



# **Ninth International Symposium on Subsurface Microbiology**

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

---

**Monday, October 6, 2014**

**POSTER PRESENTATION ABSTRACTS**

---

**POSTER SESSION A: ECOLOGY, FATE, AND BIOMES**

**7:00 PM – 8:30 PM**

**KILN ROOM**

---

Poster A1

**Screening for Heavy Molecular Weight Hydrocarbon Utilizing Bacteria from Oil Impacted, Unimpacted Soil and Natural Deposits**

Presented by Ayansina S. Ayangbenro, M.Sc. Student, Department of Microbiology, College of Natural Sciences, Federal University of Agriculture, Nigeria

Co-Authors: S.A. Balogun, A.S. Ayangbenro, S.O. Kareem, and O.S. Sojину

Contamination of soil and water by oil and its derivatives causes degradation of such environment. Thus control and treatment of hazardous effects of oil pollution are needed. This study investigated the isolation and screening of microorganisms capable of utilizing heavy oil from polluted sites. Enrichment culture technique was used to isolate bacterial species from bitumen impacted and unimpacted soil samples. The total heterotrophic bacterial counts ranged from  $1.4 \times 10^5$  CFU/g to  $2.0 \times 10^6$  CFU/g. The total oil utilizing bacterial counts varied from  $1.5 \times 10^4$  CFU/g to  $3.6 \times 10^5$  CFU/g. Twenty-four bacterial isolates capable of utilizing hydrocarbon as a carbon source were isolated and characterized using API 20E kit. The isolates belong to the genera Burkholderia, Pseudomonas, and Serratia. The degradation efficiency of the isolates petrol or Premium Motor Spirit (PMS), kerosene or Dual Purpose Kerosene (DPK) and engine oil or Low Pour Point Fuel Oil (LPFO) were carried out by a colorimetric rapid screen test using 2, 6-dichlorophenol indophenol (DCPIP) reduction test. Six of

the isolates that showed best activity on PMS, DPK and LPFO were further monitored by measuring absorbance at 600nm for 120 hours at interval of 24 hours. The order of ability of the isolates to degrade PMS: *P. aeruginosa* > *P. mendocina* > *P. borbori* > *S. rubidae* > *P. cichorii* > *B. cepacia* while for DPK is *P. cichorii* > *P. borbori* > *S. rubidae* > *P. mendocina* > *B. cepacia* > *P. aeruginosa*. The ability to degrade LPFO: *P. cichorii* > *P. borbori* > *P. aeruginosa* > *P. mendocina* > *B. cepacia* > *S. rubidae*. *P. cichorii* showed a better potential in utilizing the entire hydrocarbon tested.

Poster A2

**Organic Micropollutant Removal during River Bank Filtration: A Pilot-Scale Study**

Presented by Cheryl Bertelkamp, Doctoral Student, Department of Water Management, Delft University of Technology, The Netherlands

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

River bank filtration (RBF) in The Netherlands was originally designed as a pre-treatment step for surface water treatment (via removal of particles, NOM, pathogens, viruses etc.). However, following the detection of organic micropollutants (OMPs) in Dutch surface waters at ng/L to µg/L levels, interest has arisen in exploiting the RBF system's capability to remove OMPs. This paper investigates the sorption and biodegradation behaviour of a cocktail of 50 OMPs spanning a wide range of physico-chemical properties (molecular weight, hydrophobicity and charge) and functional groups, in laboratory soil columns under different redox conditions. Retardation factors and biodegradation rates were determined for all OMPs which enabled the development of a predictive model. The experimental set-up consisted of 3 unique RBF pilots with residence times of 4 days (oxic), 21 days (nitrate reducing) and 45 days (iron/manganese reducing). Mimicking RBF on such a scale has not been performed in literature yet. Microbial community composition and diversity was assessed in the soil to gain more insight in its relation to redox conditions and OMP removal. While most OMPs were removed to below detection level under oxic conditions, several OMPs showed redox dependent removal. Atrazine, TPPO, carbamazepine, diglyme, and bentazon showed persistent behaviour. For the oxic pilot it was observed that negatively charged OMPs were well removed followed by positively charged OMPs. For neutral OMPs no relation between removal and hydrophobicity (Log D) was obtained. The results of this study are a first step towards a predictive model that enables drinking water companies to assess what type of post-treatment is required to prevent OMPs from ending up in the drinking water.

Poster A3

**QMRACatch: A Tool to Get Microbial Indicator Input Concentrations for Transport Simulations in a Riverbank Filtration Dominated Aquifer - A Case Study**

Presented by Prof.Dr. Alfred Paul Blaschke, Institute of Hydraulic Engineering and Water Resources Management, Vienna University of Technology, Austria

Co-Authors: A.P. Blaschke, J. Derx, A.H. Farnleitner, and J. Schijven

The presented work demonstrates the application of the recently developed modelling tool QMRACatch to address complex water quality and management issues at an study area covers an alluvial groundwater aquifer (PGWA) on the left bank of the River Danube which is used as a drinking water resource. QMRACatch is designed to model pathogen infection risk for different scenarios and reference pathogens selected by using also monitoring data from faecal pollution loads and microbial source tracking for calibration (i.e. combining advanced faecal pollution diagnostics and infection risk assessment). The PGWA constitutes not only an important ground water resource

for water supply but is also a national park. To avoid aggradations at the PGWA, it is planned to bring more water directly from the Danube into the wetland and to the backwater system, especially during the dry season. However, a negative influence on the existing drinking water wells is to be avoided. The microbial groundwater quality in the PGWA is mainly influenced by the quality of the River Danube (waste water influence), another source of faecal contamination could be sources from animals in the PWGA area. The effect of different management strategies were studied using a 3D groundwater model in combination with QMRAcatch to evaluate effects of the planned management measures on the microbial groundwater quality and the water quality of the wells used for water supply.

Poster A4

**Detection of Human-Associated Bacteroidales in the Coastal Ocean Adjacent to Groundwater Seeps on Maui, Hawaii**

Presented by Alexandria B. Boehm, Ph.D., Associate Professor, Stanford University, USA  
Co-Authors: K.M. Yamahara, W. Okubo, and A.B. Boehm

Wastewater is injected into the coastal aquifer on Maui. Concerns have been raised that the wastewater is making its way through the coastal aquifer to the coastal ocean where swimmers and surfers may come into contact with the wastewater. Water samples were collected from groundwater seeps adjacent to the coastal aquifer where wastewater is injected and tested for enterococci and human-associated Bacteroidales source tracking markers on multiple days across 2 years. The results from these analyses will be presented and implications on human health discussed.

Poster A5

**Identifying Nutrient Pathways, Environmental Assimilative Capacity, and the Zone of Impact in Situations of Excess Nutrient Release, Such as Aquaculture Farming, through Changes to Pelagic and Benthic Microbial Communities**

Presented by Hannah Bradford, M.Sc. Student, St. Andrews Biological Station, University of New Brunswick, Canada  
Co-Authors: H. Bradford, S. Robinson, B. Macdonald, and T.-L. Lander

Microbial influence on the carbon cycle was historically underestimated but is now recognized as having a significant influence on the reintroduction of carbon into food webs, this understanding has important management suggestions in situations of excess nutrient release. Aerobic microbes ingest nutrients, such as dissolved organic matter (DOM), and metabolize these substrates through oxidation. The carbon conversion rates of microbial communities can be estimated using community respiration rates to quantify how sources of excess nutrients, such as aquaculture sites, affect the microbial carbon cycle. Microbial community samples were collected in the Passamaquoddy Bay temporally and spatially with horizontal distance away from an aquaculture site (0, 50, 150 & 300 m and control) from the pelagic and benthic environment. Community respiration rates were measured with a © Fibox 3 oxygen optode sensor over time using filter (0.22 µm) based incubation of samples, these rates were compared to microbial abundance estimated by epi-fluorescent staining of filtered (0.22 µm) samples using 4',6-diamidino-2-phenylindole (DAPI) and captured using a © Nikon Eclipse 80i microscope and processed using © NIS Elements imaging software. The objective of this study was to determine if the impact of aquaculture farms could be detected in microbial communities. The response of microbial communities to excess nutrients can provide insight toward nutrient pathways, environmental assimilative capacity and the zone of impact. These insights have implications toward governmental policy on fallowing period and stocking densities as well as Integrated Multi-Trophic

Aquaculture (IMTA) management strategies for complimentary extractive species placement and site design.

Poster A6

### **How Prevalent Are Transported Microorganisms to the Microbial Community of an Epigenic Cave Stream?**

Presented by Kathleen Brannen, Doctoral Student, University of Tennessee, Knoxville, USA

Co-Authors: K. Brannen and A.S. Engel

Although microbial communities in cave streams rely on allochthonous nutrients, the dogma has been that cave systems support stable subsurface ecosystems. In an epigenic cave system that receives allochthonous organic matter input from photosynthetically-derived material, as well as seasonally dynamic hydrologic changes, such as sediment transport during large flood events, we hypothesized that the cave microbial community diversity is influenced by microorganisms that are transported from the surface. Water samples were collected to evaluate potential seasonal changes in the planktonic microbial community diversity transported from the surface. Microbial communities were enriched on Nomex-bead in situ microcosms (3 replicate microcosms at an upstream and downstream location within the cave) to compare cultivated groups with those from the surface stream. Similarities between surface and subsurface diversity were assessed using ANOSIM. Between surface and subsurface stream water, there are seasonal variations in the relative distribution of Proteobacteria. Betaproteobacteria (Burkholderiales) dominate both the water column (~40%) and microcosms (15-25%). There is a higher abundance of Bacteroidetes (Flavobacteriales) in the water column (10-22%) than in the microcosms (1-2%). Alphaproteobacteria (Rhizobiales) are more abundant in the microcosms (10%) than the water column (1-3%). While Nitrospirae (Nitrospirales) is not abundant in the cave stream (<1%), they are more abundant in the downstream microcosms (14%) than the upstream microcosms (3%). Our results suggest that microbial community diversity in the cave stream is dependent on the microorganisms that are transported into the system, although the same taxonomic groups differ in abundance.

Poster A7

### **Disentangling Surface Deposited from Endemic Microbes in a Terrestrial Hot Spring**

Presented by Brandon R. Briggs, Ph.D., Postdoctoral Research Assistant, Miami University, USA

Co-Authors: B.R. Briggs, Q. Huang, and H. Dong

Hot spring ecosystems are considered windows into the deep biosphere; however, they are also influenced by surface processes. To understand microbial dynamics that occur in these hot springs, endemic microbes need to be disentangled from microbes deposited from the surface environment. To this end, daily samples of water and sediment were taken from two hot springs in Tengchong, China (Gumingquan and Diretiyan). In addition, soil was collected from near the two springs. Gumingquan was a larger pool with no visible conduit for overland water flow into the spring. The microbial community and geochemical parameters did not change for Gumingquan and remained distinct from the surrounding soils. In contrast, Diretiyan was a smaller spring situated under a cliff where water could be seen flowing into the spring during rain events and the temperature fluctuated from 48-66C depending on the amount of water flow into the spring. When the lowest temperature of the spring was recorded the sediment and water community was more similar to the surrounding soil than the community that existed at the maximum temperature. The soil samples were dominated by Actinobacteria, Acidobacteria, and Proteobacteria. The hot spring was dominated by Aquificales and Crenarchaeota when it was not raining. During heavy rain the hot spring was dominated by

Proteobacteria and Cyanobacteria. The original hot spring community recovered within 24hrs of the rain stopping. These results indicate that rain events can wash microbes into the hot spring, which can alter the community structure and influence our views of the deep biosphere.

Poster A8

**Functional Potential of Atribacteria Collected from Antarctic Marine Sediment Based on Geochemistry and Single Cell Genomics**

Presented by Stephanie Carr, Ph.D., Postdoctoral Researcher, Colorado School of Mines, USA  
Co-Authors: S. Carr, B. Orcutt, and J. Spear

Like many microorganisms of the subsurface, those classified as Atribacteria (previously known as bacterial Candidate Division OP9) have yet to be cultured and their functional potential is unclear. Organisms of this phylum are commonly found in euxinic methane-rich sediments, including those of terrestrial hot springs (>70 °C) and the cool deep-sea (~2 °C), suggesting a potential role in methane cycling. Genomic analyses have also suggested that Atribacteria may be involved in saccharide fermentation. Recently Atribacteria have been identified in sediment from the Adélie Basin (located ~100 km off shore of Antarctica's Wilkes Land Margin) collected during Integrated Ocean Drilling Program Expedition 318. Pyrosequencing of DNA extracted from these sediments revealed increasing Atribacteria abundances with depth, ranging from 4% in surface sediments to 51% at 104 meters below seafloor. To explore the functional potential of Atribacteria in these methane-bearing sediment samples, individual cells were separated from frozen but unpreserved sediment for whole genome amplification. One single amplified genome related to Atribacteria was recovered from this process, with a close phylogenetic relationship to one of the Antarctic sediment Atribacteria clades observed previously; however, the 16S rRNA gene sequence was only 82-98% similar to other sequenced Atribacteria. Here, we compare the functional potential of this deep marine sediment Atribacteria single cell genome to those recently sequenced from terrestrial hot springs. Understanding the metabolic diversity within this phylum will improve our understanding of survival mechanisms for environments of extreme temperature as well as provide valuable insight to the biogeochemistry occurring in euxinic subsurface environments.

Poster A9

**Interplay between Fe- and S-Cycling Microbial Communities, Geochemistry, and Hydrology in the Intertidal Mixing Zone of a Beach Aquifer**

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

Co-Authors: S.M. McAllister, J.M. Barnett, A. Findlay, D. MacDonald, J. Heiss, G.W. Luther III, H.A. Michael, and C.S. Chan

Fe- and S-cycling microorganisms in mixing zones of coastal aquifers have the potential to significantly influence export of C, N, P, Fe, and other metals in submarine groundwater discharge (SGD). Before discharge, fresh and saline groundwater mix in the intertidal aquifer, creating chemical gradients conducive to microbial and geochemical reactions. Previous studies showed intertidal aquifers host a reactive barrier comprised of Fe mineral-coated sands, but did not resolve the mechanisms and dynamics of mineral formation/dissolution, which are key to understanding controls on SGD chemistry. Thus, we sought to determine the roles of microbes in Fe/S mineralization, and the interactions with abiotic geochemistry and physical hydrology. Toward this, we conducted a geochemical and microbiological study at a site on Cape Henlopen, Delaware. Fresh groundwater provides Fe, which precipitates as Fe oxides, likely through a combination of biotic and abiotic

processes. Both Fe-oxidizing and Fe-reducing microbes were detected, suggesting an active microbial Fe cycle. The dominant group associated with the Fe barrier is candidate division OP3; while its metabolism is unknown, OP3 has been associated with other Fe- and S-rich environments. Saline water brings oxygen, sulfate, and organic C into the mixing zone. Organotrophs consume oxygen, creating anoxic zones for sulfate-reducers to produce sulfide. This sulfide appears to react with Fe oxides, resulting in reductive Fe dissolution, and FeS precipitation. Integrating our results with hydrologic data and previous modeling, we describe the dynamics of the Fe reactive barrier over tidal and seasonal cycles, which helps us predict its influence on SGD chemistry.

Poster A10

**Nitrate Injection into Low Pressure, High Temperature Bioreactors as a Model for Control of Microbial Sulfide Production in Hot Oil Reservoirs**

Presented by Chuan Chen, Ph.D., Postdoctoral Fellow, University of Calgary, Canada

Co-Authors: C. Chen and G. Voordouw

Injection of cold seawater (10°C) into a high temperature oil field (60-90 °C) will cause emergence of a temperature gradient from the near injection wellbore region (NIWR) to the bulk of the reservoir. To model the contribution of a cool NIWR to sulfide production by sulfate-reducing bacteria (SRB), as well as the inhibition of SRB by nitrate injection, four lab-scale high temperature bioreactors (11x2.1 cm, thermo-jacketed) with different NIWR lengths (LNIWR) from 1 to 4 cm (not thermo-jacketed) were set up. CSBA-S brine medium containing 2 mM sulfate and 3 mM each of acetate, propionate and butyrate was continuously pumped into the bioreactor after inoculation with produced water until all sulfate was reduced at room temperature (20°C). The temperature of the thermo-jacketed portion (the bulk temperature, tB) was then increased to 60 °C. With tB at 60°C, the sulfide concentration in the effluent increased with increasing of LNIWR from 0.7 to 2.0 mM, indicating a larger cool NIWR contributed more sulfide than a shorter NIWR. When 4 mM nitrate was injected, the sulfide concentration produced decreased to 0.5-1.4 mM. Further increase of tB to 90 °C, the sulfide in the effluent in bioreactors with LNIWR of 1, 2, 3 and 4 cm decreased to 0.2, 0.2, 1.0 and 1.8 mM, respectively. Hence, sulfide produced in the bioreactor with a 4 cm NIWR was only 0.2 mM less than for tB=60°C. With 4 mM nitrate injection, the sulfide production by bioreactors with 1, 2, 3 and 4 cm LNIWR was 0.1, 0.1, 0.2 and 0.4 mM, respectively. The results obtained with this simple system agree with knowledge of high temperature, souring oil fields: (i) the cool NIWR region can be the main contribution of souring. (ii) the extent of the region depends on reservoir temperature (tB) and rate of injection of cold seawater. (iii) when the NIWR region is smaller (at a given flow rate), souring control with nitrate is easier to achieve than when the NIWR region is larger. In the case of tB=23 °C (low temperature reservoir), souring control can only be obtained with 13 mM nitrate.

Poster A11

**Microbial Diversity, Isolation, and Characterization of Iodine Transforming Microorganisms from the Subsurface Sediments of a <sup>129</sup>I-Contaminated Plume at the Hanford Site, Washington**

Presented by Joshua T. Ellis, Ph.D., Postdoctoral Research Associate, Pacific Northwest National Laboratory, USA

Co-Authors: J.T. Ellis, E. Eisenhauer, A. Dodwell, D. Saunders, B. Lee, and M.H. Lee

Radioactive iodine (<sup>129</sup>I) is a major by-product of nuclear fission, and is of environmental concern due to its long half-life (~16 million years), toxicity, and mobility in the environment. The 200 area of the Hanford Site contains two separate plumes covering 1,500 acres, where <sup>129</sup>I concentrations are ~3.5 pCi/L. Speciation analysis shows that iodate comprises 70.6% of the iodine present, and organo-

iodide and iodide comprise 25.8% and 3.6% respectively. Microbial activity within these plumes may affect iodine speciation, consequently affecting iodine mobility within the subsurface. The aim of this study was to determine the microbial community within these plumes and determine the ability of environmental isolates to affect iodine speciation through oxidation or reduction of iodide and iodate respectively. T-RFLP analysis of bacterial communities within these plumes was used to determine the structure and spatial distribution of indigenous bacterial communities throughout. This analysis was performed on a range of  $^{129}\text{I}$  levels, ranging from high, low, and background  $^{129}\text{I}$  concentrations. The microbial diversity within the high and low level plumes both showed Betaproteobacteria, specifically Burkholderiales as being the most dominant taxa within these plumes, whereas the background level plume showed Alphaproteobacteria, specifically Rhizobiales, as the dominant taxa. Bacterial isolates capable of oxidizing and reducing iodine from these plumes were obtained from aquifer sediments incubated in plumes for 150 days. These data allow us to understand iodine speciation through a variety of microbial physiotypes throughout the Hanford Site, along with providing novel microbial systems for the bioremediation of iodine.

Poster A12

**Cultivation of Methanogenic Microbial Community from Deeply Buried Coalbeds in the Ocean**

Presented by Hiroyuki Imachi, Ph.D., Senior Scientist, Institute of Biogeosciences, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Japan

Co-Authors: H. Imachi, E. Tasumi, A. Ijiri, U. Konno, Y. Morono, M. Ito, K. Takai, F. Inagaki

Deep subseafloor environments associated with hydrocarbon reservoirs have been least explored by previous scientific drilling and hence the nature of deep subseafloor life remains largely unknown. In this study, we performed cultivation of deep subseafloor microbial components using a down-flow hanging sponge (DHS) reactor. The sample used for the cultivation was obtained from 2 km-deep coalbeds (i.e., lignite) off the Shimokita Peninsula of Japan during the Integrated Ocean Drilling Program (IODP) Expedition 337. The powdered coalbed samples were incubated anaerobically in the DHS reactor at near the in-situ temperature of 40°C. Synthetic seawater supplemented with a tiny amount of yeast extract, acetate, propionate and butyrate was continuously provided into the DHS reactor. After 35 days of the bioreactor operation,  $^{13}\text{C}$ -depleted microbiological methane production was observed. Phylogenetic analysis of 16S rRNA genes showed active growth of diverse bacteria and archaea in the DHS reactor. Predominant archaeal components were closely related to hydrogenotrophic methanogens within the genus *Methanobacterium*. Some predominant bacterial components were related to the spore-formers within the class Clostridia. By analyzing ion images using a nano-scale secondary ion mass spectrometry (NanoSIMS),  $^{13}\text{C}$ -bicarbonate incorporation of rod-shaped methanogens was observed. Using the reactor enrichment as inoculum, subsequent batch-type cultivation led to the successful isolation of several anaerobic microorganisms, including the hydrogenotrophic methanogen. These cultivation-based microbiological data are consistent with the geochemical indication that microbial processes contribute to the biogeochemical carbon cycle associated with the deeply buried coalbeds in the deep subseafloor biosphere.

Poster A13

**Sulfolane Biodegradation Potential in a Contaminated Subarctic Aquifer**

Presented by Christopher P. Kasanke, Doctoral Student, University of Alaska, Fairbanks, USA

Co-Authors: C.P. Kasanke and M.B. Leigh

Sulfolane, an industrial solvent that is used to de-acidify natural gas and selectively remove lighter aromatics from petroleum, has a high affinity for water and has been found in the aquifers surrounding

sites where it has been improperly handled. To date sulfolane degradation in groundwater has only been studied in three contaminated sites, which are located in western Canada, Brisbane, Australia, and Lathrop, California. A major sulfolane plume located in North Pole, Alaska, was recently identified that spans roughly a 3.5 by 2 mi area of an aquifer in discontinuous permafrost. Little is known about the biodegradation potential of sulfolane in aquifers containing permafrost. Also, many knowledge gaps remain regarding the microbial community responsible for sulfolane degradation. Aerobic and anaerobic microcosm studies were performed at 4 - 8°C using substrate from the contaminated North Pole aquifer to assess biodegradation potential. Sediment/groundwater slurries and groundwater-only biodegradation incubations were set up under aerobic conditions at different sulfolane concentrations with and without nutrient addition. Anaerobic sediment slurries were established under nitrate and sulfate reducing conditions. Sulfolane biodegradation was observed under aerobic conditions. Nutrient amendments dramatically increased the aerobic degradation rate in our high concentration slurry incubations (~100 ppm sulfolane), but didn't appear to have an effect on the rate of biodegradation at low concentrations (~500 ppb sulfolane). To date there is no evidence of anaerobic sulfolane biodegradation in our microcosms. Future work involves analyzing the microbial community associated with sulfolane biodegradation using culture-based methods and molecular tools including stable isotope probing.

Poster A14

**Bacteria and Fungi in Cold Limestone Caves of Siberia, Far East, and the Western Caucasus**

Presented by Sergey Khizhnyak, Sc.D., Professor, Krasnoyarsk State Agrarian University, Russia

Co-Authors: S. Khizhnyak, E. Nesterenko, E. Lankina, I. Ilients, S. Ovsyankina, and L. Kharlamova

Bacteria and fungi populating cold (the temperature in the homothermic zone +2°C .. +5°C) limestone caves of Siberia, Far East and Western Caucasus are presented with mesophiles which are able to grow at low temperature and with cold-adapted strains. Cold-adapted strains can be divided into two groups: "psychrophiles" with the maximal growth temperatures less then +27..+30°C and the optimal growth temperatures between 17°C and 20°C, and "psychrotolerants" with the maximal growth temperatures less then +35°C and the optimal growth temperatures between 24°C and 30°C. Proportions of psychrophiles, psychrotolerants and mesophiles as soon as total number of cultivated bacteria and fungi statistically significantly ( $p < 0.001$ ) vary among the caves and among sites within the caves. Coexistence of mesophiles and cold-adapted forms with different temperature optima can be explained using simple math model based on the idea of caves colonization by mesophiles penetrating from terrestrial ecosystems with their subsequent evolutionary adaptation to low temperature. According to this model all the microbial communities in the researched caves are at the intermediate stage of formation. Among cold-adapted cave bacteria the following genera were identified: *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Flavobacterium*, *Janthinobacterium*, *Paenibacillus*, *Pedobacter*, *Pseudochrobactrum*, *Pseudomonas*, *Sphingopyxis*, *Sporosarcina*. Cold-adapted cave fungi are presented with the genera *Pythium*, *Mucor*, *Mortierella*, *Absidia*, *Thamnidium*, *Paecilomyces*, *Penicillium*, *Chrysosporium*, *Fusarium*, *Verticillium*, *Doratomyces*, *Echinobotryum*, *Periconia*, *Thrihoderma*, *Cryptococcus*, *Rhodotorula*, with the genera *Chrysosporium*, *Mucor* and *Penicillium* the most prevalent.

Poster A15

**Cold-Adapted Cave Bacteria as the Biological Control Agents in Agriculture**

Presented by Sergey Khizhnyak, Sc.D., Professor, Krasnoyarsk State Agrarian University, Russia  
Co-Authors: S. Khizhnyak and E. Lankina

Existing biopreparations for biological control of plant diseases in agriculture are based on the mesophilic bacteria and fungi. It makes these preparations ineffective in boreal climate zone at the beginning of vegetation when soil temperature is about 5-10°C. Another problem is that even non-pathogenic mesophilic bacteria and fungi used in biocontrol are potentially dangerous for human. Our researches showed that many of cold-adapted bacteria and fungi isolated from the cold limestone caves of Siberia, Far East and Western Caucasus suppress growth of soilborne fungal pathogens belonging to the genera *Bipolaris*, *Alternaria* and *Fusarium*. High growth rate of cold-adapted strains at low temperature and their inability to grow at human body temperature makes these strains a good alternative to mesophilic microorganisms in biological control of plant pathogens, especially in the areas with boreal climate. The results of three years field tests conducted in the Krasnoyarsk State Agrarian University in collaboration with the Krasnoyarsk Research Institute of Agriculture demonstrated that cold-adapted strains isolated from caves successfully suppress root rot and leaf spot diseases of wheat and barley caused by *Bipolaris*, *Alternaria* and *Fusarium*. Effect of seed treatment with cave bacteria in the diseases symptoms reducing and in the yield increasing was comparable with the effect of commercial chemical preparations.

Poster A16

**Simple Math Model of Cave Eutrophication**

Presented by Sergey Khizhnyak, Sc.D., Professor, Krasnoyarsk State Agrarian University, Russia  
Co-Authors: S. Khizhnyak

In spite of obvious oligotrophy of the Middle Siberian caves as ecosystems, the ratio of oligotrophs to total heterotrophs in cave sediments and water ranges from 7% to 76% with the average ratio 55%. Such a low ratios of oligotrophs in the oligotrophic ecosystems as soon as a great variation in oligotrophs proportion within and among the caves can be explained using a simple model based on the Monod- Haldane's equations of substrate inhibition applied with irregular income of nutrient-rich exogenous substrate into cave ecosystem. Income of organic matter is due to bats, animals and invertebrata accidentally penetrating into the caves, cave visitors and (in the near-entrance areas) to soil and other organic substrate incoming with atmospheric water inflow.

Poster A17

**Bacterial Diversity of Mat Communities of Lava Beds, National Monument Lava Caves**

Presented by Ara Kooser, Doctoral Student, Department of Biology, University of New Mexico, USA  
Co-Authors: D.E. Northup, K.H. Lavoie, A. Kooser, K.J. Hughes, and E. Hughes

The caves of the Lava Beds National Monument (LBE) contain a rich variety of microbial mats that cover the walls, ceilings, and floors, growing in a variety of habitats and conditions. We compared microbial composition of mats of different colors (white, yellow, tan) from seven LBE caves that differed in abiotic parameters with each other and with microbial communities in overlying soils, using next gen sequencing of the 16S SSU. Recovered sequences clustered in 12 main bacterial phyla, with a scattering of "other," rarer phyla, which spanned 19 additional phyla, many of which are recently described or discovered bacterial phyla. The 12 core phyla were recovered from all seven caves, despite differences in human visitation level, elevation, length, number of entrances, or age of

the lava flow in which the caves were formed. However, caves that are longer, older, with fewer entrances, more human visitation or that occurred at higher elevations, had a larger percentage of Actinobacteria, the bacterial phylum that is often found in caves and from which we derive many of our naturally occurring antibiotics. More sequences from the phylum Nitrospirae were recovered from within the caves, while very few were recovered from soils above the caves. Microbial communities in the caves also differed from the surface soils in the sequences recovered from Protobacteria and Bacteroidetes phyla. These results will provide valuable information for understanding and interpreting the microbial diversity of subsurface cave environments in comparison to surface environments.

Poster A18

### **Microbial Communities in Arctic Sediments North of Svalbard**

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

Co-Authors: N. Straaten and M. Krüger

Major challenges for microbial life in the polar regions are low sedimentation rates and limited nutrient input caused by varying temperatures and changing ice coverage. To investigate geochemical conditions and microbial populations, sediment samples were collected using a gravity corer in three areas north of Svalbard in the Barents Sea in September 2013 and the core samples were taken from shallow (200m) areas on the Svalbard shelf as well as deep sea areas on the eastern Yermak Plateau (3200m water depths). To fill the gaps in our knowledge about the microbial communities inhabiting Arctic marine sediments, our aim was to study the seafloor geochemistry in these areas, and to quantify Bacteria and Archaea as well as important functional genes. These will target important microbial groups, like methanogens, metal and sulfate reducers, which may drive seafloor ecosystems in the Northern Barents Sea. Another objective will be the investigation of the oil biodegradation potential of the indigenous microbiota under in situ conditions. Shelf sediments showed the highest organic carbon content which decreased with increasing depths. Also, potential sulfate reduction, carbon dioxide and methane production rates were highest in these samples. Potential electron acceptors, like sulfate, iron and manganese were present in substantial amounts throughout the cores. Our first results indicate that these Arctic sediments are a suitable habitat for microorganisms despite cold temperatures and a low nutrient input. This study will provide information regarding global distributions of activities and communities of subsurface microbes.

Poster A19

### **Assessing the Potential for Anaerobic Biocorrosion of Carbon Steel by Halophilic Heterotrophs in Oil Production Facility Brines**

Presented by Christopher R. Marks, Doctoral Student, Department of Microbiology and Plant Biology, University of Oklahoma, USA

Co-Authors: C.R. Marks, V. Bonifay, B.W. Stamps, H.M. Le, B.H. Harriman, A. DeCapite, K.R. Brown, J. Sunner, B.S. Stevenson, K.E. Duncan, M.J. McInerney, D. Aktas, and J.M. Suflita

Petroleum reservoir microorganisms drastically influence the economics of energy recovery operations through deleterious activities such as souring and biocorrosion. We investigated the potential for biocorrosion using production fluid samples from 3 sites within an oil processing facility. Samples were collected before and after the flushing of a sample valve to assess possible differences between stagnant areas and bulk process fluids at each site. The production fluids were high saline brines (1.5 – 4.0 M [Cl<sup>-</sup>]) with variable pH (4.5 – 6.2) both within and between sampling sites.

Sulfate (0.1 – 8.0 mM) and acetate (0.5 – 9.3 mM) were present in all samples, but nitrate was below detection levels. Molecular surveys revealed that the microbial communities were predominantly comprised of three taxonomic groups: Halanaerobiales, Campylobacterales, and Desulfovibrionales. MPN determinations of heterotrophic fermenting, sulfate-reducing, and thiosulfate-reducing organisms were all greater than  $1 \times 10^4$  cells mL<sup>-1</sup>. Targeted mass spectral analysis of the production fluids revealed the presence of catechols, phenols, and dihydrodiols, indicative of aerobic hydrocarbon biodegradation. Metabolites associated with anaerobic hydrocarbon biodegradation were not detected. Untargeted metabolomic screening revealed over 1000 identified compounds that mapped primarily to known fatty acid and carbohydrate metabolic pathways. Laboratory biocorrosion assays revealed a generalized corrosion rate of  $<0.12$  mmpy and the formation of  $<150$  surface pits over  $\sim 78$  mm<sup>2</sup> on carbon steel coupons. Our results suggest a systemic colonization of the facility by anaerobic halophiles, commonly associated with petroleum reservoirs. These organisms were likely cross-fed by organic electron donors produced during the transformation of hydrocarbons by aerobic microorganisms.

Poster A20

### **Insights into the Physiology of *Geobacter Metallireducens* during Growth under Carbon Excess and Carbon Limitation**

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

Co-Authors: S. Marozava, W.F.M. Röling, J. Seifert, R. Küffner, M. von Bergen, and R.U. Meckenstock

Knowledge on in situ physiology of iron-reducing microorganisms which play an important role in degradation of aromatic hydrocarbons in contaminated aquifers might be helpful in designing bioremediation strategies. Of special interest is the ability of such microorganisms to consume substrates at high (polluted environments) or low carbon content (remaining contaminants). The current study examines the physiology of the anaerobic, iron-reducing delta proteobacterium *Geobacter metallireducens* at carbon excess in batch ( $\mu=0.06$  h<sup>-1</sup>) and carbon limitation in retentostats ( $\mu<0.003$  h<sup>-1</sup>). In batch experiments, acetate and ethanol were preferred over benzoate. In contrast, under carbon limitation acetate and benzoate were co-consumed. The overall physiological changes were examined via nano-LC-MS/MS. Under carbon excess the benzoyl-CoA pathway was found to be subjected to incomplete repression in the presence of acetate or on butyrate as single substrate while peripheral degradation pathways of toluene and ethanol were highly expressed only in presence of the corresponding substrates. Carbon limitation in retentostats significantly increased the abundances of several catabolic proteins involved in the degradation of substrates not present in the medium (i.e. ethanol and fatty acids). Additionally, low growth rates changed abundances of energy-, chemotaxis-, oxidative stress-, and transport-related proteins. Therefore, at carbon excess easily degradable substrates are preferred over aromatic compounds while under carbon limitation they are co-metabolized. At extremely slow bacterial growth *G. metallireducens* is prepared to consume several carbon sources simultaneously with a preference to fermentation products such as low molecular weight fatty acids and alcohols. Furthermore, it is prepared to withstand various environmental stresses.

Poster A21

**DNA Fingerprinting of Sand Indigenous Bacterial Populations from Four Great Lake Beaches by PCR-DGGE**

Presented by Maria M.F. Mesquita, Ph.D., Senior Laboratory Coordinator, Department of Earth Sciences, Ecohydrology Group, University of Waterloo, Canada

Co-Authors: M.M.F. Mesquita, R. Kovac, F. Resanezhad, and P. Van Cappellen

Sand from coastal and inland beaches is often reported as a large repository of fecal indicator bacteria (FIB; *Escherichia coli* and *Enterococci*) and human pathogens. Monitoring for FIB using traditional cultural and molecular methods such as qPCR has limitations. A positive relationship between the risk for enteric illness and beach sand-contact activities as a function of FIB has been recently confirmed. This situation generates public health concern since the sanitary quality of beach sand is not currently enforced as part of most beach monitoring programs. Results of denaturing gel electrophoresis (DGGE) for 16S rRNA targeted PCR amplified bacterial DNA gene sequences from sand bacterial populations in two rural and two urban Great Lake beaches are presented. Main contributions are: (1) recommendations for best sample size, sampling procedure, and DNA extraction protocol to ensure acceptable DNA yields from low biomass samples, (2) comparison of biodiversity and bacterial community distribution at various depths and beach locations in differently impacted beaches and (3) a better understanding of the role of sand characteristics and environmental conditions on the survival, replication and/or inactivation of sand bacterial populations, to facilitate decision making in beach management and public health protection.

Poster A22

**Removal of Bacteria from Stormwater by Geomedia Engineered from Waste Materials**

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

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

Traditional bioinfiltration systems facilitate the infiltration of urban stormwater and reduce flooding, but they do little to remove stormwater pollutants. Bioinfiltration systems can be augmented with engineered geomedia to increase the removal of pollutants including bacteria. Engineered geomedia such as activated carbon, iron oxide coated sand, and zeolite are either expensive or ineffective in removing bacteria from stormwater, partly because the attachment sites on these geomedia are quickly exhausted in the presence of natural organic matter (NOM). In this study, we evaluated the adsorption capacities of iron filings and biochar, which are cheaper and could be more effective than aforementioned geomedia to remove bacteria from stormwater. Experiments were conducted to compare the bacterial (*E. coli*) removal capacities of biochars, iron filings, iron oxide coated sand, and sand with and without NOM in stormwater. To mimic stormwater infiltration in nature, we examined the mobilization of *E. coli* from these geomedia under intermittent infiltration of stormwater. Without NOM, all geomedia retained up to 3 orders magnitude more *E. coli* than sand. NOM, however, lowered the removal capacity of all geomedia although iron filings and biochar still removed significantly more *E. coli* than sand. Intermittent flow mobilized attached *E. coli* from geomedia, but the mobilization, particularly in the presence of NOM, was least in iron filings followed by biochar, sand, and iron oxide coated sands, respectively. These results are useful to improve the design of bioinfiltration systems.

Poster A23

**Microbes Associated with Sediment and Hot Sulphidic Springs at the Archaeological Site at Roman Thermae Aquae Iasae (Varaždinske Toplice, Croatia)**

Presented by Janez Mulec, Ph.D., Researcher, Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Slovenia

Co-Authors: J. Mulec, V. Krištufek, A. Chroňáková, A. Oarga, J. Scharfen, and M. Šestauberová

At the archaeological site of Roman Spa in Varaždinske Toplice are natural hot sulphidic springs (~57°C) which fill the ancient Roman pool. The retention time of water in the underground which emerges at springs is estimated at 40,000 years. At the bottom of the pool is a sediment named fango which serves as a medicinal and beauty treatment. In fango are buried different metal, stone and wood archaeological artefacts which are generally well preserved even after more than 1500 years exposure to such conditions. Fango is characterized by pH close to neutral, a high level of organic C, elevated concentration of heavy metals and high total microbial biomass having more than 10<sup>8</sup> cells per g of dry weight. The dominance of four Proteobacterial classes ( $\alpha$ -,  $\beta$ -,  $\delta$ -, and  $\epsilon$ -Proteobacteria) in fango was confirmed by fluorescent in situ hybridisation. The dominant microbial groups were assessed using DGGE and were affiliated with *Thiobacillus denitrificans*, *Sulfuricurvum*, *Polaromonas* and *Bdellovibrio*. PCR for microbial functional guilds proved the presence of sulphur oxidisers and methanogens. Sulphate reducers which are otherwise largely involved in anaerobic iron corrosion in habitats rich in sulphate, were not proven. Up to 23.4% of the total bacterial counts represented cultivable bacteria including the genera *Acinetobacter*, *Aeromonas*, *Arthrobacter*, *Comamonas*, *Ewingella*, *Flavobacterium*, *Pseudomonas*, *Rahnella* and *Staphylococcus*. The heterogeneous structure of fango of neutral pH creates various microniches which largely support microbial life based on sulphur-driven autotrophic denitrification. Microbes involved in sulphur oxidation in such settings might contribute to biological cleaning of various materials, including historical artefacts.

Poster A24

**Characterization of a Deep Subsurface Viral Community within Basement Fluids of the Juan de Fuca Ridge Flank**

Presented by Olivia D. Nigro, Ph.D., Postdoctoral Scholar, Department of Oceanography, University of Hawaii at Mañoa, USA

Co-Authors: O.D. Nigro, J. Mueller, S.P. Jungbluth, H.-T. Lin, C.-C. Hsieh, M.S. Rappé, and G. Steward

Microbial communities in fluids percolating through the basaltic basement rock of the Juan de Fuca Ridge (JdFR) flank are distinct from those inhabiting the overlying sediments and seawater. The ecology of the bacteria and archaea that dominate these communities is likely affected by viruses, which are present everywhere that life is found. Although viruses have been observed in samples of oceanic basement fluids, their identities and ecological significance are not yet known. In order to better understand how viruses contribute to the ecology of deep subsurface communities, crustal fluids containing viral and microbial communities were collected via Circulation Obviation Retrofit Kits (CORKs) placed into drilled boreholes located on the eastern flank of the JdFR. Samples were analyzed using transmission electron microscopy (TEM), epifluorescence microscopy, and genomic and metagenomic techniques. Viruses were detected by TEM and epifluorescence microscopy from samples collected from 2010 to 2013. Viral abundance, determined by epifluorescence counts, indicated that the highest concentrations of viruses in subsurface basement fluids (~10<sup>5</sup> per ml) are low compared to sediments and seawater, but are an order of magnitude higher in abundance than microbial cells in the same samples. An analysis of two metagenomes sequenced from JdFR flank

fluids has revealed virus-like sequences integrated into the genomes of deep subsurface microorganisms. Together, these data suggest that viruses contribute to mortality of deep subsurface bacteria and archaea through cell lysis, and that these viruses may alter the genetic potential of their hosts through the processes of lysogenic conversion and horizontal gene transfer.

Poster A25

**Pristine Moonmilk Microbiomes within an Alpine Cave and the Dominance of Archaea**

Presented by Christoph Reitschuler, Doctoral Student, Institute of Microbiology, Leopold-Franzens University of Innsbruck, Austria

Co-Authors: C. Reitschuler, P. Lins, and P. Illmer

Moonmilk describes secondary CaCO<sub>3</sub> precipitations, which can be found in caves all around the world and in all climate zones. Limited studies on this unusual habitat revealed that microorganisms are associated with these speleothems and are likely to play an active forming and reshaping part within. Extensive molecular- (DGGE, qPCR, cloning and sequencing) and cultivation-based analyses of different moonmilk deposits, derived from the touristy not accessible part of the alpine Hundsalm cave (Tyrol, Austria), indicated that Archaea constitute a significant part within these moonmilk microbiomes. While bacteria and fungi formed heterogeneous and highly diverse communities within all investigated samples, a limited number of archaeal species was found to dominate the Hundsalm microbiomes (up to 108 organisms mL<sup>-1</sup>). These Archaea were intrinsic to all investigated samples and could be closely related to the low abundant Thaumarchaeon *Nitrosopumilus maritimus* (99% identity) and highly abundant undescribed Euryarchaeota, only distantly related to *Aciduliprofundum* sp. (86% identity) and *Thermogymnomonas* sp. (89% identity), which, in contradiction to the cold cave, are typically found at high temperatures and under acidic conditions. Although a fraction of the Hundsalm moonmilk Archaea was closely affiliated to globally trackable ammonium-oxidizers and methanogens (only detectable after enrichment), the archaeal majority itself and their physiological properties are completely unknown and demand further research. Moonmilk is not only an interesting habitat to study the contribution of Archaea, bacteria and fungi to geomorphological processes, but moreover an ideal study object to investigate supposed non-extremophilic Archaea and possible distribution patterns of this ubiquitous group.

Poster A26

**Cultivability of Unusual and Undescribed Non-Extremophilic Archaea from Alpine Moonmilk**

Presented by Christoph Reitschuler, Doctoral Student, Institute of Microbiology, Leopold-Franzens University of Innsbruck, Austria

Co-Authors: C. Reitschuler, P. Lins, and P. Illmer

So far Archaea were regarded as typical inhabitants of extreme environments, basically because previous research was carried out in habitats characterized by extreme temperature, pH or salinity regimes. However, in recent years it became obvious that Archaea are almost ubiquitous in marine, freshwater, terrestrial and also subsurface habitats and occasionally outnumber other microbial groups and hold key positions in globally relevant energy and nutrient cycles. The lower part of the alpine Hundsalm cave in Tyrol (Austria) offered the unique opportunity to study a pristine cave habitat, which is separated from the touristic part of the ice cave. A special focus of our research was laid on the archaeal community within moonmilk deposits (secondary calcite speleothems). To reveal the physiology of these Archaea the first goal was to enrich them in co-culture with bacteria and fungi, but also trying to isolate Archaea as a whole and single species. Altogether, a combination of different cultivation and separation strategies, physiological and molecular analyses were applied to assess

community compositions and alterations and to reveal the microbial functions within the moonmilk biome. So far we were able to enrich the archaeal community reproducibly, representing the naturally occurring in situ Hundsalm community, and at the same time highlighting unusual Archaea in moonmilk and demonstrating that so far uncultured or as 'not cultivable' regarded organisms are accessible. Further research is focusing on the physiological properties of these Archaea, their role within moonmilk and shall contribute to unravel the distribution patterns of non-extremophilic Archaea on earth.

Poster A27

**Investigation of Coal-Associated Microbial Communities from a Diffusive Microbial Sampler (DMS)**

Presented by Hannah D. Schweitzer, Ph.D., Research Graduate Fellow, Center for Biofilm Engineering, Montana State University, USA

Co-Authors: H.D. Schweitzer, E.P. Barnhart, and M. Fields

The Powder River Basin (PRB), located in southeastern Montana and northeast Wyoming, is the largest coal mining region in the United States but the majority of the coal in this region is too deep to be conventionally mined. Within the deep coal beds microbial ecosystems produce methane which can be used for electrical generation, heat and transportation fuel and compared to other hydrocarbon fuels, methane produces less carbon dioxide per unit of heat released. How this microbial community produces methane within coal beds is poorly understood. To begin to investigate this process the microbial ecology from four PRB coal seams was sampled with a diffusive microbial sampler (DMS). Native core samples from each of the four seams were used in the DMS. The native core samples were sequenced with Illumina MiSeq® sequencing to compare the in situ community from the cores with the community obtained from DMS. These results will provide novel insights into the microbial ecosystems producing methane within PRB coal beds and potentially lead to new techniques for harvesting this energy resource.

Poster A28

**Online Monitoring of Enzymatic Activity in Water and Water Resources**

Presented by Philipp Stadler, Doctoral Student, Center for Water Resource Systems, Vienna University of Technology, Austria

Co-Authors: P. Stadler, T. Lendenfeld, G. Ryzinska-Paier, A.P. Blaschke, H. Stadler, R. Mach, K. Brunner, J. Appels, M. Zessner, and A.H. Farnleitner

On-site detection of enzymatic activities has been suggested as a rapid surrogate for microbiological pollution monitoring of water resources (e.g. using glucuronidases, galactosidases, esterases). Due to the possible short measuring intervals enzymatic methods have high potential as near-real time water quality monitoring tools. This presentation describes the successful realization and application of a novel concept for automated on-line monitoring of enzymatic activities. During a two years field study at karstic and porous ground water resources the new method proved sensitive and robust for enzymatic on-line determination. In addition the device was tested for surface water monitoring in an agricultural catchment. It is shown that rapid and on-site enzymatic detection can successfully be operated from a technical point of view in diverse catchments. Selection of the type of measured enzymatic activities has to be done on a catchment-specific basis and further work is needed to learn more about its detailed information characteristics in different habitats. The results of this study highlight that automated on-line monitoring devices for microbial or biochemical parameters to support water quality monitoring is a realistic task. The application of such automated field systems

will likely become increasingly important for sustainable and pro-active water management in the near future.

Poster A29

### **Microbial Diversity Dynamism of Volcanic Soils under Sulfate-Reducing Conditions**

Presented by Orsolya Szedlacsek, Doctoral Student, Tohoku University, Japan

Co-Authors: O. Szedlacsek, Y. Shibuya, K. Suto, and C. Inoue

In this study we investigated the activity and diversity of sulfate reducing bacteria in various volcanic soils. Four volcanic soils obtained from different volcanic areas in Miyagi Prefecture, Japan: Zao, Aoso, Izumigatake and Nanatsumori, were used in this study. Cultivation experiments using modified Postgate's medium indicated that the sulfate reducing activities of these soils were different. The Zao and Aoso soils, which contain a higher amount of TOC showed high sulfate reducing activities, while the Izumigatake and Nanatsumori samples showed less activity and produced no sulfide. Bacterial diversities in both the original soils and enrichment cultures were determined using clone library method based on 16S rRNA gene. Every original soil's bacterial diversity consisted of mainly Proteobacteria and Acidobacter. The Zao soil sample was the most diverse. Phylogenetic analysis was performed on the 16S rRNA gene sequences likely to be related to sulfate reducing bacteria. However, none of the clones proved to be SRB species. In all the enrichment cultures, clones belonging to the Firmicutes group were dominant. The Zao, Aoso and Izumigatake enrichment samples contained Desulfotomaculum clones, while the Aoso enrichment sample also had some SRB clones belonging to the  $\delta$ -proteobacteria group. In the Nanatsumori enrichment sample clones of sulfate reducing bacteria were not detected. This representation is largely dependent on the laboratory culturing conditions, and further studies are needed to show a more accurate picture of the differences in diversity in each soil.

Poster A30

### **Identifying Groundwater under the Direct Influence of Surface Water (GWUDI) by Using an Analysis of Bacterial Communities**

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

Co-Authors: J.C. Underwood, R.W. Harvey, D.W. Metge, and D.R. LeBlanc

Public water-supply systems that utilize groundwater as a source of drinking water are required to evaluate their water sources to determine if they should be classified as Groundwater Under the Direct Influence of Surface Water (GWUDI). Without additional water treatment, GWUDI used as drinking water could be at risk of contamination from surface-water pathogens. To test the hypothesis that the analyses of unattached bacterial communities would be a sensitive and accurate measure of GWUDI, a 20-m-deep kettle pond was studied. The kettle pond is solely recharged by precipitation and groundwater inflow on the North side whereas the pond water recharges the aquifer on the South side creating what is referred to as a "pond-water shadow." Water samples were compared between the pond water, an up-gradient control well, and wells at various depths and distances within the pond-water shadow. In addition, samples of groundwater inflow and pond-water outflow were collected by using small microwells that were driven less than 1 m into the pond bottom near the shoreline. The water samples were analyzed for differences in bacterial communities by using 16S rRNA gene sequences, and also for dissolved organic carbon, selected anions and cations, ammonium and total dissolved nitrogen concentrations. GWUDI downgradient from the pond was confirmed by the presence of bacterial communities and organisms in the pond-water shadow that were present in

Ashumet Pond but not present in groundwater samples collected from the control well or the shallow pond-bottom microwells located upgradient of the pond.

Poster A31

### **Factors Influencing H<sub>2</sub>O<sub>2</sub> Decomposition in Sand Filters**

Presented by Feifei Wang, Doctoral Student, Department of Water Management, Delft University of Technology, The Netherlands

Co-Authors: F. Wang, D. van Halem, and J.P. van der Hoek

The combination of AOP and MAR is expected to provide a multiple barrier system for OMP removal. The fate of H<sub>2</sub>O<sub>2</sub> residuals from AOP within MAR is of concern. The top 0.5-2.0 cm (schmutzdecke) of sand filtration system has diverse microbial communities and greatly contributes to the removal of NOM by biodegradation process. Besides, this layer also acts as a barrier filtration role. The mechanism of H<sub>2</sub>O<sub>2</sub> decomposition in sand, especially schmutzdecke layer, had not been understood well and is interesting. The objectives of this study were to investigate H<sub>2</sub>O<sub>2</sub> decomposition kinetics and the influences of different factors, such as sand adsorption, the catalytic decomposition microorganism and the chemocatalysis of mineral substance on the surface of sand, on H<sub>2</sub>O<sub>2</sub> decomposition react in schmutzdecke sand of slow sand filter. The batch reactors filled with 100g schmutzdecke sand and 500ml prepared water were placed in a dark and temperature (25 and 11.5) controlled room. The decomposition of H<sub>2</sub>O<sub>2</sub> at pH=7.8 was represented by firstorder model. The first-order rate constants ranged from 0.32/h to 0.71/h at 1-5mgH<sub>2</sub>O<sub>2</sub>/L. H<sub>2</sub>O<sub>2</sub> decomposed completely in two to six hours for all the treatment groups. Among the three factors, sand, microbial community and mineral substance, the microbial communities in sand were primarily responsible for the decomposition of H<sub>2</sub>O<sub>2</sub> had significantly positive correlation with bacterial population at pH=7.8. Mineral substance promoted H<sub>2</sub>O<sub>2</sub> decomposition at some level. However, virtually pure sand treated by pH=2 and autoclave had no impact on H<sub>2</sub>O<sub>2</sub> decomposition.

Poster A32

### **Seasonal Variation of Microbes in Dripping Water, Heshang Cave, Central China**

Presented by Hongmei Wang, Ph.D., Professor, School of Environmental Studies, China University of Geosciences, PR China

Co-Authors: Y. Yun, H. Wang, X. Xiang, L. Gong, Q. Liu, Q. Dong, and R. Wang

A pilot molecular survey was conducted here on microbial communities of dripping water at two sites in Heshang Cave of Hubei province in central China for a period of 2008 to 2013. The samples were subjected to genome DNA extraction, 16S rRNA gene amplification, clone library construction and phylogenetic analysis to explore the seasonal variation of microbial communities and their association with environmental factors including temperature, pH, conductivity and dripping rate of the water samples. Seasonal variations were clearly observed in both the component and diversity of microbial communities at 1D and 3D. Bacterial community was dominated by Gammaproteobacteria in autumn and winter, whereas Betaproteobacteria became dominant in samples collected in summer and spring. Among the environmental factors investigated, temperature was found to show a strong impact on microbial community and their biodiversity. Ecologically, the bacteria identified in dripping water are either of oligotrophs or able to acquire nutrients from minerals under oligotrophic conditions. They might also be able to induce calcite precipitation in cave systems. Our data shed light on the ecological understanding of microbial communities harboring in caves.