



# **Ninth International Symposium on Subsurface Microbiology**

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

---

**Thursday, October 9, 2014**

**POSTER PRESENTATION ABSTRACTS**

---

**POSTER SESSION B: CONTAMINANTS, CHEMISTRY, AND METHODS**

**7:00 PM – 8:30 PM**

**KILN ROOM**

---

Poster B1

**Chemotaxis toward NAPL Sources Localized within a Sand-Packed Column Impacts the Residence Time Distribution of Bacteria**

Presented by Joanna S. Adadevoh, Doctoral Student, Department of Chemical Engineering, University of Virginia, USA

Co-Authors: J.S. Adadevoh and R.M. Ford

The use of chemotactic bacteria to clean up chemical pollutants present in groundwater environments could potentially improve bioremediation strategies. However, the transport of chemotactic bacteria within contaminated aquifers is not fully understood. This study investigates the impact of localized contaminant sources within a sand column on the migration and retention of chemotactic bacteria. A laboratory-scale sand column saturated with artificial groundwater and subjected to continuous flow was used to simulate features of the saturated subsurface environment. Naphthalene crystals were distributed uniformly within the column to mimic localized contaminant sources within a natural aquifer. A pulse of a mixture of a chemotactic bacterial strain, *Pseudomonas putida* G7, and its non-chemotactic mutant, *Pseudomonas putida* G7 Y1, was injected into the column and the resulting bacterial breakthrough curves (BTCs) were analyzed to determine chemotaxis transport parameters. In addition to the non-chemotactic mutant as a control for chemotaxis, the sand column experiment was

repeated in the absence of naphthalene. We expected the BTCs of *P. putida* G7 Y1 to follow a Gaussian distribution in the presence and absence of naphthalene. A similar trend was expected for the BTC of *P. putida* G7 in the absence of naphthalene and preliminary experiments confirmed this. Furthermore, we anticipate that in the presence of naphthalene the BTC of *P. putida* G7 will exhibit a tailing behavior accounting for the prolonged bacterial residence time within the column due to chemotaxis. The results of this project will help elucidate the role of chemotaxis on bacterial retention in the subsurface.

Poster B2

**Chemotaxis toward Hydrogen Gas by *Methanococcus Maripaludis***

Presented by Kristen A. Brileya, Ph.D., Postdoctoral Research Assistant, Portland State University, USA

Co-Authors: K.A. Brileya, J.M. Connolly, R. Gerlach, and M.W. Fields

Chemotaxis toward hydrogen gas (H<sub>2</sub>) or “hydrogenotaxis” represents a potential strategy of motile microorganisms that compete for H<sub>2</sub> in subsurface environments. Although the ability for biological cells to sense and swim toward H<sub>2</sub> has been hypothesized for many years, this capacity was only recently demonstrated. H<sub>2</sub> is a crucial substrate for methanogens, and is a common source of energy for other archaea and bacteria in both anaerobic and aerobic environments. After a brief starvation, average swimming velocity of *Methanococcus maripaludis* increased toward a H<sub>2</sub> source, in a modified capillary assay with anoxic gas-phase control and time-lapse microscopy. This indicates that a methanogen couples motility to H<sub>2</sub> concentration sensing and is the first direct observation of hydrogenotaxis in any domain of life. The ability to move toward higher concentrations of H<sub>2</sub> could incur an advantage to methanogens that are otherwise outcompeted by those that are able to use H<sub>2</sub> at lower concentrations and/or utilize terminal electron acceptors that are more energetically favorable. The demonstrated chemotactic response would also allow cells to maintain desirable positions with respect to the major energy source as well as allow for proximity to H<sub>2</sub>-producers in mixed communities. Thus, hydrogenotaxis could play a crucial role in the establishment and maintenance of microbial interactions at the population- and community-level. The observed hydrogenotaxis could represent a widespread eco-physiological strategy of methanogens and potentially other hydrogen-utilizing microbes that are important to subsurface processes such as bioremediation, and to overall carbon, sulfur, and nitrogen cycling.

Poster B3

**Charge-Regulated Variation in Local pH and Its Effects on the Bioenergetics of Attached Bacteria under Non-Growth Conditions**

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

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

Since the first reported observations by Zobell in 1943, it has been recognized that the metabolic activity of adhered bacteria can differ from that of their planktonic counterparts. Many studies have been performed and the overwhelming evidence is that bacterial adhesion to surfaces can result in changes to cellular metabolic activity. However, the mechanism that results in these observations has remained elusive. We are approaching this problem by focusing on cellular bioenergetics, which describes how bacteria obtain, store and use energy, and how adhesion can affect this process. Using a combination of experimental and numerical studies, we will present our hypothesis linking cellular bioenergetics to the physicochemical charge-regulation effect, which causes variations in surface pH

as a surface containing acid/base functional groups (e.g., the bacterial cell surface) approaches another surface. Specifically, the local pH change during adhesion affects the proton gradient across the cell's cytoplasmic membrane. This proton gradient controls the concentration of the energy carrier adenosine triphosphate (ATP); thus, changes in local pH at the cell surface can affect cellular ATP levels and metabolic activity. The hypothesis predicts that a decrease in local pH should result in higher ATP levels, while an increase in pH should result in lower ATP levels. Here, we will present our hypothesis and provide supporting numerical and experimental results indicating a direct link between local pH variations due to the charge-regulation effect and ATP levels of attached bacteria.

Poster B4

**Investigating Microbial Dependent Redox Environments within a Mussel Shell Bioreactor Used for the Treatment of Acid Mine Drainage (AMD)**

Presented by Zach DiLoreto, M.Sc. Student, Great Lakes Institute for Environmental Research (GLIER), University of Windsor, Canada

Co-Authors: Z. DiLoreto, J. Pope, D. Trumm, and C. Weisener

Acid Mine Drainage (AMD) is an environmental issue prominent at mine sites and is cause for environmental concern. The oxidation of sulfide minerals leads to the generation of an acidic effluent enriched in metals that are prone to microbial biotransformation. The Stockton Coal Mine, located on the west coast of the south island of New Zealand, is no exception to AMD and its effluent contains elevated concentrations of Al, Fe, Ni, Zn, and Tl. A remediation approach being explored uses a passive bioreactor composed of mussel shell waste to treat the acidic effluent. The mussel shell bioreactor is able to remove  $\approx 99\%$  of toxic metals, as well as raise the pH of the effluent from 2 to 7. This research will investigate microbial community structure and its codependence on the stratified redox chemistry formed within the reactor. Specifically the role or influence of iron oxidizing bacteria and sulfate reducing bacteria on the mechanisms governing metal deportment within the reactor will be discussed. . Correlations between the microbial activity and diversity within redox dependent chemical zones identified in the bioreactor will be presented.

Poster B5

**Impact of Microbes Associated with Hydraulic Fracturing of Shale Gas Formations**

Presented by Alexander Elliott, M.Sc. Student, Department of Biological Sciences, University of Calgary, Canada

Co-Authors: A. Elliott, G. Voordouw, and Y. Shen

Hydraulic fracturing is now used routinely for the production of natural gas from low porosity shale formations. Following injection of a large volume of polymer and sand containing fracturing fluid, gas and flow-back water are produced. Flow-back water is a mixture of fracturing fluid and formation water, of which the fraction increases with time. Microbes may negatively impact shale gas extraction by degrading polymers such as guar gum, reducing sulfate to sulfide leading to souring, contributing to corrosion, or by fracture plugging through biomass growth. We have obtained flow-back water samples from several shale gas fields from the Montney formation in Western Canada. These have high salt concentrations (equivalent to 2 M KCl), low pH (5.5) and low counts of sulfate-reducing and acid-producing bacteria. Microbial community analyses indicated that thermophilic and halophilic microbes, which would be expected in the shale gas environment, are absent. Analysis of a timed sequence indicated that initial microbial numbers and extractable DNA were much higher than those found in source waters, which then rapidly declined reflecting the increasingly harsh conditions due to increasing ionic strength and decreasing pH. Overall, our data suggest that microbes are not native to

Western Canadian shale gas formations and that they are introduced with the fracturing fluid but are largely eradicated when hot, saline, acidic formation waters are produced.

Poster B6

**Investigating Phyio-Chemical Properties of Biogenic Zinc Sulfide Precipitates**

Presented by Nick Falk, M.Sc. Student, Great Lakes Institute for Environmental Research (GLIER), University of Windsor, Canada

Co-Authors: N. Falk and C. Weisener

In anaerobic environments, sulfate-reducing bacteria (SRB) may precipitate sparingly-soluble, fine-grained metal sulfides as by-products of their metabolism. This bio-mechanism is of huge interest to environmental research for its ability to remove harmful metals from contaminated sites. This research focuses on the effectiveness of this mechanism within a novel bioreactor treatment method currently employed at the Stockton coal mine in New Zealand. The method uses locally supplied mussel shells to intercept and neutralize acidic mine tailing runoff while also serving as an organic substrate to sustain SRB that enhance removal of harmful dissolved metals. Material collected from the bioreactor has been used to provide bacterial enrichments in the lab to investigate their ability to form biogenic zinc-sulfide (ZnS) and sequester and stabilize potentially toxic trace metals in the sub-surface. The fate of Thallium (Tl) is of particular interest in this study due to its persistence on the west coast of New Zealand. This study will use a combination of scanning and transmission electron microscopy and atomic force/Raman microscopy techniques (SEM/TEM; AFM/RAMAN) to characterize and track key morphological and bacterial relationships during ZnS formation. This research is the first to provide detailed investigation linking the microbial causal relationships related to early nucleation of biogenic ZnS and its ability to sequester Tl.

Poster B7

**Characterization of Methanogens and Methane-Utilizing Archaea and Bacteria in Sediments of the Northern South China Sea by PCR Amplification of *mcrA* and *pmoA* Genes**

Presented by Ji-Dong Gu, Ph.D., Associate Professor, School of Biological Sciences, University of Hong Kong, PR China

Co-Authors: Z. Zhou and J.-D. Gu

Methanogens and anaerobic methane-oxidizing archaea (AMA) were investigated by profiling *mcrA* and *pmoA* genes encoding by methyl-coenzyme M reductase alpha subunit and particulate methane monooxygenase alpha subunit, respectively, in sediments of the northern South China Sea (nSCS). Community structures representing by *mcrA* gene based on clone libraries from nSCS were studied and those from surface and subsurface sediments were separately clustered together indicating the niche specificity. Novel clusters specific to the SCS were also identified and the phylogeny of *mcrA* harboring archaea was completely updated. Quantitative PCR were used to detect *mcrA* gene abundance in all samples with high abundance ( $6.9 \times 10^6$  to  $1.02 \times 10^8$  copies per gram dry weight) in subsurface samples. Aerobic methanotrophic bacteria were more abundant in surface layers ( $6.7 \sim 11.1 \times 10^5$  copies per gram dry weight) than the subsurface layers ( $1.2 \sim 5.9 \times 10^5$  copies per gram dry weight) based on *pmoA* gene. This study investigated methane cycling prokaryotic communities in marine ecosystems, suggesting distribution features of *mcrA* harboring communities in nSCS.

Poster B8

**Investigation of Diesel Biodegradation in Decommissioned Rock-Vaults**

Presented by Lotta Hallbeck, Ph.D., Senior Scientist, Microbial Analytics Sweden AB, Sweden

Co-Authors: A. Chukharkina, A. Blom, L. Eriksson, L. Johansson, B. Hallbeck, K. Pedersen, and L. Hallbeck

In Sweden, unlined rock vaults are used for storage of petroleum products. The Swedish government had until the nineties, 45 storage facilities for civil purposes. After decommission, pumping of groundwater has stopped and many vaults are almost filled up. Remaining petroleum products are a threat to the environment and it is important to investigate the natural degradation and estimate the degradation rates. Microbiological and chemical characterizations of three depths in three storage facilities were done. With a discrete interval sampler operated with nitrogen gas, samples for total number of cells (TNC), cultivable heterotrophic aerobic bacteria (CHAB), most probable number of nitrate-, iron-, and sulfate-reducing bacteria were determined. Occurrence of diesel hydrocarbons and oxidized degradation products by selective extraction of groundwater samples with three different kinds of SPE-cartridges: C18, polymer phase and ion-exchange, and different solvents, was determined. Analyzes were done on GC-MS and HPLC. The facilities were different regarding the groundwater chemistry and microbiology. The TNC was highest at the shallowest depth close to the water surface and at the bottom the vaults in all three facilities. One facility had approximately one magnitude higher TNC and CHAB than the other and was almost anoxic. This facility had the highest concentrations of both non-polar and polar hydrocarbons. Presence of polar hydrocarbons increased the solubility of alkanes in the water. Some uneven alkanes were missing and there were measurable concentrations of fatty acids with even number of carbons. Availability of electron acceptor is the most plausible explanation for the different degradation intensity in the three storage facilities.

Poster B19

**Impact of Biofiltration and Dissolved Organic Carbon on Nitrosamine Biotransformation**

Presented by Carissa L. Homme, Doctoral Student, Division of Civil and Environmental Engineering, Colorado School of Mines, USA

Co-Authors: C.L. Homme, D. Li, and J.O. Sharp

Prior work within our group and by others has revealed that alterations in bioavailable dissolved organic carbon (DOC) concentrations and composition result in changes to microbial community diversity and metabolic capabilities. These changes have the capability to enhance xenobiotic biodegradation during managed aquifer recharge scenarios. Here we evaluate the effect of a biostimulation strategy employing manipulations of DOC on microbial community composition and nitrosamine removal in columns containing biologically active carbon (BAC) characteristic of filters used in drinking water treatment. Nitrosamines, which are ubiquitously present as trace organics in drinking and environmental water supplies, were selected due to their toxicological relevance and a mechanistic understanding of biodegradability under laboratory conditions by bacterial strains expressing monooxygenase-type enzymes. Nitrosamine removal by a laboratory strain of rhodococcus was shown to follow a tiered hierarchy where lighter, less aromatic nitrosamines were degraded at faster rates with the following hierarchy (NDMA>NDEA>NDPA>NPYR>NMOR). BAC systems containing a natural microbial assemblage were exposed to influents with different DOC levels and sources as well as an influent biostimulated with propane to enhance for the presence of monooxygenase-containing microorganisms capable of nitrosamine biotransformation. After a conditioning phase, columns were exposed to a pulse of mixed nitrosamines in order to monitor attenuation during transport. Our project aims to document the effects of DOC manipulation on 1)

microbial community composition, 2) the relative presence of enzymes applicable to nitrosamine biodegradation and 3) nitrosamine biodegradation behavior in a system representative of biologically-based drinking water treatment.

Poster B10

**Effects of Hydraulic Frac Fluids and Formation Waters on Groundwater and Gas Shale-Associated Microbial Communities**

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

Co-Authors: N. Jimnez and M. Krger

Hydraulic fracturing operations can influence and be influenced by subsurface microbial communities. To investigate the potential impacts of changing environmental reservoir conditions (temperature, salinity, oxygen content, etc.), and the introduction of frac or geogenic chemicals on subsurface microbial communities, laboratory experiments under in situ conditions (i.e. with high pressure and salinity, etc.) are being conducted. Enrichment cultures with samples from subsurface environments (e.g. shale and coal deposits, gas reservoirs, geothermal fluids and groundwaters) have been set up using different carbon sources, including hydrocarbons and typical frac chemicals. Classical microbiological methods and molecular analyses are used to determine changes in the microbial abundance, community structure and function after the exposure to different single frac chemicals, "artificial" frac fluids or produced fluids. On the other hand, potential transformation reactions of frac or geogenic chemicals by subsurface microbiota and their lifetime are investigated. In our "fracking simulation" experiments, an increasing number of hydrocarbon-degrading or halophilic microorganisms is to be expected after exposure of subsurface communities to artificial produced fluids, whereas the introduction of freshwater and of easily biodegradable substrates might favor the proliferation of fast-growing generalistic heterotrophs in shale-associated communities. Nevertheless, toxicity of some of the frac components cannot be excluded.

Poster B11

**Biom mineralization of Carbonate Minerals by *Proteus Mirabilis***

Presented by Serku Kang, Ph.D., Researcher, Chonnam National University, South Korea

Co-Authors: S. Kang, Y. Kim, and Y. Roh

Formation of carbonate minerals by biological processes may play an important role in CO<sub>2</sub> fixation and solid phase capture of metal including inorganic contaminants in environments. This study focused on investigation of microbial precipitation of carbonate minerals using *Proteus mirabilis* and to identify mineralogical characteristics of the precipitates. *Proteus mirabilis* were enriched from rhodoliths sampled at Wu do, Jeju, S. Korea. *Proteus mirabilis* were aerobically cultured in D-1 media containing Ca, Mg, Sr and Cd-acetate concentration at 25°C for 15 days. Mineralogical characteristics of the precipitates mediated by *Proteus mirabilis* were identified by SEM-/TEM-EDS and XRD analyses. The growth of *Proteus mirabilis* gradually increased for 16 days. The pH decreased from 6.5 to 5.3 for 4 days by oxidation of organic acids, and then increased up to 8.5 for 25 days of incubation. It may be attributed to NH<sub>4</sub><sup>+</sup> generated from microbial metabolism. Sr<sup>2+</sup> concentration sharply decreased from 2,953 to 5.68 mg/L for 29 days. SEM-/TEM-EDS and XRD analyses showed the precipitates formed by *Proteus mirabilis* were identified as ca. 140 µm sized calcite, 100 ~ 300 µm sized huntite, 20 ~ 70 nm sized strontianite and ca. 100 nm sized X-ray amorphous. These results suggested that formation of sparingly soluble Ca, Mg, Sr and Cd-precipitates mediated by *Proteus mirabilis*,

sequesters Ca, Mg, Sr, Cd and CO<sub>2</sub> into more stable and less toxic form. Therefore, microbial precipitation of carbonate minerals may play one of important roles in metal including inorganic contaminants and carbon biogeochemistry as well as carbon sequestration in environments.

Poster B12

**Mechanical Property of Iron-Reduction and Characterization of Magnetite Nanoparticles by *Clostridium sp.***

Presented by Yumi Kim, Postdoctoral Researcher, Chonnam National University, South Korea  
Co-Authors: Y. Kim and Y. Roh

The objectives of this research were to investigate mechanical property of iron reduction by *Clostridium sp.* and characterization of biogenic magnetite nanoparticles formed by them. The iron-reducing bacteria (*Clostridium sp.*) were enriched from intertidal flat sediments in S. Korea. In order to investigate the favorable conditions and mechanical properties of iron reduction for magnetite biomineralization, the bacteria were grown with poorly crystalline iron-oxides such as akaganeite and ferrihydrite as electron acceptors, and glucose, lactate, and pyruvate as electron donors. Mineralogical characterization of biogenic minerals was performed by XRD and TEM-EDS analyses, and chemical characterization was determined by UV-vis, FT-IR and XPS analyses. The iron-reducing bacteria reduced akaganeite and ferrihydrite via glucose fermentation, and transformed them to more stable phases such as magnetite and goethite. The biogenic magnetite nanoparticles had around 10 nm in size and were spherical in shape. Unlike chemically synthesized magnetite, the biogenic magnetite was coated with organic matter containing an abundance of reactive carboxyl groups (-COOH) and showed that the biogenic magnetite has high potential for serving as a useful and applicable material in relevant medical technologies. These results indicate that the iron-reducing bacteria (*Clostridium sp.*) transform akaganeite and ferrihydrite to magnetite via glucose fermentation, and such microbial processes may facilitate simple preparation of functional magnetite-organic complex nanoparticles which have benefits for biomedical applications.

Poster B13

**Growth and Activity of Thermophilic *Petrotoga sp.* under Carbon Capture and Storage Conditions**

Presented by Martin Krüger, Ph.D., Senior Scientist, Federal Institute for Geosciences and Natural Resources (BGR), Germany

Co-Authors: J. Rakoczy, S. Kümmel, C. Gniese, H.H. Richnow, M. Schlömann, and M. Krüger

Carbon capture and storage is a technology set out to decelerate global warming by reducing CO<sub>2</sub> emissions into the atmosphere. To ensure safe long-term storage of CO<sub>2</sub> in the underground a number of factors need to be considered. One of them is microbial activity in storage reservoirs, which can lead to the formation of acidic metabolites or carbonates which then might affect permeability and pressure build-up in reservoir and cap rock. Our research focused on the effect of high CO<sub>2</sub> concentrations on growth and activity of *Petrotoga sp.* (Thermotogae), a thermophilic fermenting and thiosulphate-reducing bacterium which had been isolated from the gas reservoir Schneeren (Germany). To dissect the effects induced by CO<sub>2</sub> treatment, we also studied the effects of high gas pressure and low pH value individually. The highest growth yield, sulfide production and maltose consumption rate was observed under control conditions (100 bar hydrostatic pressure, pH 7) and likewise at 100 bar N<sub>2</sub> and at pH 6 (at 100 bar hydrostatic pressure) which demonstrated that neither the gas pressure nor a slightly lowered pH would affect the viability of the cells. However, CO<sub>2</sub> treatment (100 bar, 10 bar) and low pH (pH 5, 100 bar hydrostatic pressure) fully inhibited both

growth and activity, indicating that the CO<sub>2</sub> effect might actually be a pH effect. When provided with a surface for attachment, CO<sub>2</sub>-inhibited cells restarted growth after CO<sub>2</sub> release. Currently, protein expression patterns are analysed to elucidate the observed effects on a molecular level, e.g., metabolic pathways, membrane transport systems.

Poster B14

**Shifting of Microbial Community Structures Related to Different Levels of Organic Contamination in Arctic Groundwater**

Presented by Mengyan Li, Ph.D., Postdoctoral Research Fellow, Rice University, USA

Co-Authors: M. Li, Y. Yang, and P.J.J. Alvarez

The Arctic tundra is a unique ecosystem of great global relevance to carbon storage and cycling. The tundra is particularly vulnerable to climate change and anthropogenic pollution, which is increasing with oil production and related activities. A site at Prudhoe Bay, Alaska has been impacted by chlorinated solvents spills since the 1980s. One particular concern is co-contamination of the tundra by the toxic stabilizer 1,4-dioxane. Here, we use pyrosequencing-based metagenomics to understand how dioxane contamination incidents along with chlorinated solvents and other hydrocarbons have affected the indigenous microbial communities and the microbes that are critical to carbon cycling and degradation at the site. Among the obtained 5600 to 14243 eukaryote 16S sequences, Actinobacteria and Proteobacteria were the dominant bacterial phyla. DCA and Bray-Curtis clustering analysis unveiled three different patterns of microbial community structures among samples from source zone, mid-plume and un-impacted area at the Arctic site. Proteobacteria is the most abundant phylum at the source zone and unimpacted area, while Actinobacteria dominates the mid-plume. Members of the family Comamonadaceae and Geobacteraceae are only prevailing near the source zone, suggesting their enrichment related to the high level of contamination. CCA analysis also demonstrated that the microbial communities were significantly influenced by groundwater contaminants near the source zone. However, the mid-plume was controlled by high TOC and methane contents. All the lines of evidence suggest that the indigenous microbes have adapted to the local environment due to long term of acclimation, implying the presence of natural attenuation.

Poster B15

**Antibiotic Resistance of *E. Coli* and *Enterococcus* Collected from Monitoring Wells on Dairies and from Domestic Wells in San Joaquin Valley, California**

Presented by Xunde Li, Ph.D., Research Microbiologist, Western Institute for Food Safety and Security, University of California, Davis, USA

Co-Authors: X. Li, E.R. Atwill, E. Antaki, and T. Harter

Human consumption of untreated groundwater contaminated with wastewater containing antibiotic resistant bacteria can result in the transmission of antibiotic resistance genes into the microflora of the human gut. Therefore, antibiotic resistant genes may potentially transmit to pathogens infectious to humans and negatively affect health treatment. Patterns of antibiotic resistance of indicator bacteria can be used for to predict antibiotic resistance of pathogenic bacteria, as they may exhibit the same or similar antibiotic susceptibilities when exposed to specific antibiotics. *E. coli* and *Enterococcus* isolates from monitoring wells on dairies and from domestic wells within and also outside dairy influence were analyzed for profiles of antibiotic resistance. *E. coli* and *Enterococcus* were isolated and archived from groundwater wells of multiple field sampling projects conducted between 2007 and 2011. Fourteen *E. coli* isolates and 36 *Enterococcus* isolates were tested for susceptibility to antibiotics using a Minimum Inhibitory Concentration (MIC) method. The antibiotics that *E. coli* and

Enterococcus most often resisted to were Azithromycin and Tigecycline, respectively. E. coli isolates from 4/5 (“four of five”) dairy monitoring wells and from 4/5 domestic wells near dairies exhibited multi-antibiotic resistance. E. coli isolates from 2/4 (“two of four”) domestic wells outside dairy influence exhibited multi-antibiotic resistance (most domestic well samples were free of E. coli). Enterococcus isolates from 17/18 dairy monitoring wells, from 10/12 domestic wells near dairies and from 5/6 domestic wells outside dairy influence exhibited multi-antibiotic resistance. Findings indicate that bacterial antibiotic resistance is an emerging issue from surface to subsurface water.

Poster B16

**Isolation of a Halophilic Bacterium from a Hydraulically Fractured Shale and Its Role in Pipeline Corrosion**

Presented by Renxing Liang, Doctoral Student, Department of Microbiology and Plant Biology, University of Oklahoma, USA

Co-Authors: R. Ling, I.A. Davidova, C.R. Marks, B.W. Stamps, B.S. Stevenson, K.F. Duncan, and J.M. Suflita

Return waters from the hydraulic fracturing of shale formations contain a variety of chemicals and a characteristic microflora. Microbial activity in such waters can be associated with deleterious consequences including the corrosion of carbon steel pipelines. We examined production water from a shale gas facility in the Barnett formation. The microbial community was predominated by halotolerant, sulfidogenic bacteria within the Order Halanaerobiales, which reflected the geochemical conditions of highly saline water (11.7% Cl<sup>-</sup>) containing sulfur species (S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, SO<sub>4</sub><sup>2-</sup> and HS<sup>-</sup>). Most probable number determinations revealed that acid-producing bacteria were the numerically dominant cultivable organisms. A halophilic bacterium was subsequently isolated and identified as a Halanaerobium sp. The isolate could ferment guar gum (0.5%), a polysaccharide polymer used in fracture fluids, to acetate (11.81±0.75 mM) and small amounts of formate and lactate in a 10% NaCl medium at 37°C. The strain could also couple guar gum oxidation with thiosulfate reduction, producing sulfide (7.12±0.31 mM) and roughly twice the amount of acetate (27.31±0.68 mM) than fermentative growth conditions. Unexpectedly, the degree of carbon-steel coupon dissolution in Halanaerobium incubations was less than sterile controls. Abiotic experiments revealed that the equivalent amount of sulfide and acetate produced in Halanaerobium cultures caused more pronounced pitting corrosion than the corresponding incubations without these components. Therefore, we suggest that sulfide and acetate produced during anaerobic polysaccharide biodegradation by subsurface microorganisms might be transported in pipelines and cause pitting corrosion in other locations where microbial activity might be minimal or absent due to various control measures.

Poster B17

**Anaerobic Degradation of Polycyclic Aromatic Hydrocarbons by a Highly Enriched Iron-Reducing Culture**

Presented by Sviatlana Marozava, Sc.D., Postdoctoral Researcher in Microbiology, Institute for Groundwater Ecology, Helmholtz Zentrum München, Germany

Co-Authors: S. Marozava and R.U. Meckenstock

Polycyclic aromatic hydrocarbons (PAHs) (naphthalene, anthracene, etc.) are among the most frequently detected pollutants in groundwaters contaminated with leakages from pipelines, oil tanks or gas stations. Natural microbial communities are able to remove these pollutants from the ecosystems.

To date, only few iron-reducing enrichments have been described to be able to degrade polyaromatic hydrocarbons in groundwater under anaerobic conditions. We enriched an iron-reducing culture (1MN) which is able to degrade naphthalene, 1- and 2-methyl-naphthalene. Besides, the enrichment grows well on 1- and 2-naphthoic acids, suggesting that degradation of naphthalene proceeds via carboxylation. Surprisingly, metabolite such as 1-naphthoic acid was detected in cultures cultivated on 1-methylnaphthalene indicating that the initial activation reaction is a fumarate addition to the 1-methylgroup. This is in contrast to all other cultures described so far where activation is always in position 2 of the naphthalene ring and degradation is through the central metabolite 2-naphthoic acid. Terminal restriction fragment length polymorphism and 16S rRNA gene sequence analyses clustered the dominant organism in the Deltaproteobacteria which differs from the so far known iron-reducing PAH-degraders that belonged to the Firmicutes. The 1MN enrichment culture depicts a new type of iron-reducing microorganisms that can degrade polycyclic aromatic compounds in groundwater.

Poster B18

### **Microbial Methane Production and Carbon Cycle in Deep Aquifer Associated with Accretionary Prism**

Presented by Makoto Matsushita, M.S. Student, Department of Geosciences, Shizuoka University, Japan

Co-Authors: M. Matsushita and H. Kimura

Idiosyncratic combinations of reductive dehalogenase (rdh) genes are a distinguishing genomic feature of closely related organohalogen-respiring bacteria. This feature can be used to deconvolute the population structure of organohalogen-respiring bacteria in complex environments and to identify relevant subpopulations, which is important for tracking interspecies dynamics needed for successful site remediation. Here we report the development of a nanoliter qPCR platform to identify organohalogen-respiring bacteria by quantifying major orthologous reductive dehalogenase gene groups. The qPCR assays can be operated in parallel within a 5184-well nl-qPCR chip at a single annealing temperature and buffer condition. We also developed a robust bioinformatics approach to select from thousands of computationally-designed primer pairs those that are specific to individual rdh gene groups and compatible with a single condition. We validated hundreds of the most selective qPCR assays and examined their performance in two pilot applications: (i) the quantitative analysis of biostimulated aquifer pore water from a 1,2-dichloroethane-contaminated site and (ii) a trichloroethene-degrading bioreactor. Both revealed sub-population abundance shifts and unexpected community dynamics. We also discuss the use of ongoing bioreactors operated to mimic slow flux of substrate in aquifer environments.

Poster B19

### **A Nanoliter qPCR Platform for Highly Parallel, Quantitative Assessment of Reductive Dehalogenase Genes and Populations of Dehalogenating Microorganisms in Complex Sediment Environments**

Presented by Koshlan Mayer-Blackwell, Doctoral Student, Department of Civil and Environmental Engineering, Stanford University, USA

Co-Authors: K. Mayer-Blackwell, M. Azizian, C. Machak, E. Vitale, G. Carpani, F. de Ferra, L. Semprini, and A.M. Spormann

Idiosyncratic combinations of reductive dehalogenase (rdh) genes are a distinguishing genomic feature of closely related organohalogen-respiring bacteria. This feature can be used to deconvolute the population structure of organohalogen-respiring bacteria in complex environments and to identify

relevant subpopulations, which is important for tracking interspecies dynamics needed for successful site remediation. Here we report the development of a nanoliter qPCR platform to identify organohalogen-respiring bacteria by quantifying major orthologous reductive dehalogenase gene groups. The qPCR assays can be operated in parallel within a 5184-well nl-qPCR chip at a single annealing temperature and buffer condition. We also developed a robust bioinformatics approach to select from thousands of computationally-designed primer pairs those that are specific to individual rdh gene groups and compatible with a single condition. We validated hundreds of the most selective qPCR assays and examined their performance in two pilot applications: (i) the quantitative analysis of biostimulated aquifer pore water from a 1,2-dichloroethane-contaminated site and (ii) a trichloroethene-degrading bioreactor. Both revealed sub-population abundance shifts and unexpected community dynamics. We also discuss the use of ongoing bioreactors operated to mimic slow flux of substrate in aquifer environments.

Poster B20

**Biodegradation of Petroleum Hydrocarbons in Soils Co-Contaminated by Petroleum Hydrocarbons and Heavy Metals Derived from Petroleum**

Presented by Obioma Mejeha, Doctoral Student, School of Civil Engineering and Geochemistry, Newcastle University, United Kingdom

Co-Authors: O. Mejeha, I. Head, M. Jones, and N. Gray

The bioremediation of sites co-contaminated by organic pollutants and Heavy metals is often a challenge due to the inhibition of microbial activities by heavy metals. Trace Metals such as arsenic, cadmium, chromium, lead, nickel, etc. are often constituents of petroleum and petroleum spills result in the release of such metals into the environment. Subsequent spills will result in increased concentrations of the heavy metals building to concentrations above intervention values. This research investigates the effects of different metals on degradation of petroleum hydrocarbon and the microorganisms which mediate degradation of the pollutant in soils. So far, the effect of the heavy metals Nickel (Ni<sup>2+</sup>), Cadmium (Cd<sup>2+</sup>) and Lead (Pb<sup>2+</sup>) on biodegradation of petroleum hydrocarbon in the soil environment are being studied. Preliminary results indicate that low concentrations of nickel stimulated the biodegradation of petroleum hydrocarbons. However, high concentrations of nickel and high and very low concentrations of cadmium inhibited the degradation. In addition to this, Pb<sup>2+</sup>, at high and low concentration levels, inhibited the biodegradation of petroleum hydrocarbon. Interestingly, the chemical form of added metals is an important factor. These microcosms are now being investigated by culture independent microbial ecological approaches combined with rigorous analysis of the fate of the added metals in soils and the fate of individual hydrocarbon components. Such analyses will provide a full understanding of the diversity, activity, function and interactions of hydrocarbon degraders within the experimental framework.

Poster B21

**Reduction of Cr(VI) in the Context of Chromate Conversion Coatings by *Shewanella Oneidensis***

Presented by Robert B. Miller, M.S. Student, Department of Biology, University of Akron, USA

Co-Authors: R.B. Miller, M. Iannuzzi, and J.M. Senko

Chromate conversion coatings (CCCs) have been used extensively to prevent localized corrosion of aluminum alloys and various ferrous and non-ferrous materials. The application of CCCs produces large volumes of carcinogenic chromate-containing (i.e. Cr(VI)) liquid waste that may contaminate aquifers underlying application facilities. While microbially-mediated reductive immobilization of chromates as sparingly soluble Cr(III) has been exploited as a means of limiting Cr(VI) transport in

contaminated aquifers, the influences of other constituents of CCCs on Cr(VI) reduction have not been evaluated. Besides Cr(VI), CCC waste streams contain other chemical species including salts of ferricyanide, fluorides, borates, and zirconates, which may inhibit Cr(VI)-reducing activity. We evaluated the potential for *Shewanella oneidensis* MR-1 to reduce Cr(VI) at concentrations relevant to CCC waste streams as well as the influence of other CCC constituents on this activity. While Cr(VI) concentrations  $\geq 1$  mM inhibited aerobic growth of *S. oneidensis*, that organism could reduce Cr(VI) in anoxic solutions containing up to 1 mM Cr(VI) under non-growth conditions. Cr(VI) reduction by *S. oneidensis* proceeded in solutions containing all CCC additives except ferricyanide at rates comparable to that observed in CCC additive-free solutions. The inclusion of ferricyanide in Cr(VI) containing solutions inhibited Cr(VI) reduction by *S. oneidensis*, likely due to disruption of electron transfer to Cr(VI) by ferricyanide. Our results suggest that microbiologically-mediated reductive immobilization of Cr in CCC-contaminated systems may be achieved, provided ferricyanide is present at sufficiently low concentrations to limit its inhibition of Cr(VI) bioreduction.

Poster B22

**Characterization of Depth-Related Changes in Structural and Functional Microbial Community Associated with Arsenic Reduction in Soil Profile of Northern Utah**

Presented by Babur S. Mirza, Ph.D., Postdoctoral Researcher, Utah Water Research Laboratory, USA  
Co-Authors: B.S. Mirza, X. Meng, D.L. Sorenson, R.R. Dupont, and J.E. Mclean

Microorganisms play an important role in the direct or indirect reduction of As(V) to As(III), which is more mobile in soil and groundwater environments. Basin-fill aquifers of the Southwest U.S. are associated with elevated concentrations of arsenic (As) in groundwater. Many private domestic wells in the Cache Valley Basin, UT, have As concentrations in excess of the U.S. EPA drinking water limit. In the current study, two continuous soil cores (NP-9 and NP-13) were collected from the Cache Valley basin and analyzed for As speciation and biogeochemical parameters associated with As reduction in relationship to the shifts in the microbial communities. The highest concentration of As(III) in the pore water extracted from NP-9 and NP-13 cores at the depth of water table was 102 and 60  $\mu\text{g/L}$ , respectively. Microbial communities (both structural and functional) were characterized through the 16S rRNA and *arrA* gene sequencing. Overall, the structural microbial community at the phylum level was predominated by the Actinobacteria, Proteobacteria, and Firmicutes in the both soil profiles. However, at the lower taxonomic levels, Acidobacteria for the NP-9 and Thiobacillus, Desulfuromonas, Trichococcus, for the NP-13 site were detected as the dominant bacterial genera associated with the samples containing the highest As concentrations, respectively.

Poster B23

**Early Steps of the Anaerobic Naphthalene Degradation Pathway by the Sulfate-Reducing N47**

Presented by Housna Mouttaki, Ph.D., Postdoctoral Researcher, Institute for Groundwater Ecology, Helmholtz Zentrum München, Germany  
Co-Authors: H. Mouttaki, J. Kölschbach, and R.U. Meckenstock

Aromatic hydrocarbons such as benzene and naphthalene are highly recalcitrant environmental pollutants that are only slowly metabolized by bacteria under anoxic conditions. We have recently brought biochemical evidence that at least for naphthalene the degradation is initiated via direct carboxylation. Naphthalene carboxylase converts naphthalene and  $^{13}\text{C}$ -labelled bicarbonate to 2-[carboxyl- $^{13}\text{C}$ ]naphthoic acid at a rate of 0.12  $\text{nmol min}^{-1} \text{mg}^{-1}$  of protein in crude cell extracts of N47. The enzyme catalyzes a much faster exchange of  $^{13}\text{CO}_2$  with the carboxyl moiety of [ $^{13}\text{C}$ ]-2-naphthoic acid at a rate of 3.2  $\text{nmol min}^{-1} \text{mg}^{-1}$  protein, indicating the formation of free reversible

intermediates. Additionally, the enzyme is reversible and catabolizes the formation of naphthalene from 2-naphthoic acid. Comparative native proteomic studies were done on blue native PAGE coupled to LC-MS/MS -LTQ OrbitrapXL mass spectrometer analysis and were conducted on N47 proteins extracted from naphthalene versus 2-methylnaphthalene grown cells. Results have indicated a clear up-regulated big protein complex. Subunits of this complex have been previously identified by proteogenomic in N47 (Bergmann et al, 2011) and in NaphS2 (DiDonato et al, 2010). They belong to a highly conserved gene cluster identified as coding for the naphthalene carboxylase subunits, and they were shown to be encoded in an operon structure. Results have indicated that the protein complex has a size of about 750 kDa. The new carboxylation reaction is unprecedented in biochemistry and opens the door to understand the anaerobic degradation of polycyclic aromatic hydrocarbons which are among the most hazardous environmental contaminants.

Poster B24

**Laboratory Simulation of a Geochemical Disturbance in the Sedimentary Subsurface Environment**

Presented by Toru Nagaoka, Ph.D., Senior Research Scientist, Bioengineering Sector, Central Research Institute of Electric Power Industry (CRIEPI), Japan

Co-Authors: T. Nagaoka, S.-I. Hirano, Y. Sasaki, Y. Amano, and H. Yoshikawa

In order to assess the microbial impacts on the geochemical processes around the nuclear waste repository, the laboratory jar experiments were conducted using the deep sedimentary rock and groundwater in the Horonobe URL, Japan. In the experiments, pulverized rock and groundwater were suspended in the jar and the redox changes were induced by aeration and discontinuation to sediment slurry, which simulated the redox process occurring during operation of nuclear waste repositories. During the experiments, redox potential, pH and dissolved oxygen in the slurry were monitored, and also the concentrations of dissolved ions and head space gasses in the jar were analyzed. In addition, microbial DNA was extracted from the slurry, and analyzed the response of microbial communities toward the geochemical changes. As a results, after discontinuation of air exposure with lactate and acetate amendments as an electron donor, redox potentials decreased from ca. +100 mV (vs. Ag/AgCl) to -600 m V for lactate and -300mV for acetate, and the microbial communities were changed with the redox potentials of the slurry, and also the sequential terminal electron-accepting process (TEAPs) such as aerobic respiration, iron reduction was observed. These results indicated that the microbial activities would affect the geochemical changes around nuclear waste repositories. This study was performed as a part of “Project for Assessment Methodology Development of Chemical Effects on Geological Disposal System” funded by Ministry of Economy, Trade and Industry, Japan.

Poster B25

**Phylogenetic Diversity of Microorganisms in the Carbonate Petroleum Reservoir**

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

Co-Authors: T.N. Nazina, N.K. Kostryukova, D.S. Sokolova, B. Clement, T.L. Babich, T.P. Tourova, A.B. Poltarau, S.S. Belyaev, and M.V. Ivanov

Microbial communities of carbonate oilfields are commonly studied less extensively than microorganisms of terrigenous petroleum reservoirs. Carbonate oil-bearing rocks are characterized by significant variations in pore size. This study was undertaken to characterize the abundance and phylogenetic diversity of prokaryotes, including ultramicroforms, in formation water of a carbonate petroleum reservoir. The studied US-based carbonate petroleum reservoir was characterized as highly

fractured carbonate with average porosity 20.3% and permeability varying from 3 to 760 mD. Formation water in the studied reservoir was 28°C, with pH of 6.8–8.2, low mineral content (6–13 g/l), and low dissolved organic acid content (<29 mg/l C); sulfate and H<sub>2</sub>S content was high. Using cultivation-based methods, anaerobic fermentative, sulfate-reducing, and methanogenic prokaryotes were detected in the microbial community. Libraries of 16S rRNA genes were constructed from DNA extracted from biomass obtained by successive filtration of water through 0.22 and 0.09 µm pore-sized-filters (E1 and E2 libraries, respectively). A comparative phylogenetic and statistical analysis of 3813 fragments of 16S rRNA genes of Bacteria and 745 genes of Archaea was performed. Diversity of major groups 16S rRNA gene sequences of bacteria in the both libraries were similar and affiliated with the Candidate divisions JS1 & OD1, Deltaproteobacteria (Desulfovibrionales, Desulfobacterales), Bacteroidetes (Proteiniphilum), Epsilonproteobacteria (Sulfurospirillum), Thermotogae, Chloroflexi (Anaerolineae) and Spirochaetae. Archaeal 16S rRNA gene libraries were dominated by sequences classified within the Terrestrial Miscellaneous Group (TMEG) of the order Thermoplasmatales; minor phylotypes belonged to methanogens and ANME-1; ANME-1b groups. The data obtained demonstrate a unique microbial community inhabiting the carbonate oilfield.

Poster B26

### **Interconnected Cycling of Fe, S, and C in the Terrestrial Subsurface: New Paths and Opportunities for Coupling Biotic and Abiotic Processes**

Presented by Edward J. O'Loughlin, Ph.D., Biogeochemist, Argonne National Laboratory, USA

Co-Authors: T.M. Flynn, D.A. Antonopoulos, K.M. Handley, M.J. Kwon, D. Bartels, M.I. Boyanov, F. Meyer, B. Mishra, W.L. Trimble, K.M. Kemner, P. Long, K. Williams, T.J. DiChristina, and E.J. O'Loughlin

Coupled biotic and abiotic processes responsible for the biogeochemical cycling of Fe, S, and C in the terrestrial subsurface affect contaminant mobility, nutrient availability, and groundwater chemistry. We show that at pH 9, dissimilatory metal-reducing bacteria (DMRB) can respire S(0) but not goethite because goethite reduction is not thermodynamically favorable under alkaline oligotrophic conditions. However, Fe(III) reduction can proceed via S(0)-mediated electron-shuttling pathways through a mutualistic partnership between DMRB and SRB rather than the direct enzymatic reduction of Fe(III) minerals by DMRB alone. In separate experiments, we investigated how the nature of organic C plays a critical role in determining the mechanism by which Fe(III) minerals are reduced. In microcosms containing both ferrihydrite and sulfate as electron acceptors and amended with acetate or lactate, we found that both acceptors were reduced simultaneously; however, when glucose was provided ferrihydrite was reduced without concomitant sulfate reduction. Because elements such as Fe, S, and C can cycle rapidly between different valence states in subsurface environments, actual pathways of electron flow are difficult to determine from geochemical measurements alone. By reconstructing genomes of microorganisms that catalyze these processes from a metagenome, we can begin to unravel metabolic networks active in these environments. We reconstructed the complete 2.4 Mb genome of a previously uncultivated epsilonproteobacterium by assembling short-read shotgun metagenomic data using a complexity reduction approach. Genomic evidence suggests that the organism is a chemolithoautotrophic diazotroph capable of deriving energy for growth by microaerophilic or nitrate-/nitric oxide-dependent oxidation of S(0), sulfide, sulfite, or hydrogen.

Poster B27

**Biomining and Metal Reduction by Metal-Reducing Bacteria from a CO<sub>2</sub> Repository Candidate Site**

Presented by Yul Roh, Ph.D., Professor, Department of Earth and Environmental Sciences, Chonnam National University, South Korea

Co-Authors: Y. Roh, S.K. Kang, and Y. Kim

The objective of this study was to examine metal reduction and biomining by metal-reducing enrichment cultures from groundwater and rock samples collected from the deep subsurface environments. The sampling site is a candidate site for CO<sub>2</sub> geologic sequestration and is geologically and hydrologically isolated Eocene-age sedimentary basins in the deep (>950 meters below land surface) terrestrial subsurface. The enriched bacterial strains from rocks and groundwater were used to examine physiological characteristics. These metal-reducing bacteria were able to use acetate, glucose, hydrogen, lactate, pyruvate as electron donors while reducing Fe(III)-citrate and iron oxyhydroxide at 25°C. These bacteria exhibited diverse mineral precipitation capabilities, including the formation of magnetite (Fe<sub>3</sub>O<sub>4</sub>), siderite (FeCO<sub>3</sub>), and rhodochrosite (MnCO<sub>3</sub>). The gas composition of the incubation headspace and the ionic composition of the incubation medium exerted profound influences on the types of minerals formed.

Poster B28

**Microtopography Controls Methanogenesis in Polygonal Tundra as Revealed by mcrA Gene Abundance in Anoxic Incubation Study**

Presented by Taniya Roy Chowdhury, Ph.D., Postdoctoral Research Associate, Oak Ridge National Laboratory, USA

Co-Authors: T. Roy Chowdhury, E.M. Herndon, T.J. Phelps, D.A. Elias, S.D. Wulfschleger, and D.E. Graham

Large reserves of organic carbon (C) in the active layer and permafrost affected soils of the Arctic tundra ecosystems are vulnerable to accelerated microbial degradation and release as greenhouse gases like methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>). In the present microcosm study, we determine dynamics and fluxes of CH<sub>4</sub> and CO<sub>2</sub> from depressed, elevated and subsided areas of low- and high-centered polygons from interstitial tundra on the Barrow Environmental Observatory (Barrow, AK). Temperature sensitivities of anaerobic respiration and methanogenesis were determined for organic, mineral and permafrost horizons incubated at -2, +4, or +8 °C upto 60 days. Production rates for both CO<sub>2</sub> and CH<sub>4</sub> were substantially higher for organic horizons (20 to 40 % wt. C) than the mineral horizons (< 18 % wt. C). Permafrost soils (~12 % wt. C) produced CO<sub>2</sub> but negligible CH<sub>4</sub>. A characteristic lag phase, temperature threshold for methanogenesis, and temporal dynamics indicated that a constant Q<sub>10</sub> relationship is inadequate to explain temperature responses from a range of -2 to +8 °C. Temperature response of methanogen mcrA genes (encoding the alpha subunit of methyl coenzyme M reductase) were correlated to the relative abundance of active methanogens in the active layer vs. permafrost. qPCR analysis revealed absence of mcrA gene copies in the permafrost. Time course measurements in this study were used to estimate continuous, differentiable functions for CH<sub>4</sub> and CO<sub>2</sub> production at relevant temperatures. These functions enable rate calculations that will help parameterize Arctic terrestrial ecosystem models for anaerobic biogeochemical processes.

Poster 29

**Field-Scale Tracking of Active Methane-Oxidizing Communities in a Landfill-Cover Soil**

Presented by Martin H. Schroth, Ph.D., Professor, Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Switzerland

Co-Authors: R. Henneberger, E. Chiri, P.E.L. Bodelier, P. Frenzel, C. Lüke, and M.H. Schroth

In landfill-cover soils, aerobic methane-oxidizing bacteria (MOB) attenuate emissions of the greenhouse gas methane (CH<sub>4</sub>), which is produced during anaerobic waste degradation. We characterized spatial and seasonal differences in active MOB communities at the field scale in a Swiss landfill-cover soil that is subject to high variability in environmental conditions. Field-based measurements of CH<sub>4</sub> oxidation activity and stable isotope probing of polar lipid-derived fatty acids (PLFA-SIP) were complemented by microarray analysis of *pmoA* genes and transcripts extracted from soil samples, linking diversity and function at the field scale. In situ CH<sub>4</sub> oxidation rates (up to 53 mmol CH<sub>4</sub> L<sup>-1</sup> d<sup>-1</sup>) varied between sites and were generally one order of magnitude higher in summer (soil temperature 17 – 19°C) compared with winter (~2°C). Results obtained from PLFA-SIP and *pmoA* transcript-based microarrays were largely congruent and revealed distinct spatial and seasonal clustering. Overall, active MOB communities were highly diverse, and type Ia MOB, specifically *Methylomonas* and *Methylobacter*, were identified as key drivers for CH<sub>4</sub> oxidation, in particular at the site showing highest activity. Type II MOB were mainly active at a site showing substantial fluctuations in CH<sub>4</sub> soil-gas concentrations and soil-moisture content. Spatial separation was less distinct in winter, with *Methylobacter* species and uncultured MOB mediating CH<sub>4</sub> oxidation. Notably, USG-gamma related *pmoA* transcripts were detected in both seasons at the site showing lowest in situ activity. We propose that the high diversity of active MOB communities in this cover soil is promoted by high variability in environmental conditions, facilitating efficient CH<sub>4</sub> removal.

Poster B30

**Long-Term Chemostat Kinetics of Multiple TCE-Dehalogenating Anaerobic Consortia under Electron Limited Conditions**

Presented by Lewis Semprini, Ph.D., Professor, School of Chemical, Biological, and Environmental Engineering, Oregon State University, USA

Co-Authors: L. Semprini, M. Azizian, K. Mayer-Blackwell, and A.M. Spormann

Two cultures – the Victoria Strain (VS) and the Evanite Strain (EV), that are highly enriched with the organohalide respiring bacteria *Dehalococcoides mccartyi* – were grown in chemostats for more than 4 years at a mean cell residence time of 50 days. The slow doubling rate achieved represents growth conditions likely experienced in subsurface environments. Formate, as an electron donor, was fed in excess and limited for complete dehalogenation of TCE to ethene. Under excess formate conditions, stable operation was observed with respect to TCE transformation, steady-state hydrogen (H<sub>2</sub>) concentrations, and the structure of the dehalogenating community. TCE was transformed to ethene, with minor amounts of vinyl chloride (VC) observed. Acetate was produced at aqueous H<sub>2</sub> concentrations ranging from 14 to 17 nM. Dehalogenation, acetate formation, and biomass production represented 59 to 61 %, 22 to 28%, and 11 to 12% of electron donor utilization. When formate was limited, markedly different dynamics were observed. TCE was transformed incompletely to ethene (40-60%) and VC (60- 40%), and H<sub>2</sub> concentrations ranged from 1 to 3 nM. The acetate concentration dropped below detection. Dehalogenation and biomass production represented 83 to 88% and 14% of the electron donor utilization, respectively. Batch kinetic studies of TCE transformation were performed with chemostat harvested cells. Transformation rates of c-DCE and

VC were greatly reduced when the cells were grown with limited formate. Molecular analysis is being performed to quantify the multiple unique strains of *Dehalococcoides mccartyi* present when the rate tests were performed.

Poster B31

### **Use of the Subsurface as a Bio-Filter for Removing Sulfate from Water Injected into an Oil Field**

Presented by Yin Shen, Technician, Petroleum Microbiology Research Group, Department of Biological Sciences, University of Calgary, Canada

Co-Authors: Y. Shen, T. Jack, R. Clark, A. Benko, K. Miner, R. Pederzoli, and G. Voordouw

The Medicine Hat Glauconitic C (MHGC) field produces heavy oil through produced water re-injection (PWRI) from a reservoir 850 m below the surface. Sulfate-containing make-up water (1000 m<sup>3</sup>/day; 3.5 mM sulfate) is mixed with produced water in a water plant prior to re-injection. Reduction of injected sulfate (350 kg/day) to sulfide by resident sulfate-reducing bacteria is referred to as souring. Souring, determined as the volume-weighted average aqueous sulfide concentration in monitored production wells, is controlled by continuous field-wide nitrate injection since early 2007. Sulfide increased from 0.08 to 0.15 mM in the first 2 years of injection and decreased back to 0.08 mM in the next 3 years. This was associated with breakthrough of nitrate and nitrite in several production wells. A further decrease in produced sulfide was associated with the start-up of the polymer pilot plant for enhanced oil recovery in 2012. All sulfate-containing make-up water was used for polymer dissolution, amended with nitrate and injected in a limited number of injection wells. Pilot produced water had 0 mM sulfate and maximally 0.3 mM sulfide with the balance (3.2 mM) likely being retained in the subsurface as metal sulfides. Hence, the subsurface removed about 90% of injected sulfate. Using the polymer pilot produced water as make-up water in the water plant decreased injected sulfate and subsequent souring. This has allowed injected nitrate to be lowered by 2- to 4-fold. Hence, in addition to nitrate, injection of all sulfate-containing water in a single location is a good strategy to control souring in fields from which oil is produced by PWRI.

Poster B32

### **Subseafloor-Incubated Mineral Metagenomes**

Presented by Amy R. Smith, Doctoral Student, College of Earth, Ocean, and Atmospheric Sciences, Oregon State University, USA

Co-Authors: A.R. Smith, F. Colwell, M. Fisk, O. Mason, A. Thurber, G. Flores, and R. Popa

Crustal mineralogy has been proposed as a driving force for determining deep life community structure and diversity. Recently, we found that minerals characteristic of the igneous crust harbored unique communities when incubated in the Juan de Fuca Ridge flank borehole IODP 1301A. Based on pyrotag sequencing of the 16S rRNA gene for archaea and bacteria, dominant organisms were related to heterotrophs and autotrophs, hydrogen oxidizers, methane cyclers, iron and sulfur metabolizers, and hydrocarbon degraders. Differences in mineral communities were reflected in biofilm morphologies, with olivine having thick, carbon-rich biofilms often containing secondary minerals, glass biofilms containing encrusted cells and tubes, and plagioclase having only small clumps of cells. We also found that the attached communities from incubated borehole minerals were taxonomically more similar to native, attached communities from marine and continental crust than to communities from the aquifer water that seeded it. Here we present the unique metagenomes associated with olivine, plagioclase, and glass from the subseafloor incubation experiment. We also describe the functional differences between the mineral-attached subseafloor metagenomes and those

of other deep crustal or seawater sites. Our findings have important implications for resolving ecosystem function and microbial distributions in igneous crust, the largest deep habitat on Earth.

Poster B33

**Methanogenic Biodegradation of a Three-Ringed Polycyclic Aromatic Hydrocarbon**

Presented by Courtney R.A. Toth, M.Sc. Student, Department of Biological Sciences, University of Calgary, Canada

Co-Authors: C.R.A. Toth and L.M. Gieg

Environmental contamination by polycyclic aromatic hydrocarbons (PAHs) is of great concern because of their recalcitrant nature and known toxic properties. Bioremediation is a desirable method for PAH removal in situ however PAH degradation under anaerobic, and in particular methanogenic conditions, is poorly understood. Only a handful of reports have documented methanogenic PAH biodegradation; the mechanisms for PAH activation and subsequent degradation under these conditions are largely unknown. In order to better understand methanogenic PAH metabolism, we have enriched a methanogenic culture able to biodegrade phenanthrene, a three-ringed PAH. Enhanced levels of methane production and hydrocarbon loss were measured in the culture relative to controls, providing evidence for phenanthrene utilization. Metabolomic analysis using GC-MS has revealed the presence of p-toluic and benzoic acid, inferring ring opening and that the final ring may be biodegraded in a similar manner to an anaerobic decay pathway known for p-xylene. p-Carboxybenzaldehyde was also detected, which may transform into p-toluic acid by an aldehyde dehydrogenase gene. Methyl-substituted naphthalenes and cyclohexane carboxylic acid have also been identified. None of these putative metabolites were detected in controls. Ongoing work with <sup>13</sup>C-labeled phenanthrene will help to confirm these compounds as metabolites and identify the mechanism of phenanthrene activation under methanogenic conditions. Microbial community and gene analyses also in progress will help to identify the key organisms and genes associated with the methanogenic degradation of unsubstituted PAHs.

Poster B34

**Similarity in Subsurface Microbial Community Dynamics at Two Crude Oil Spill Sites**

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

Co-Authors: D.M. Akob, B.B. Bekins, I.M. Cozzarelli, and E. Warren

Microbial community structure was compared at crude oil spills in two glacial outwash aquifers near Bemidji, MN, USA (sites: Bemidji and Cass Lake). The Bemidji site was contaminated by a 1979 pipeline rupture, whereas at the Cass Lake site, a pipe coupling leaked for an unknown period during 1971-2002. At both sites, hydrocarbons are actively degraded, with benzene degradation linked to iron reduction occurring at similar rates. However, nitrate concentrations are higher (4-18.5 mg/L) at Cass Lake. The oil at Bemidji shows spatial variability in degree of degradation, whereas oil at all Cass Lake locations is highly degraded. Sediment microbial communities were characterized in uncontaminated areas (4 samples), within the oil (7 samples), and within the hydrocarbon plume (10 samples) using pyrosequencing and group-specific qPCR. The Bacteria communities clustered into three groups independent of spill site but corresponding to contamination characteristics: (1) oil source zones, (2) groundwater plumes, and (3) uncontaminated areas. At Cass Lake, plume-impacted samples had the highest diversity, whereas at Bemidji the uncontaminated samples had the highest diversity. The lowest diversity at both sites was in the oil, consistent with previous analyses near the oil at Bemidji. Sulfate reducer (dsr), Geobacter, and methanogen (mcrA) abundance was higher in the oil and plume samples compared to the uncontaminated locations, with higher overall abundance of

functional groups in the Cass Lake plume. Despite differences in the oil degradation state, subsurface microbial communities were similar between the sites, suggesting that crude oil is an important selective driver on community structure.

B35

### **Viral Metagenomes from the Terrestrial Deep Biosphere**

Presented by Xiaofen Wu, Doctoral Student, Systems Biology of Microorganisms Research Group, Linnaeus University, Sweden

Co-Authors: X. Wu, K. Holmfeldt, S. Bertilsson, A. Andersson, and M. Dopson

The existence of viruses in deep granitic groundwater at the Äspö Hard Rock Laboratory (HRL), Sweden has been reported, and the presence of bacteriophages suggests that viruses might control the bacterial population in deep subsurface and potentially mediate gene transfer. Despite this, the knowledge of phage diversity and ecological effects is highly limited, and no viral metagenomes in deep subsurface have been reported. This study was conducted at the Äspö HRL, which is located north of Oskarshamn on the east coast of Sweden. Äspö HRL consists of a 3.6 km long tunnel extending to 460 m underground and is the world's largest bedrock research facility for investigating natural processes in the deep sub-surface and the potential for nuclear waste storage. Three viral metagenomes have been prepared directly from deep granitic groundwater corresponding to the dominant water types: meteoric, marine, and a mixture thereof. The viral metagenomes will provide taxonomic and functional information regarding the viral communities at these sites. Of special importance is the detection of auxiliary metabolic genes, through which viruses can have a direct impact on the biogeochemical cycles. Additionally, comparison to bacterial metagenomes from the same site will provide information on how the viruses drive microbial diversification and the potential of virus mediated DNA gene transfer events in deep subsurface. Overall, this study will give a better understanding of the role of viruses in deep biosphere and their impacts on this unique ecosystem.

B36

### **Towards Souring Control with Continuous Nitrate and Pulsed Biocide in Bioreactors Simulating Oil Fields**

Presented by Fiona (Yuan) Xue, M.Sc. Student, University of Calgary, Canada

Co-Authors: F. Xue

Souring, the reduction of sulfate to sulfide by sulfate-reducing bacteria (SRB) in oil fields can be remediated by nitrate injection. However, continuous amendment of sulfate-containing injection water with nitrate can lead to zonation, where nitrate reduction occurs in the near injection wellbore region followed by sulfate reduction deeper in the reservoir. Once microbial zonation has been established, nitrate is no longer effective in removing sulfide. Although biocide application is limited to above-ground infrastructures to control corrosion and fouling, the possibility that the combination of biocides and nitrate can successfully break zonation needs to be investigated. The combination of nitrate and biocide may control SRB more effectively, if synergy between these two agents can be achieved. Souring up-flow bioreactors injected with excess volatile fatty acids (acetate, propionate and butyrate) and 2 mM sulfate were treated with biocide pulses of long duration (5 days) at low concentration or of short duration (1 h) at high concentration. The success of these strategies was determined by the recovery time needed to re-establish sulfide. The short-high strategy was better with glutaraldehyde and benzalkonium chloride, whereas the long-low strategy was better with cocodiamine in obtaining souring control. When 2 mM nitrate was also continuously injected into the bioreactors, together with 2 mM sulfate, pulses of biocide were synergistic, antagonistic or indifferent

depending on the type of biocide, its concentration and the length of the pulse. Hence, pulsed biocide injections can improve souring control with continuous nitrate injections, if the appropriate product and injection strategy are chosen.