methanogens tightly correlated with the presence of methane in the groundwater. Deviation from this relationship was found in two samples that contained taxa related to anaerobic methane oxidizers, suggesting different chemical conditions within these groundwaters. These results provide baseline data for groundwater quality and microbiology in an area of active hydraulic fracturing, and suggest that microbial community studies may be useful in assessing future anthropogenic impacts on groundwater.

Isotopic and Molecular Analysis for Evaluation of In Situ Degradation of Brominated Compounds in Contaminated Fractured Aquifer

Presented by Zeev Ronen, Ph.D., Professor, Zuckerberg Institute for Water Research, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Israel

Co-Authors: N. Balabn, K. Kuntze, A. Bernstein, F. Gelman, L. Halicz, H.H. Richnow, I. Nijenhuis, and Z. Ronen

Brominated organic compounds (BOCs) have a broad spectrum of applications in form of flame retardants, pharmaceuticals or herbicides and many of these compounds are considered to have toxic, carcinogenic or mutagenic effects. Concepts to quantify in situ degradation of BOCs in the environment are lacking and degradation pathways and reaction mechanisms have not yet been fully elucidated. Therefore, the aim of this study is to determine the effect of in situ microbial activities on the fate of BOCs in a contaminated fractured chalk aquitard, in the Northern Negev, Israel. To assess the in situ biodegradation of brominated compounds, we apply compound-specific isotope analysis (CSIA) in combination with molecular biological approaches. Laboratory reference experiments with either enrichment cultures incubated under oxic and anoxic conditions were done to generate isotopic enrichment factors (ϵ) for carbon and bromine for selected compounds. Aerobic degradation experiment of Tribromoneopentyl alcohol (TBNPA) or Dibromoneopentyl glycol (DPNPG) with enrichment cultures showed a debromination with stoichiometric accumulation of bromide and significant isotopic effects on carbon only. Although, anaerobic reductive debromination of these compounds has not yet been observed, molecular analysis indicated the presence of dehalogenating bacteria like *Dehalococcoides* spp. in the site water. Preliminary investigations under anoxic conditions showed debromination activity of enrichments for ethylene dibromide, whereas no activity was observed in enrichments supplemented with dichloroethane. Further analysis of the overall diversity and CSIA during biodegradation will contribute to assess whether dehalogenation is taking place and which organisms are involved in this process.

2:00 PM – 3:20 PM
MERRILL HALL

SESSION A2 FATE: MICROBIAL FATE AND TRANSPORT

Moderated by:

- **Thomas Harter, Ph.D.**, Robert M. Hagan Endowed Chair, Water Management and Policy, Department of Land, Air and Water Resources, University of California, Davis, USA
- **Prof. Dr. Jack F. Schijven**, Senior Scientist, National Institute of Public Health and the Environment (RIVM), The Netherlands, and Professor, Environmental Hydrogeology Group, Department of Earth Sciences, University of Utrecht, The Netherlands



Non-Linear Particle Removal by Granular Media Filtration Attributable to Media Surface Roughness

Presented by Chao Jin, Ph.D. Candidate, Microfluidics Laboratory, University of Waterloo, Canada
Co-Authors: M.B. Emelko, C. Jin, T. Glawdel, and C. Ren

BACKGROUND: Natural and engineered filtration can be very effective at removing colloids and waterborne pathogens from water. Significant discrepancies between theoretical predictions and experimental observations are commonly reported when surface roughness is present on collector surfaces. Consensus regarding the contribution of surface roughness to particle deposition is currently not definitive. Surface roughness classification and mechanistic investigations are still lacking. **EXPERIMENTS:** Phase I: A bench-scale experiment was conducted to assess the impacts of macro- and micro-scale roughness on particle deposition in a packed column at favorable conditions for deposition. Normalized effluent concentration, retained particle distribution profile and total mass recovery ratio were utilized to evaluate the impact of roughness on particle deposition in packed media. Phase II: To confirm Phase I experimental observations and exclude the impacts from other mechanisms, experiments were conducted using a parallel plate chamber and rigorous quality assurance. The deposition rate was used to compare the particle removal difference between different roughness patterns. Numerical solutions of Convective-Diffusion Equation were calculated to interpret the experimental results. **FINDING:** Phase I results suggest that presence of surface roughness can not only enhance particle deposition, but also decrease it. A critical roughness size, associated with a dynamic equilibrium between physical /chemical mechanisms, exists that drives particle deposition impacts on roughness and therefore the observed deposition outcomes. Phase II results confirm those from Phase I, indicating that there is a non-linear relationship between surface roughness and particle deposition flux. Numerical solutions were able to describe particle deposition behavior at the conditions applied.

Size-Excluded Transport of Bacteria through Unsaturated Porous Media in the Presence of Surfactants

Presented by Derick G. Brown, Ph.D., Associate Professor, Department of Civil and Environmental Engineering, Lehigh University, USA
Co-Authors: D.G. Brown, S. Tripathi, and G.P. Lennon

A key assumption often made with groundwater is that pathogenic organisms from on-site wastewater treatment systems are retained at the soil/water and air/water interfaces as they pass through soil and ultimately die without reaching drinking water wells. In practice however, this assumption can fail and pathogens are often identified in groundwater surveys. Here we present results on the effects of surfactants – the largest anthropogenic component of domestic wastewater – on the transport of bacteria through unsaturated porous media. Specifically, we examined the effects of the nonionic surfactants Brij 30 and Brij 35 and the anionic linear alkylbenzene sulfonate (LAS) on the transport of *Escherichia coli* through laboratory columns packed with unsaturated Ottawa sand. Through a systematic series of experiments, we found that the time required for the bacteria to pass through the column decreased and the effluent bacterial concentration increased with increasing surfactant concentrations under all conditions examined. LAS impacted the transport to much greater levels than the Brij surfactants, and when present in mixtures, LAS was the dominant surfactant. Most interestingly, the decrease in transport time to cell breakthrough was not due to changes in flow conditions, as the breakthrough of conservative tracer was not affected by the presence of surfactants. Rather, the results suggest that the decreased transport time is due to an enhancement in the size-excluded transport of the bacteria, where the bacteria are forced into the higher velocity flow paths due to enhanced repulsion because of the sorbed surfactants.

Factors Affecting Transport of Bacteria and Microspheres through Biochar-Amended Soils

Presented by Carl H. Bolster, Ph.D., Research Hydrologist, U.S. Department of Agriculture, Agricultural Research Service, USA

Co-Authors: C.H. Bolster, S.M. Abit, K.B. Cantrell, J.Q. Flores, and S.L. Walker

We have investigated the role of biochar feedstock type (poultry litter extract and pine chips), biochar pyrolysis temperature (350 and 700 °C), biochar application rate (1, 2, and 10%), soil moisture content (saturated and 50% saturation), soil texture (1 and 12 % clay content), and surface properties on the transport of bacteria and microspheres through biochar-amended soils. Under most conditions we found that biochar addition to soils could significantly reduce bacterial and microsphere transport – in some cases up to five-orders-of-magnitude reduction in transport was observed. In general pine chip biochars were much more effective than poultry litter biochars at increasing microbial retention in our columns. Indeed, in some cases we observed an increase in microbial transport following addition of poultry litter biochars. High temperature biochars were generally more effective at increasing microbial retention in soils than low temperature biochars. Biochar addition to soils was observed to be more effective in partially saturated soils than fully saturated soils. We also found that bacteria with contrasting surface properties responded to biochar additions differently. Our results show that the addition of biochar can affect the retention and transport behavior of bacteria and that biochar application rate, biochar feedstock source, biochar pyrolysis temperature, soil moisture content, soil texture, and bacterial surface characteristics were all important factors determining the transport of bacteria through soil. Our results also suggest that the interactions between biochar and bacteria are complicated and need further investigations to determine the mechanisms involved.

Biotin- and Glycoprotein-Coated Microspheres as Surrogates for *Cryptosporidium Parvum* in a Granular Limestone Aquifer

Presented by Margaret E. Stevenson, University Assistant and Ph.D. Candidate, Centre for Water Resource Systems, Vienna University of Technology, Austria

Co-Authors: M.E. Stevenson, A.P. Blaschke, S. Toze, J. Sidhu, A.K.T. Kirschner, R. Sommer, A.H. Farnleitner, I.H. van Driezum, and L. Pang

Subsurface filtration is often used as a primary step for drinking water treatment because it is effective and cost efficient. One of the possible microbial contaminants in groundwater is *Cryptosporidium parvum*, entering groundwater through agricultural activities such as dairy farming or the spreading of fertilizer. *C. parvum* is of particular interest because of its risk of persisting in groundwater for long periods of time due to its thick cell wall in spore form and resistance to disinfection. Therefore, removal by filtration is preferable and many studies have attempted to find an appropriate surrogate for *C. parvum* so that experiments in the laboratory and in the field can be conducted. Recent research has looked at the possibility of biotin- or glycoprotein-coated microspheres as being an appropriate surrogate because glycoproteins are present on the oocysts' surface. Additionally, the surface charge potential (zeta potential) of the microspheres using a protein coating is more similar to the pathogen's zeta potential, compared to the common carboxylated coating used for most microsphere surrogates. Our work elaborates on this idea by testing the protein-coated surrogates in aquifer material taken from a managed aquifer recharge site in Adelaide, South Australia. It was found that biotin-coated polystyrene microspheres mimicked the transport of *C. parvum* well in the porous medium, and that glycoprotein-coated polystyrene microspheres seemed to be the best surrogate, due to the minimal breakthrough of glycoprotein-coated microspheres and *C. parvum* in column tests using the aquifer material. The tests also showed that granular limestone material is effective in removing *C. parvum* as a pre-treatment step for drinking water consumption.

2:00 PM – 3:20 PM
FRED FARR FORUM



SESSION B2 CONTAMINANTS: BIORESTORATION
Moderated by **Terry C. Hazen, Ph.D.**, UT/ORNL Governor's Chair Professor,
University of Tennessee and Oak Ridge National Laboratory, USA

Assessing Anammox and Denitrification in Wastewater-Contaminated Groundwater Using In Situ Natural Gradient Tracer Tests with 15N

Presented by Richard L. Smith, Ph.D., Lead Scientist, U.S. Geological Survey, USA
Co-Authors: R.L. Smith, B. Song, C.R. Tobias, and J.K. Böhlke

Anaerobic ammonium oxidation (anammox) is a process that couples the oxidation of ammonium with the reduction of nitrite, producing N₂. Previously, groundwater studies usually attributed the disappearance of nitrate coupled with an increase in N₂ solely to denitrification and would base C/N budgets and transport simulations on that assumption. However, the presence of anammox activity in groundwater could significantly alter that viewpoint. In this study, natural gradient experiments were conducted in a wastewater contaminant plume located on Cape Cod, Massachusetts using 15NO₂⁻ as tracer to determine the relative rates of anammox and denitrification. Tests were conducted in ammonium- and nitrate-containing zones and two tests with and without added acetate in a zone containing both constituents. Acetate was used to examine the competition between the processes when electron supply was not limiting. Rates of denitrification and anammox were determined by quantifying 28N₂, 29N₂, and 30N₂, changes in 15N/14N of nitrate, nitrite, and ammonium with time, and applying reaction-coupled transport simulations. Anammox contributed 44-62% of potential N₂ production in this aquifer, with rates on the order of 10-4 μmol N L⁻¹ hr⁻¹. Nitrate was produced via anoxic nitrite oxidation in some tests, indicative of autotrophic carbon fixation by anammox, while denitrification was preferentially stimulated during the acetate tests. These results demonstrate that anammox activity in groundwater can rival that of denitrification. Differential transport rates for

nitrate and ammonium, the potential for ammonium production from DOC degradation, and the competition with denitrification indicate that subsurface N-cycling is more complex than is commonly considered.

Effect of Microbial Interactions with Zero Valent Iron in Chlorinated Methane Dechlorination

Presented by Matthew Lee, Ph.D., Senior Research Associate, University of New South Wales, Australia

Co-Authors: M. Lee, J. Koenig, L. Adrian, and M. Manefield

Chlorinated methanes (CMs) are problematic in sub-surface environments as they are highly toxic and inhibitors anaerobic microbiological processes including organohalide respiration. Consequently, bioremediation of organochlorine polluted sites is severely hampered by the presence of CMs. Recent discoveries of chloroform (CF) and dichloromethane (DCM) dechlorinating bacteria have made the bioremediation of these sub-surface environments possible. Another approach to treating CMs is to install zero valent iron (ZVI) barriers in the path of the CM polluted ground water. ZVI transforms CMs to harmless chlorine free products. However, CF reacts slowly with ZVI and DCM not at all. In the current study we observed the effect of combining ZVI with CM dechlorinating Dehalobacter (DHB) species on the dechlorination of carbon tetrachloride (CT), CF and DCM. Our results showed that CF transformations in the presence of ZVI and DHB were five times faster than ZVI alone and three times faster than CF respiration where hydrogen was supplied as the electron donor. However, unreactive DCM was the major product (90%) from CF where DHB was present compared with 10% where ZVI alone was employed. DCM fermentation was three times faster in the presence of ZVI. We employed isotope ratio mass spectroscopy (IRMS) to observe carbon stable isotope fractionation during throughout all transformations. Different isotope enrichment factors were observed in DCM and CF when comparing the effect of ZVI with DHB with either ZVI or DHB alone. This suggests that direct ZVI to bacteria electron transfer maybe a possible mechanism in the dechlorination of these compounds.

Dichloromethane Dehalofermentation by a Novel Group of Peptococcaceae

Presented by Sara Kleindienst, Ph.D., Postdoctoral Fellow, Joint Institute for Biological Sciences, Oak Ridge National Laboratory, USA

Co-Authors: S. Kleindienst, S. Higgins, K. Chourey, B. Hettich, and F. Löffler

The C1-chlorinated aliphatic hydrocarbon dichloromethane (DCM) is produced naturally and is an anthropogenic contaminant. While aerobic DCM degradation under oxic conditions is fairly well understood, the microbiology and pathways contributing to DCM fate in the absence of oxygen are largely unexplored. A DCM-degrading enrichment culture was obtained from Rio Mameyes (Puerto Rico) freshwater sediment. To identify pathways, organisms, genes and proteins involved in DCM degradation, we applied 16S rRNA gene amplicon sequencing, metagenomics, metaproteomics, and mass balance approaches. Chloromethane (CM) was not detected during DCM degradation and the culture failed to grow with CM, indicating that reductive dechlorination was not involved. In bicarbonate-buffered medium amended with DCM as the sole energy source, the enrichment culture formed acetate, chloride, methane and biomass as products. Hydrogen was formed transiently and consumed in hydrogenotrophic methanogenesis and acetogenesis. 16S rRNA gene clone library and amplicon sequencing revealed a diverse community comprising members of the Firmicutes, Proteobacteria, Bacteroidetes, Spirochaetes and Euryarchaeota. A bacterium with <95% 16S rRNA gene sequence similarity to *Dehalobacter restrictus* was responsible for DCM degradation, presumably utilizing a fermentative pathway (“dehalofermentation”). Detailed phylogenetic analyses of full-length

16S rRNA gene sequences indicated that the dehalofermenter affiliates with a novel, unclassified cluster within the Peptococcaceae, possibly representing a new genus. Metagenome analysis and assembly of a draft genome revealed differences between Dehalobacter spp. and the DCM-degrading bacterium supporting the classification as a new genus. Furthermore, proteomic analysis of cultures grown with and without DCM identified proteins and pathways implicated in DCM dehalofermentation.

Clues from Geochemical and Proteomic Investigations of Metal-Linked Anaerobic Methane Oxidation

Presented by Jeffrey Marlow, Ph.D. Candidate, Division of Geological and Planetary Sciences, California Institute of Technology, USA

Co-Authors: J. Marlow, J. Steele, C. Skennerton, C. Pan, K. Chourey, Z. Li, R. Hettich, O. Sivan, G. Antler, A. Turchyn, and V. Orphan

The use of iron or manganese as electron acceptors for the anaerobic oxidation of methane (AOM) are two of the most prominent “missing metabolisms” that appear to be energetically possible. Past studies in search of metal-based AOM have revealed methane oxidation in the presence of ferrihydrite and birnessite, but the mechanism remains uncertain. In particular, can microorganisms “directly” couple methane oxidation to metal reduction, or does “traditional” sulfate-based AOM persist through the regeneration of sulfate by abiotic metal-sulfide reactions? In this study, we present geochemical and isotopic data in support of a sulfur-based metabolism whose reactants are produced abiotically. In a set of methane seep sediment incubations, reduced metals and activated methane were tracked over a two-year period, and we propose a mechanism for both kinetic and geochemical inhibition of methane oxidation in the presence of iron and manganese. Another approach for investigating metabolic processes involving metal-based anaerobic methane oxidation utilizes proteomic surveys of ferrihydrite- and vernadite-amended incubations. The analysis of these proteomes is ongoing, but analogous experiments with sulfate as the electron acceptor have populated baseline metagenomes and metaproteomes, revealing gene products that, if found in the newly synthesized protein fraction, could point to “direct” metal reduction. Through this complement of experiments, we are well positioned to offer multiple new lines of evidence regarding the feasibility of metal-based methane oxidation.

2:00 PM – 3:20 PM
NAUTILUS ROOM

SESSION C2 BIOMES: SUBSEAFLOOR AND COASTAL MICROBIOLOGY

Moderated by:

- **Prof. Dr. Kenji Kato**, Department of Geosciences, Graduate School of Science, Shizuoka University, Japan
- **R. John Parkes FLSW, FSB, FAAM, FRS**, Head of School and Distinguished Research Professor, School of Earth and Ocean Sciences, Cardiff University, United Kingdom



Putting Age into the Equation: A New Look at Microbial Distribution in Subseafloor Sediments

Presented by Jens Kallmeyer, Ph.D., Researcher, German Research Centre for Geosciences (GFZ),
Helmholtz Zentrum Potsdam, Germany

Author: J. Kallmeyer

Cell abundance in subseafloor sediments varies by up to six orders of magnitude. To a large extent this variability correlates with mean sedimentation rate and distance from land and can therefore be predicted (Kallmeyer et al., 2013). Usually, changes in subseafloor microbial abundance with depth can be described by power-law function. However, at some sites, cell distribution strongly deviates from this trend. To better understand why cell distribution at some sites exhibits unusual patterns it is necessary to include sedimentation rates and therefore sediment ages into the model. IODP Exp. 320 and 321 recovered a continuous Cenozoic record of the paleoequatorial Pacific by coring above the paleoposition of the highly productive equatorial upwelling zone at successive crustal ages on the Pacific plate. Although some unusual cell distribution are caused by diagenetic alteration fronts, the significant differences in sedimentation rates between within and outside the upwelling zone appear to be the major cause for the strong deviations in cell abundance from the expected power-law decline with depth. Subseafloor microbial communities have to subsist with organic matter that becomes increasingly recalcitrant with increasing age. Cells vs. age correlations show less variability between sites than correlations vs. depth. These observations suggest that subseafloor microbial abundance is controlled by organic matter reactivity, which is largely controlled by sediment age. Plotting cells vs. age instead of depth might provide important clues about the finer details of subseafloor cell abundance, especially in areas that have not fitted into previous models.

An Active Microbial Community in Fluids Circulating through the Cold Oceanic Crust beneath the Mid-Atlantic Seafloor

Presented by Julie A. Huber, Ph.D., Associate Scientist, Marine Biological Laboratory, USA

Co-Authors: J.A. Huber, J.L. Meyera, U. Jaekelb, B.T. Glazer, C.G. Wheat, S.M. Hulme, T. Dittmar, and P.R. Girguisb

The cold, basalt-hosted, oceanic crustal aquifer is one of the largest ecosystems on Earth, yet little is known about its indigenous microorganisms. Recently installed subseafloor observatories on the western flank of the Mid-Atlantic Ridge penetrate hundreds of meters into the crust and provide an unprecedented opportunity to investigate microbial life in this understudied realm. Here, we present the first evidence for an active microbial community in fluids that circulate through the crustal aquifer beneath North Pond, a ~200-m-thick sediment-filled basin overlying relatively young basaltic crust (8 Ma). Microbial biomass in fluids from multiple depth horizons of the subseafloor observatory was similar to deep Atlantic bottom seawater (1×10^4 cells/ml) and dominated by bacteria. Chemical analysis of this fluid showed little differentiation from deep seawater. Incubations of basaltic formation fluids with ^{13}C -labeled bicarbonate or acetate at 5°C and 25°C revealed potential rates of autotrophy that exceeded those of heterotrophy by an order of magnitude. Autotrophy in fluid samples from the deepest horizon of the observatory at 25°C exceeded rates of carbon transformation under 5°C conditions. 16S rRNA Illumina tag sequencing showed that the microbial community of these formation fluids is dominated by Proteobacteria, but unlike deep Atlantic seawater, the dominant genera detected in both DNA and RNA fractions of formation fluids include Colwellia, Acidiferrobacter, and Sulfurimonas, which are likely seeded from deep seawater, basaltic rocks, and marine sediment, respectively. Our results provide the first snapshot of an active bacterial community geared toward autotrophic processes in a cold, oxygenated crustal aquifer.

Deep Seawater Circulation through Oceanic Crust Fuels Sulfate-Dependent Anaerobic Oxidation of Methane at ~400 Meters below the Seafloor in the Nankai Trough, Southwest Japan

Presented by Toni L. Cox, Ph.D. Candidate, School of Earth Sciences, University of Melbourne, Australia

Co-Authors: T.L. Cox, M.E. Torres, and J.W. Moreau

We investigated the microbiology of a sulfate-methane transition zone (SMTZ) in the Nankai Trough (IODP Expedition 322), south-western Japan. Sediment pore fluid chemistry revealed sulfate delivered to the SMTZ through subseafloor circulation of seawater. Diffusional exchange with overlying sediments supports the anaerobic oxidation of methane at ~400 mbsf, with oxidation rates being similar in magnitude to those we estimate using data from the Juan de Fuca Ridge Flank. Whole community genomic DNA was extracted from sediment cores from ~300-450 meters below seafloor (mbsf), and sequenced for bacterial and archaeal diversity. Bacterial sequences were most closely related to a suite of uncultured marine bacterial clones. Archaeal sequences were closely related to *Methanosarcina mazei* (99%), *Methanolobus psychrophilius* (98%) and *Methanospirillum hugatei* (98%), which previous studies have shown to be close relatives to archaea detected in subseafloor anaerobic methane oxidation zones. Deep SMTZ may therefore represent a reactivated subsurface biosphere, isolated from surface influences, but dependent on the hydrogeology of the subseafloor crust.

Diversity and Transport of Microorganisms in Intertidal Sands of the California Coast

Presented by Alexandria B. Boehm, Ph.D., Associate Professor, Stanford University, USA

Co-Authors: A.B. Boehm, K.M. Yamahara, and L.M. Sassoubre

Forced by tides and waves, large volumes of seawater are flushed through the beach daily. Organic material and nutrients in seawater are remineralized and cycled as they pass through the beach. Microorganisms are responsible for most of the biogeochemical cycling in the beach, however, very few studies have characterized their diversity in intertidal sands, and little work has characterized the extent to which microbes are transported between different compartments of the beach. The present study uses next generation massively parallel sequencing to characterize the microbial community present at 49 beaches along the coast of California. In addition, we characterize the transport of microorganisms within intertidal sands using laboratory column experiments. We identified immense diversity in the beach sands. Nearly 1000 unique taxa were identified in sands from 10 or more unique beaches suggesting the existence of a group of 'cosmopolitan' sand microorganisms. A biogeographical analysis identified a taxa-distance relationship among the beaches. In addition, sands with similar grain size, organic carbon content, exposed to a similar wave climate, and having the same degree of anthropogenic influence tended to have similar microbial communities. Column experiments identified microbes readily mobilized by seawater infiltrating through unsaturated intertidal sands. The ease with which microbes were mobilized suggests intertidal sands may represent a reservoir of bacteria that seed the beach aquifer where they may partake in biogeochemical cycling.

3:40 PM – 5:20 PM
MERRILL HALL

SESSION A3 FATE: AQUIFER RECHARGE MICROBIOLOGY

Moderated by:

- **Menu Leddy**, Principal Scientist, Orange County Water District, USA
- **James G. Mitchell, Ph.D.**, Professor, School of Biological Sciences, Flinders University, Australia



Diurnal Changes in Carbon Cycling Dynamics in an Oligotrophic Karst Aquifer under Base Flow Conditions

Presented by Kathleen Brannen, Ph.D. Candidate, Department of Earth and Planetary Sciences, University of Tennessee, USA

Co-Authors: T. Brown, K. Brannen, A.S. Engel, and S. Pfiffner

Water flowing into and through epigenic caves hydrologically links the surface to the subsurface and delivers allochthonous dissolved organic matter (DOM) that can support groundwater-dependent ecosystems. To assess DOM compositional changes due to photodegradation or microbial processes, diurnal DOM composition under base flow conditions was evaluated from a 2-km long sinking stream using UV-Vis, fluorescence, and infrared absorbance spectroscopy, and assessment of microbial communities using 16S rRNA gene sequences and phospholipid fatty acid (PLFAs) profiles. Dissolved organic carbon concentrations (<5 mg/L) fluctuate slightly along the flowpath, and chromophoric DOM does not significantly differ from day to night. However, DOM aromaticity and humification differ between upstream and downstream sites diurnally, and aromatic DOM present at night and in the cave is absent at the surface (in daylight), suggestive of photodegradation. From FTIR spectra of DOM isolates, some macromolecules ubiquitous in the cave were only present at the surface at night, and others increased from day to night along the flowpath. Betaproteobacteria (Burkholderiales) and Bacteroidetes (Flavobacteriales) dominated microbial communities, with increased relative abundances of Flavobacteriales at night. PLFA profiles reflected diverse bacterial assemblages in both surface and cave waters; however, day and night ratios of bacterial-to-eukaryotic PLFAs were 5:1 for surface water and 15:1 for the cave. Whereas community structure and biomass varied nominally along the flowpath under base flow conditions, these data indicate that microbial carbon cycling processes that operate at all times in the cave system produce macromolecules similar to processes only operating at the surface during dark hours.

Use of Aquifers as Natural Treatment Barriers during Managed Aquifer Recharge

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

Co-Authors: S. Toze, J. Sidhu, L. Hogers, K. Barry, D. Page, and P. Dillon

Managed Aquifer Recharge (MAR) employs the capture, recharge and storage of water into an aquifer for later recovery and beneficial use. While a range of water types can be used as the recharged water, MAR has particular potential in aiding the capture and reuse of environmental and wastewater

sources. These water types can contain a range of contaminants with microbial pathogens being of immediate concern due to the potential to cause chronic illness and wide spread contamination of environmental water and wastewater. Considerable research has been undertaken in Australia demonstrating that pathogen decay in aquifers during MAR does occur. The ability to validate a natural system such as aquifers as active treatment barriers, however, is difficult due to a lack of processes that can enable an appropriate validation protocol to be accepted by health regulators. This presentation provides outcomes of the comparison of different MAR schemes in varying geographical regions, recharging different water sources, and using a variety of recharge methods. Analysis of the results from the different sites found that when there is oxygen present, pathogen removal is not influenced by geographical location, recharge water type or recharge method. When there is little or no oxygen present and the redox is low, pathogen decay slows and can even cease. The outcomes demonstrate that a validation process can be established as long as aquifer conditions and water residence times are well understood. The validation processes could even help design robust MAR schemes as treatment barriers or assist in development of guidelines on MAR.

Detecting Low Concentrations of MS2 Coliphage to Demonstrate the Efficacy of Soil-Aquifer Treatment of Secondary Effluent

Presented by Richard Danielson, Ph.D., Laboratory Director, BioVir Laboratories, Inc., USA
Co-Authors: R. Danielson, J. Truscott, and R. Cooper

The State of California Department of Public Health (CA DPH) is requiring wastewater utilities utilizing groundwater recharge and extraction operations through soil-aquifer treatment (SAT) to demonstrate a 99.999 percent (5-log) reduction of the plaque-forming units (pfu) of male-specific (MS) bacteriophage (phage). The secondary effluent of many treatment plants discharge phage concentrations below the sensitivity level of standard protocols. Analysis of large volumes of extracted water are necessary to show that the 5-log reduction is being met. In this study, a modification of the presence-absence format of EPA Method 1601 was validated for use in demonstrating MS phage concentration, in a most probable number (MPN) format, for volumes from 30 L to 300 L. For the larger volumes (300 L), three electro-positive filters processing 100 L each were used. A 3-tube, one dilution MPN calculation was made to demonstrate a detection limit of 0.0004 MPN/100 mL. This method was applied to two sites where utilities were attempting to demonstrate 5-log reduction by SAT. One utility consistently demonstrated >5-log reduction by SAT, while the other was sporadically in compliance with the 5-log reduction goal. This method can be applied where tertiary filtration through percolation to groundwater can be used by utilities to avoid unnecessary construction costs associated with advanced water treatment for reclaimed water production.

Nonpoint Source Loading of Fecal Bacteria to a Shallow Heterogeneous Aquifer: Stochastic Forcing Versus Stochastic Transport

Presented by Thomas Harter, Ph.D., Robert M. Hagan Endowed Chair, Water Management and Policy, Department of Land, Air and Water Resources, University of California, Davis, USA
Co-Authors: T. Harter, S. Cook, X. Li, E.R. Atwill, A.I. Packman, C. Puente, and N. Watanabe
Manure and wastewater irrigation presents a microbiological risk to shallow groundwater quality putting domestic wells at risk, particularly in rural areas. Multiple and persistent sources of fecal contamination suggest extensive groundwater contamination. Yet, monitoring of fecal bacteria in groundwater indicates erratic prevalence and - if detectable - highly variable concentrations. We present a conceptual and modeling framework to determine the degree to which nonpoint source loading variability, aquifer heterogeneity, and variability in microbial transport dynamics explain

observed spatio-temporal patterns of pathogen occurrence in groundwater subject to application of manure or wastewater. Manure loading heterogeneity is assessed by incorporating stochastic nonpoint source loading functions. Aquifer heterogeneity is represented by hydrostratigraphic simulations of a range of heterogeneous alluvial aquifer types. Simulations indicate that breakthrough to wells is generally limited to shallow depths, suggesting limited risk to domestic wells screened several tens of meters below the water table. The presence of aquifer heterogeneity significantly extends the transport distance and tailing of breakthrough curves due to remobilization and preferential flow paths. Owing to macro-dispersion and in-well mixing, simulated breakthrough curves are relatively smooth and consistent with observed longitudinal studies. Spatio-temporally variable source loading of manure or wastewater effluent across a field or catchment, in contrast, contributes significantly to the sporadic occurrence measured in shallow groundwater wells. This suggests that the highly erratic and variable nature of microorganism detection may be due to highly transient processes, including but not limited to spatio-temporal variations in source variability.

Bacteria Removal by Biochar: Recent Results and Unexplored Opportunities

Presented by Sanjay K. Mohanty, Ph.D., Postdoctoral Researcher, Department of Civil and Environmental Engineering, Stanford University, USA

Co-Authors: S.K. Mohanty, K. Cantrell, K.L. Nelson, and A.B. Boehm

Numerous studies have shown that biochar—a carbonaceous geomeedia made out of waste biomass—has the potential to remove heavy metal, organic chemicals, and nutrients from contaminated water. However, few studies have examined the removal of microbial contaminants from water by biochar. We examined the removal of fecal indicator bacteria (*E. coli*) from synthetic stormwater with and without natural organic matter (NOM) by columns containing sand and sand mixed with biochar. To evaluate the effect of biochar properties on bacterial removal, we used three types of biochar: a commercially available biochar, and two steam-activated biochars produced in the laboratory via pyrolysis of wood chips at 350 and 700 °C, respectively. Compared to sand, biochar not only retained up to 3 orders of magnitude more *E. coli* during injection of contaminated stormwater, but also prevented mobilization of the attached *E. coli* during successive intermittent flows with sterile stormwater. NOM lowered the removal capacity of biochar. Biochars with lower volatile matter and polarity appear to be more effective in removing bacteria. These results suggest that biochar is efficient in removing bacteria in the short term, but the removal could vary with the properties of biochar and the composition of water. Although the current study has improved the understanding of bacterial attachment to biochar, the long-term removal and fate of attached bacteria need further studies.

3:40 PM – 5:20 PM
FRED FARR FORUM

SESSION B3 **CHEMISTRY: MICROBE-MINERAL INTERACTIONS (MOLECULAR FOCUS)**

Moderated by:

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



Diverse Arsenic- and Iron-Cycling Microbial Communities in Arsenic-Contaminated Drinking Water Wells in Bangladesh

Presented by Md. Zahid Hassan, Ph.D. Candidate, Department of Molecular Cell Physiology, VU University Amsterdam, The Netherlands

Co-Authors: Z. Hassan, M. Sultana, M. van Breukelen, S.I. Khan, and W.F.M. Röling

The availability of “safe” drinking water is a big issue in Bangladesh. Natural high concentrations of arsenic in groundwater are a major concern, threatening 77 million people and killing nearly 43,000 people per year. Recently, a cost-effective Subsurface Arsenic Removal (SAR) technology has been introduced. SAR contributes to the in-situ removal of arsenic by the injection with oxygenated water, through the oxidation of ferrous iron to solid ferric iron minerals to which arsenic adsorbs. While the oxidation reaction is considered to be abiotic, metal-oxidising or reducing microorganisms may potentially have positive or negative contributions to arsenic removal, respectively. We hypothesized that the potential for microbial iron- and arsenic-cycling is widespread in arsenic-containing groundwater in Bangladesh. We conducted a combined cultivation-independent molecular and culturing-based survey covering 24 different sites in several geographical regions in Bangladesh and assessed microbial community structure and diversity in general, and addressed specific functional groups capable of the oxidation and reduction of arsenic and iron, in relation to hydrochemistry. The specific groups were indeed widespread, 83% of samples contained arsenic reducing microorganisms (based on *arrA* detection), at low diversity, while all samples revealed arsenic oxidizing microorganisms (based on the *aioA* gene), at relative high alpha- and beta-diversity. 79% of samples harbored iron oxidizing *Gallionella*-like 16S rRNA genes, and 83% was positive for iron-reducing *Geobacteraceae* and *Desulfuromonadaceae*. Our data suggest that there are microorganisms that may have positive roles (arsenite-oxidisers and microaerophilic ironoxidisers), but also potential negative contribution (anaerobic arsenate reducers and iron-reducers) for arsenic mobilization.

Anthropogenic CO₂-H₂S-H₂ Injection in Deep Basalts Stimulate Biofilm Development and Microbially-Induced Mineralizations that Favor Reservoir Clogging

Presented by Rosalia Trias, Ph.D., Postdoctoral Researcher, Institut de Physique du Globe de Paris, France

Co-Authors: R. Trias, E. Gérard, P. Le Campion, E.S. Aradóttir, I. Gunnarsson, S.R. Gíslason, and B. Ménez

Carbon capture and storage in the deep subsurface is proposed as a mitigating solution for reducing anthropogenic CO₂ emissions in the atmosphere. The efficiency for mineral storage in mafic rocks is being evaluated by the CARBFIX consortium (<https://www.or.is/en/projects/carbfix>) at the Hellisheidi pilot site (Iceland). In 2012, two injections were performed (i.e. pure CO₂ or H₂S/CO₂/H₂ mix from gas separation station associated to the power plant). The last injection was interrupted because of a decrease of one order of magnitude of the injectivity index. Back-flushing of the injection well by airlift pumping was then performed for remediation. We have studied the microbial diversity and the mineralogy of the collected materials in order to understand the causes of clogging. Molecular analyses revealed an increase of gene copy numbers (detected by qPCR) and a loss of diversity, in comparison to pre-clogging communities. The endemic sulfur-oxidizing *Thiobacillus* and the iron oxidizing/reducing *Geobacter* were the main representatives of the bacterial communities, as revealed by 454-tagged pyrosequencing. Microscopic observations evidenced that H₂S reacted with the Fe-bearing basaltic minerals to abiotically form submicrometric Fe-sulfides, then oxidized by *Thiobacillus* that formed to adhere to this substrate, a dense biofilm aggregating individual Fe-

sulfides. Oxidation byproducts were in turn used by iron-metabolizing bacteria, forming large crusts of cell-entombing Fe-oxi-hydroxides. The overall process, interpreted as the result of the stimulation at depth of specific activities, converted submicrometric Fe-sulfides into compact microbially-induced mineralizations, up to hundreds of micrometers in size, likely filling the basalt porosity, with a deleterious impact on the injectivity.

Fe-Oxidizing Microorganisms in Microscopic Model Aquifer Systems: Feedbacks between Flow and Biomineralization

Presented by Clara Chan, Ph.D., Assistant Professor, Department of Geological Sciences, University of Delaware, USA

Co-Authors: C. Chan, K. Cabaniss, K. Williams, M. Moore, H. Michael, J. Caplan, and C. Lin

Fe oxidation and (bio)mineral formation are important in aquifers because the highly-reactive oxides can control the mobility of nutrients (e.g. phosphate, C) and metals (e.g. arsenic). Mineral formation also has the potential to affect hydrology, depending on the volume and distribution in pore spaces. In this study, we sought to understand how microbial Fe-oxidizers and their biominerals affect, and are affected by groundwater flow. As part of work at the Rifle aquifer in Colorado, we initially hypothesized that Fe-oxidizers were contributing to aquifer clogging problems associated with enhanced bioremediation. To demonstrate the presence of Fe-oxidizers in the Rifle aquifer, we isolated a novel chemolithotrophic, microaerophilic Fe-oxidizing Betaproteobacteria, *Hydrogenophaga* sp. P101. This organism was grown in a “mini-aquifer,” a sand-filled flow-through microscope chamber that we followed using confocal microscopy. Fe oxide biofilms formed on sand grains, but the biominerals did not clog the mini-aquifer. Instead, Fe biofilm distribution was dynamic: they grew as coatings, then periodically sloughed off sand grains, with some flocs later caught in pore throats. This has implications for both physical hydrology and element transport. The changes in pore scale architecture diverted fluid flow, with implications for dispersion. While intact biomineral coatings retain Fe and associated elements (P, As, C), when biofilms slough, these elements are transported through the aquifer in the particulate phase. Our work shows that microbial mineralization impacts in aquifers are dynamic, and that the fate and transport of biomineral-associated elements depend not only on geochemical conditions, but also physical pore-scale processes.

Biogeochemistry of Sulfate-Reducing Bioreactors Treating Acid Rock Drainage

Presented by Jonathan O. Sharp, Ph.D., Assistant Professor, Department of Civil and Environmental Engineering, Colorado School of Mines, USA

Co-Authors: R. Almstrand, D.M. Drennan, I. Lee, J. Ladderud, L. Landkamer, S.M. Webb, L. Figueroa, and J.O. Sharp

A favorable approach for mitigating acid rock drainage impact is construction of passive sulfate-reducing bioreactors (SRBRs) to allow for microbially catalyzed sulfate reduction. In order to test long-term effects of different organic substrates on process efficiency (Zn-removal) and microbial community composition, eight pilot-scale SRBRs were constructed and monitored for one year. Canonical correspondence analysis showed that Zn-removal was positively correlated with organic substrates containing alfalfa hay, whereas the opposite was shown for woodchips. A novel approach combining scanning-electron microscopy and energy dispersive X-ray spectrometry (SEM-EDS) for in situ analysis of column precipitates revealed that Ca and S were preferentially associated in poorly performing columns (low Zn-removal), as opposed to Zn and S in well performing ones. Moreover, a bench-scale column experiment was initiated to couple microbial community spatial distribution with

detailed redox-chemistry of metal sulphide precipitates by combining Illumina sequencing and synchrotron techniques (X-ray Absorption Fine Structure). Examination of the sulfate-reducing clades in the pilot-scale columns, revealed that the genera *Desulfosporosinus* and *Desulfurispora* coincided with high Zn-removal. In contrast, columns where the major sulfate-reducers were related to *Desulfosporomusa* consistently removed less Zn while the presence of the metabolic generalist *Desulfobulbus* did not show any correlation with process performance. Preliminary data suggest that a similar pattern may exist for key cellulose degraders, such as *Dysgonomonas*. The relative abundance of cells related to this genus was negatively correlated with Zn-removal. Phylogenetic affiliation of the major bacterial populations could be an important indicator and predictive tool for sulfate-reducing bioreactor potential.

Electrode Cultivation of Subsurface Microorganisms

Presented by Yamini Jangir, Ph.D. Candidate, University of Southern California, USA

Co-Authors: Y. Jangir, L.M. Momper, J.P. Amend, and M.Y. El-Naggar

A comprehensive characterization of the subsurface biosphere is hindered because the majority of the resident archaea and bacteria appear to be ‘unculturable.’ We here report recent progress in developing electrochemical platforms, based on physical electrodes that function as electron donors and acceptors for respiration, to cultivate slow-growing subsurface microbes. We describe two electrode cultivation platforms: (1) a multi-liter volume, bioelectrochemical cultivation platform based on carbon fiber cloth working electrodes and (2) a microfluidic ‘on-chip’ platform based on microfabricated electrodes. Both platforms mimic interfacial electron transport as a central mechanism for microbe-mineral energy transfer in the subsurface, while allowing the simultaneous exploration of multiple redox potentials in a single experiment. Preliminary characterization was performed on samples from Nevares deep well-2 (NDW2) in Death Valley, CA. This site is an artesian well drilled into dolostone rock, intersecting fault-associated, deeply sourced water. Collected well water was used to enrich for subsurface microorganisms at four different redox potentials. Preliminary 16S rRNA analyses point to the growth of metal reducers, including *Rhodoferrax ferrireducens* which are currently being isolated. Electrochemical cyclic voltammetry reveals redox activity at -200 mV vs Ag/AgCl, providing clues into the interfacial electron transfer mechanisms dominant in these reactors. Molecular biological measurements are ongoing to assess the microbial diversity dominant in the reactor. This study demonstrates an alternate method for culturing subsurface microbes, while using physical electrodes to emulate the microhabitats, redox and geochemical gradients, and the spatially dependent interspecies interactions encountered in the subsurface.

3:40 PM – 5:20 PM

NAUTILUS ROOM

SESSION C3 BIOMES: SUBSEAFLOOR MICROBIOLOGY

Moderated by:

- **Prof. Dr. Kenji Kato**, Department of Geosciences, Graduate School of Science, Shizuoka University, Japan
- **R. John Parkes FLSW, FSB, FAAM, FRS**, Head of School and Distinguished Research Professor, School of Earth and Ocean Sciences, Cardiff University, United Kingdom



Geographical Molecular Analysis of the Deep Subseafloor Sedimentary Microbiomes

Presented by Tatsuhiko Hoshino, Ph.D., Senior Researcher, Geomicrobiology Group, Kochi Institute for Core Sample Research, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Japan

Co-Authors: T. Hoshino, Y. Morono, and F. Inagaki

To date, the total microbial cell number of marine subseafloor environments was estimated to be 2.9×10^{29} cells, corresponding to approximately 0.6% of the total living biomass carbon on Earth [1]. Accumulating evidences suggest that activity of subseafloor microbes may play significant roles in biogeochemical elemental cycles over geological timescales. However, spatial distribution of subseafloor microbial communities has still remained largely unknown: i.e., what is the geographical distribution pattern of the taxonomic composition, community size and structure, phylogenetic and functional diversity of subseafloor sedimentary microbiomes? How do these patterns correlate to the environmental factors such as physical properties and geochemical characteristics? Using over 200 deep-frozen sediment core samples that have been collected by previous ODP/IODP and other drilling expeditions from a variety of oceanographic settings, we extracted microbial DNA by a hot-alkaline extraction method [2] and a commercial kit, followed by molecular quantification of archaeal and bacterial 16S rRNA genes using microfluidic digital PCR (dPCR) [3]. Preliminary results of dPCR showed that the total molecular amount of subseafloor microbes generally decreases with increasing depth and the current average ratio of archaeal and bacterial 16S rRNA genes is in good agreement with our previous estimate using real-time PCR and slot-blot hybridization analyses [4]. In this project, all PCR products, including some new and upcoming sampling opportunities, will be sequenced by next generation sequencers, providing a comprehensive geographical view of the deep subseafloor sedimentary microbiomes. References: [1] Kallmeyer et al. (2012) PNAS 109:16213-16219. [2] Morono, Y., et al. (2014) Appl Environ Microbiol 80:1985-1994. [3] Hoshino, T. and Inagaki, F. (2012) Syst Appl Microbiol 35:390-395. [4] Lipp, J.S., et al. (2008) Nature 454:991-994.

Breaching the Subsurface: Potential Cell Transport Pathways as Revealed by the Biogeography of Spore-Forming Thermophiles in Marine Sediments

Presented by China Hanson, Ph.D., Postdoctoral Research Associate, Civil and Engineering Geosciences, Newcastle University, United Kingdom

Co-Authors: C. Hanson, A. Müller, A. Loy, J.R. de Rezende, B.B. Jørgensen, and C. Hubert

Subsurface habitats are leaky due to geological features such as hydrocarbon seeps, hydrothermal vents, discharging seamounts, and mud volcanoes. Geofluids may thus passively transport deep biosphere-inhabiting microbes both through and out of the subsurface. If these “hitchhiker” organisms can survive this transport and persist outside their preferred habitats, then they should be detected in surface environments near leakage point sources. In marine systems, subsequent dispersal by ocean currents might result in a decreasing abundance of cells away from the source. In this way, biogeography in surface environments may provide novel information about the diversity and ecology of deep subsurface-adapted microbes. Consistent with this is the discovery of thermophilic Firmicutes present as dormant endospores in marine surface sediments, where in situ conditions are too cold to support their germination and growth. These “thermospores” are physiologically and phylogenetically diverse and are closely related to Clostridia commonly found in deep anoxic habitats such as petroleum reservoirs or mid ocean ridges. The distribution of thermospores in marine sediments is spatially variable, both globally and regionally. For example, in Arctic sediments near Svalbard, certain thermospore phylotypes appear to be unique to certain fjord sediments, while others are more

widespread. This suggests that not only are some thermospores likely hitchhiking out of the deep biosphere, but that their fate may also be shaped by different geofluid transport pathways. Ongoing work to quantify biogeographic variation in thermospore abundance can therefore elucidate previously unconsidered dispersal mechanisms for deep biosphere organisms in particular, and for marine microorganisms in general.

Exploring Microbial Growth at High Pressure

Presented by Karyn L. Rogers, Ph.D., Assistant Professor, Department of Earth and Environmental Sciences, Rensselaer Polytechnic Institute, USA

Author: K.L. Rogers

A majority of the Earth's prokaryotes reside in high-pressure, subsurface environments(9) and piezophiles have been isolated from deep boreholes, deep ocean basins and deep-sea hydrothermal vents(1). Furthermore, deep-sea hydrothermal systems have been posited as locations for the emergence of life on Earth(3,4), and the potential for life in high-pressure, subsurface environments on Mars and Europa, has also received considerable attention(5-8). Despite the fact that most of Earth's prokaryotes reside in high-pressure environments and the potential for extraterrestrial high-pressure habitats(2), the effect of pressure on habitability is not well understood. In deep-sea hydrothermal systems, pressure plays a multifaceted role in microbial survival, stabilizing biomolecules that are otherwise susceptible to thermal degradation and allowing aqueous concentrations of volatile species that far exceed what can be found in surface systems where partial pressures are limited to the total surface pressure. Increased aqueous activities of volatiles can have significant impacts of metabolic energy yields and subsequent microbial growth, however the physiologic and bioenergetics effects of pressure on microbial growth are poorly understood and high-pressure growth apparatus are not common. Here we will describe the recent development of a new high-pressure, high-temperature microbial growth chamber, as well as the development of a new, community facility that accommodates subsampling and transport of high pressure samples. 1. Trends Biot (2001), 19(3)108; 2. Trends Microb (1999), 7(11); 3. OLEB (1985), 15; 4. OLEB (1992), 22; 5. JGR (1998), 103(E8); 6. JGR (1999), 104; 7. JGR (1997), 102(E10); 8. Astrobiol. (2003), 3(2), pp.407–414; 9. PNAS (1998), 95(12), p.6578.

Metabolic Characteristics of Microorganisms Inhabiting Basement Basalt of the Juan de Fuca Ridge Flank Revealed by Metagenomics

Presented by Michael S. Rappe, Ph.D., Associate Research Professor, Hawaii Institute of Marine Biology, University of Hawaii at Mañoa, USA

Co-Authors: M.S. Rappe, S.P. Jungbluth, H.-T. Lin, and B.N. Orcutt

Microbial communities that are distinct from both overlying sediments and bottom seawater inhabit the subsurface fluids circulating within the basaltic crust of the Juan de Fuca Ridge flank. In order to characterize the metabolic potential and genomic characteristics of microbes residing in this region of the crustal oceanic subsurface, we sequenced environmental DNA extracted from fluid samples obtained from advanced Circulation Obviation Retrofit Kit (CORK) observatories installed at two recently drilled boreholes, U1362A and U1362B. From the deep (204 m below the sediment-basement interface) basement horizon of Borehole U1362A and shallow (40 m below the sediment-basement interface) basement horizon of Borehole U1362B, 503 and 705 million base-pairs of genomic DNA sequence data was generated, respectively. A survey of phylogenetically informative genes revealed that the community structure recovered via metagenomics was generally consistent with that obtained previously by 16S rRNA gene sequencing and was dominated by uncultivated lineages of

Proteobacteria, Nitrospirae, Candidate Division OP8 and Euryarchaeota. Significant metagenome assembly resulted in 72 and 105 contigs of >100 Kbp from U1362B and U1362A, respectively, including 1137, 977 and 356 Kbp long contigs from Candidate Division OP8 residing in U1362B. These assemblies facilitate the functional characterization of abundant community members, and revealed novel physiologies adapted for survival in the deep oceanic crust.

Development of Analytical Technologies for Low-Biomass Microbial Communities in the Subseafloor Sedimentary Biosphere

Presented by Yuki Morono, Ph.D., Senior Researcher, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Japan

Co-Authors: Y. Morono, T. Hoshino, M. Ito, and F. Inagaki

One of the primary scientific objectives in the deep biosphere is to understand the factors that controls biomass, activity and diversity of microbial communities in marine subsurface sediments. To address the issue regarding the limits and habitability of subseafloor life, development of analytical technologies that customizes for low-biomass samples is necessary. To date, an improved cell separation [1] followed by fluorescence image-based cell count [2] have lowered the quantification limit by a factor of 500 from that of the standard microscopic direct count, enabling us to study activity of individual cells by using cell sorter and nano-scale secondary mass spectrometry (NanoSIMS) [1,3]. In addition, a new hot-alkaline DNA extraction method has significantly improved cell disruption efficiency [4]. These new analytical technologies can be used systematically to explore extreme subseafloor environment such as ultra-oligotrophic South Pacific Gyre (IODP Expedition 329) and deep coalbed biosphere off Shimokita (IODP Expedition 337). References: [1] Morono, Y., et al. (2013) *Environ Microbiol* 15:2841-2849. [2] Morono, Y., et al. (2009) *ISME J* 3:503-511. [3] Morono, Y., et al. (2011) *PNAS* 108:18295-18300. [4] Morono, Y., et al. (2014) *Appl Environ Microbiol* 80:1985-1994.