Perchlorate (ClO$_4^-$) is a major contaminant of soil/groundwater and surface water. It is used in the manufacture of propellant, rocket fuel, fireworks, automobile air bag, explosives, and many other products. Aquifer in alluvial soil is a good source for drinking water. However, alluvial aquifer in estuary is susceptible to seawater intrusion and chemical contamination such as perchlorate. Biological treatment of perchlorate is method of choice since perchlorate-reducing bacteria (PRB) can convert perchlorate to harmless end products. Cleanup of perchlorate-contaminated alluvial aquifer in estuary requires salt-tolerant PRB. In this regard, salt-tolerant PRB were enriched in a fed-batch reactor that inoculated with activated sludge. NaCl concentration was increased stepwise up to 5% (w/v) while perchlorate was spiked into the reactor after every disappearance of perchlorate. The enrichment culture completely removed about 100 mg ClO$_4^-$/L under 5% NaCl as analyzed by IC. DGGE analysis revealed that change of microbial community composition during the enrichment culture and microbial profiles of the inoculum and enrichment culture were different from each other. The enriched salt-tolerant PRB will be useful to remove perchlorate in alluvial aquifer in estuary.

Polyhydroxyalkanoates (PHAs) are the only known natural thermoplastic polymers that are completely synthesized and decomposed via microorganisms' metabolism. PHAs can be divided into two classes depending on the number of carbon atoms in their monomer units; short-chain-length (PHAscl) and medium-chain-length (PHAmcl). PHAscl are thermoplastics with a high degree of crystallinity, while PHAmcl are elastic materials with a low degree of crystallinity and a low melting temperature. Because of its properties, PHAmcl may be much more suitable biomaterial for biomedical applications. Aiming the bioprospection of microorganisms from the composting process of organic residues adopted at São Paulo Zoo Park (Brazil), our research group is looking for microbial strains with ability to produce PHAmcl. São Paulo Zoo Park is located in an area of 824,529 m$^2$ of native Atlantic rainforest, and it has a collection of different animal species from many habitats around the world. At the Park was established a Composting System - self-sustaining, aerobic solid-phase biodegradative process of organic materials under controlled conditions, where organic residue from dead animals, native Brazilian Atlantic Forest, animal feed and excrements, generate humus, which is later used as fertilizer on the farm. A sustainable cycle is completed when the food produced on the farm returns to the zoo. The composting process is rich in microbial diversity and because of this it is considered as an excellent spot to be explored. In the present work it was isolated 540 bacterial strains, which were screened by Sudan Black B staining for their polyester accumulating ability, using octanoic acid as the carbon source as well as nitrogen limiting condition. It was obtained 49 isolates with the required ability and these strains were cultured in shaking flasks (150 rpm, 30 $^\circ$C, 72 h) for determination of total biomass, octanoic acid consumption and PHA contents. The isolated named as FPZSP 498 showed the highest production of PHAmcl (13.7 %). Of these, 60 mol% was attributed to monomer with 8 carbons (Hydroxyoctanoate). The cell concentration was also determined, and the isolated FPZSP 439 presented values close to 1.5 g L$^{-1}$, when PHAmcl content of 10 % was formed. The data presented in this work, could show Sao Paulo's Zoo composting process as an important site for bio-prospecting microorganisms with biotechnological potential to produce polyhydroxyalkanoates containing medium-chain-length monomers.
**388B**  The impact of solid retention time on bacterial community diversity in laboratory-scale sequencing batch reactors: revealed by 16S rDNA amplicon pyrosequencing

Samik Bagchi*, Berenice Garcia Tellez, Hari Anandarao, Pascal Saikaly

*King Abdullah University of Science and Technology (KAUST), Saudi Arabia*

Bacterial amplicon pyrosequencing analysis of 16S rRNA genes was used to investigate reproducibility and stability of the bacterial community structure and to assess the impact of solid retention time (SRT) on the bacterial community diversity of replicate laboratory-scale sequencing batch bioreactors (SBRs). Four sets of duplicate SBRs were operated for a period of 100 days under identical COD and ammonia loading rate of 751 ± 21 mg/m³.d and 46 ± 5 mg-N/m³, respectively. Two sets of SBRs were operated at an SRT of 2 days where one set was inoculated with an acclimated activated sludge and another set was inoculated with a non-acclimated sludge. Another two sets were operated at an SRT of 10 days with similar operational strategy. Samples for pyrosequencing analysis were collected from all eight reactors periodically throughout the experimental period. Sequences of bacterial 16S rRNA gene amplicons were filtered for quality, trimmed and processed using the Quantitative Insights Into Microbial Ecology (QIIME v1.5.0) pipeline. Additionally, sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using uclust algorithm and a representative sequence from each OTU was phylogenetically assigned to a taxonomic identity using the RDP Naïve Bayesian rRNA classifier at a confidence threshold of 80%. Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria were the predominant classes at almost all sampling periods, though the relative abundance varied between acclimated and non-acclimated replicate SBRs. Hierarchical clustering analysis using the algorithm of Ward showed that the bacterial community structure was dynamic in all eight SBRs. Despite identical operating conditions and stable process performance in terms of COD removal (95 ± 3%), no convergence in community structure between replicate reactors was observed after 100 days of operation. Measures of alpha diversity (Shannon diversity index (H) and species richness estimator of Chao1) revealed that SBRs operated at an SRT of 10 days have significantly higher diversity than SBRs operated at an SRT of 2 days. This was well corroborated with better nitrification in SBRs operated at an SRT of 10 days. Overall, high-throughput pyrosequencing approach was successful in characterizing the microbial community structure in lab-scale SBRs and the results revealed that the bacterial community structure was not stable and replicate reactors evolved differently while SRT could positively affect diversity in activated sludge process.

**390B**  Metaproteomic and metagenomic characterisation of floccular and granular phosphorus removal biofilms

Jeremy Barr*, Bas Dutířh, Andrew Cook, Toshikazu Fukushima, Marcus Hastie, Jeffery Gorman, Chongle Pan, Robert Hettich, Gene Tyson, Philip Bond

1San Diego State University, United States, 2Centre for Molecular and Biomolecular Informatics, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Netherlands, 3The University of Queensland, Advanced Water Management Centre (AWMC), Australia, 4Division of Environmental Engineering, Faculty of Engineering, Japan, 5Protein Discovery Centre, Queensland Institute of Medical Research (QIMR), Australia, 6Chemical Sciences Division, Oak Ridge National Laboratories, United States

Conventional wastewater treatment plants utilise floccular sludge biofilms, which form small aggregates of micro-organisms (~150 μm) to degrade and remove wastewater nutrients and facilitate the separation of the biomass from the treated wastewater. A novel alternative to conventional floccular sludge is the use of aerobic granular sludge, which offers numerous operational and economic advantages. Aerobic granules form large (~1 mm), dense, spherical aggregates, embedded by large amounts of extra-cellular polymeric substances. However, use of aerobic granule technology is not typical in full-scale plants due to a lack of fundamental microbial knowledge relating to their formation and stability. As a result, granular biofilm systems are difficult to cultivate and maintain. Our research uses metaproteomic techniques to identify the fundamental microbial functions, interactions and metabolism involved with granular formation and stability.

Floccular and granular sludge biofilms were obtained through the operation of two identical laboratory-scale bioreactors performing enhanced biological phosphorus removal over a period of 160 days. Operational conditions of one reactor were manipulated to induce the change from a floccular biofilm to the larger granular biofilm aggregates. The biofilm morphology changed, however, both sludges were found to be dominated by upwards of 80% Accumulibacter phosphatis, albeit potentially by
different strains. Cellular and extra-cellular protein samples were extracted throughout the operational period and classified as either; flocular, granular or transitional, before analysis by liquid-chromatography with dual parallel mass spectrometry.

Our proteomic analysis, based on current publicly available metagenomic sequences, resulted in fewer than expected protein identifications. Consequently, it was discovered that both the floccular and the granular reactors contained strains divergent from the Accumulibacter phosphatis reference genome. This contributed to the reduced number of unique metaproteomic peptides being matched. To investigate the presence of different strains of Accumulibacter phosphatis potentially divergent flanking microbial communities, the metagenomes of both granular and flocular reactors have been sequenced. This will provide the first annotated granular metagenome and a much improved reference sequence to significantly increase the identifiable proteins.

Nevertheless, the proteomic analyses reveal unique metabolic differences between stable flocular and granular sludges and identify potential proteins responsible for the transition from flocular to granular sludge. Granular sludge was found to have increased amino acid uptake and metabolism compared to flocular sludge, indicating a potential shift from easily accessible fatty acids to stored extra-cellular material, potentially due to mass transfer limitations. Transition samples revealed; increased pilus, flagellin and hook-associated proteins for the development of tertiary biofilm structure; increased periplasmic polysaccharide transport, glycosyltransferase and secretory systems highlighting the importance of extra-cellular polymer synthesis; levels of methyl-accepting chemotaxis proteins and response regulators, CheA and CheY, were increased, these play potentially crucial roles in regulating chemotaxis, biofilm sessility and granular biofilm formation. These findings detail some of the first fundamental proteomic observations relating to the formation, stability and metabolic differences between flocular and granular sludge biofilms, contributing towards the full-scale application of the technology.

391B Acetoclastic methanogenesis in a microbial community associated with a sub-bituminous coal seam in NSW, Australia
Sabrina Beckmann*, John Webster, Torsten Thomas, Michael Manefield
University of New South Wales (UNSW), Australia

Coal seam gas is increasingly considered a valuable energy resource worldwide. Australian coal deposits have a large, unexplored potential for methane generation via microbial biogasification, however little is known about the microbiology of Australian coal seams. This study investigated the natural microbial communities associated with a sub-bituminous coal seam. Coal and groundwater samples were collected from a coal mine in the Western Coal Fields of NSW, Australia. The identity and abundance of potentially active microorganisms were assessed by direct cultivation and cultivation-independent molecular techniques, such as pyrosequencing and FISH (fluorescence in situ hybridization). Phylogenetic studies showed the presence of a broad spectrum of facultative anaerobic bacteria belonging to the Alphaproteobacteria, Deltaproteobacteria, Firmicutes, Bacteroidetes and Actinobacteria in the seam associated groundwater and on the coal. Due to high sulfate concentrations in the groundwater, sulfate-reducing bacteria (Desulfovibrio spp.) represented a numerically significant fraction of the bacterial community. Molecular analysis also detected the methanogenic Methanoseta sp. and Methanosarcina sp. and ex situ they could produce methane from acetate. Furthermore, members of the Thermococcales (Euryarchaeota) and Nitrososphaerales (Thaumarchaeota) were detected, which might have a potential role in sulfur and nitrogen cycling, respectively. The presence of methanogenic archaea in situ and the stimulation of acetoclastic methane formation in vitro confirmed the methanogenic potential of these indigenous communities.

392B Interactions between autochthonous microbial community and invading microbial strains in biofilm
Micol Bellucci*, Kim Milferstedt, Renaud Escudié, Gaëlle Gévaudan, Nicolas Bernet, Jean-Jacques Godon, Jean-Philippe Steyer, Jérôme Harmand
INRA-Laboratoire de Biotechnologie de l'Environnement, France

Biofilms have a major impact on human health, environment, and industry as they can drive either fundamental ecosystem services or detrimental processes. The type of these biological functions is dependent on the activity, composition and diversity of the microbial communities forming the biofilm.
Therefore, natural and/or induced alterations of the community structure by abiotic (for example environmental conditions, shear forces) or biotic (for example predation, grazing, invasion of allochthonous species) factors could have severe consequences on the biofilm functionality. Addition and colonization of genetically modified or unmodified microbial strains were often attempted with the aim of enhancing a desired biological function (for example bioaugmentation). On the contrary, pathogen invasion and survival in beneficial biofilms could be catastrophic, and it is thus trying to be avoided. Nevertheless, the biological interactions between the colonizers (positive or negative) and the existing microbial community in the biofilm are still unclear, though their understanding could have widespread implications and potentials in environmental, industrial and human wellbeing.

In this study, we provide insights into the microbial community dynamics of mature biofilms in contact with allochthonous bacterial strains by combining experimental and theoretical approaches. Biofilm growth was initially promoted on polyethylene coupons inserted into bubble column reactors with a volume of 4.6 liters. The reactors were inoculated with activated sludge from a domestic wastewater treatment plant. One bioreactor was used as a control. After 20 days of operation 130 ml of pure microbial suspensions, with known concentration, of Aquabacterium spp., Escherichia coli, Pseudomonas putida, Lactococcus lactis and Leuconostoc mesenteroides were injected to the other reactors over a period of two hours. The capacity of these strains to adhere and colonize the existing biofilm was then surveyed over time and compared against the biofilm in the control unit by quantitative Polymerase Chain Reaction (qPCR). The inclusion of the allochthonous strains added a new competitor for space and resources in the multispecies biofilm, thereby perturbing the naturally occurring microbial community dynamics. The observed biofilm changes in composition and diversity, which were regularly monitored by single-strand conformational polymorphism (SSCP), together with the specific growth kinetics of the allochthonous strain in the biofilm, were then included as assumptions in a conceptual multi-species model. This model will provide a valuable tool for predicting the ecological behavior of a complex biofilm community when colonized by an external species.

Consequently, this study will help future manipulation of biofilm diversity for the improvement of a desired biological process and/or the protection of the existing microbial community against adverse pathogens.

393B  Efficient nitrifying bioreactors operated at extremely low dissolved oxygen concentrations produce low N2O emissions

Micol Bellucci*1, Tomoko Yamamoto2, Shohei Riya2, Shohei Mizuma2, Ryo Kanai2, Kohei Kamimura2, Keisuke Hojo2, Thomas Peter Curtis3, Masaaki Hosomi2, Akihiko Terada2

1INRA-Laboratoire de Biotechnologie de l’Environnement, France, 2Tokyo University of Agriculture and Technology, Japan, 3Newcastle University, United Kingdom

In wastewater treatment plants, nitrification is the two-step biological oxidation of ammonia to nitrate via nitrite. Ammonia oxidizing bacteria (AOB) convert ammonia to nitrite, which is then transformed to nitrite by nitrite oxidizing bacteria (NOB). For many years, these bacteria have been considered to have a strict aerobic metabolism and to be poor competitor for oxygen. Consequently, the systems have been operated with extensive aeration, with the drawback of high energy consumption and carbon footprint. Recent studies have reported that AOB have been found active in several anoxic and oxic environments, including bioReactors. However, in such conditions AOB seem to produce high amount of gaseous N2O, a potent greenhouse gas. Therefore, the advantage of selecting AOB able to survive in low aerated systems could be paradoxically offset by substantial emissions of N2O. Also, practitioners are still skeptic in reducing aeration in nitrifying systems as they are afraid of nitrification failures.

Here, we aimed at investigating (i) the efficiency and reliability of nitrifying systems supplied with extremely low oxygen, (ii) their N2O emissions, and (iii) the dominant AOB populations responsible for the process. To achieve this, nitrification performance and N2O productions of four continuous flow bioreactors operated with differing oxygen supplies were monitored for 80 days. The nitrifying communities selected were then identified by molecular tools. The systems were initially inoculated with activated sludge (3 L) from a municipal WWTP, and aerated was provided at fixed flow rate of 0.1 liter per minute using air in one reactor and gas mixtures with different percentages of O2 (4%, 2%, and 1%) in the other units. The resulting dissolved oxygen (DO) concentrations ranged between 1.1 mg/L and 0.01 mg/L. Such low DO did not influence nitrification efficiency which was higher than 70%
PS13 – Managing Microbial Communities

Tuesday 21 August

in all systems after 30 days. The high oxidation rate concurred with the shift from the dominant AOB population *Nitrosomonas europaea* to *Nitrosomonas communis* in all systems as revealed by T-RFLP. However, the nitifying community structure changed over time, but irrespective of the amount of oxygen supplied. This, in turn, influenced the overall emissions of N₂O as the production of this gas was observed only in the reactors with DO concentrations lower than 0.2 mg/L. However, the N₂O emissions decreased to the level of the reactor supplied by air once the ammonia oxidation rate stabilized.

In conclusion, reliable ammonia oxidation can be achieved in bioreactors with extremely low DO without N₂O emissions after an acclimation time, in which specific AOB populations are selected. This study will help to reduce energy cost and carbon footprint related to the nitrification process by encouraging practitioners to reduce aeration.

394B  Correlation of enterococci community structure to microbial and physico-chemical parameters in surface water in the North West Province, South Africa
Carlos Bezuidenhout, Lesego Molale*
North-West University: Potchefstroom Campus, South Africa

Water from the North West province of South Africa support gold, platinum and chrome mining as well as related support manufacturing industries. The same water sources also support growing urban and rural populations. However, the waters within the catchment areas of various rivers in the province are exposed to pollution from mining, agricultural and industrial activities. Additionally sewer leakage, faulty septic tank operation and landfill leachate cause sewage pollution. In this study the physico-chemical (temperature, pH, electrical conductivity, nitrates, phosphates) and microbial quality (total coliforms, faecal coliforms, E. coli, entrococci) of the water at selected sites were assessed. Diversity of enterococci based on 16S rDNA was also determined. Multivariate analysis was used investigate potential impacts of the physico-chemical and microbial indicator bacterial parameters on enterococci community structures at various sites. The average physico-chemical and microbial levels measured at some sites in the surface water systems were elevated and exceeded the acceptable South African target water quality ranges. These included full and intermediate recreational contact, livestock watering and irrigation. Faecal indicator bacterial levels in the surface water systems indicated that faecal pollution is occurring and that activities surrounding these systems may have an influence on the quality. A total of 6 enterococci species were identified and these included Enterococcus faecium, E. faecalis, E. mundtii, E. casseliflavus, E. gallinarum and E hirae. Redundancy analysis indicated that positive correlations existed between (i) electrical conductivity and entrococci levels (ii) faecal coliforms and E. coli levels on the one hand and temperature and phosphates measurements on the other. Furthermore, based on community structure data the various sampling sites from the selected river systems formed groups. These groups could be attributed to physico-chemical and microbial dynamics at the sites. In conclusion, results from this study indicated that faecal pollution in the river systems is a major challenge. Results also demonstrated that correlations existed between indicator bacterial levels and physico-chemical parameters. Another aspect that was demonstrated by redundancy analysis is that enterococci community data based on 16S rDNA data could be correlated to microbial and physico-chemical levels in selected river systems in the North West province of South Africa.

395B  Anaerobic microbial community response to crude oil spill in the marsh sediments of southeast Louisiana
Raj Boopathy*
Nicholls State University, United States

The significant challenges presented by the April 20, 2010 explosion, sinking, and subsequent oil spill of the Deep water Horizon drilling platform in Canyon Block 252 about 52 miles southeast of Venice, Louisiana, USA greatly impacted Louisiana’s coastal ecosystem including the sea food industry, recreational fishing, and tourism. The short term and long term impact of this oil spill are significant and the Deep water Horizon spill is potentially both an economic and an ecological disaster. Microbes present in the water column and sediments have the potential to degrade the oil. Oil degradation could be enhanced by biostimulation method.
The conventional approach to bioremediation of petroleum hydrocarbon is based on aerobic processes. Anaerobic bioremediation has been tested only in a very few cases and is still considered experimental. The currently practiced conventional in-situ bioremediation of petroleum-contaminated soils, and ground water relies on the supply of oxygen to the sub-surface to enhance natural aerobic processes to remediate the contaminants. However, anaerobic microbial processes can be significant in oxygen-depleted sub-surface environments and sediments that are contaminated with petroleum-based compounds such as oil-impacted marshes in Louisiana. The goal of this work was to identify the right conditions for the indigenous anaerobic bacteria present in the contaminated sites to enhance degradation of petroleum hydrocarbons. We evaluated the ability of microorganisms under a variety of electron acceptor conditions to degrade petroleum hydrocarbons. Researched microbial systems include sulfate-, nitrate-reducing bacteria, and fermenting bacteria. The results indicated that anaerobic bacteria are viable candidates for bioremediation. Enhanced biodegradation was attained under mixed electron acceptor conditions, where various electron-accepting anaerobes co-existed and aided in degrading complex petroleum hydrocarbon components of marsh sediments in the coastal Louisiana. Significant degradation of oil also occurred under sulfate reducing and nitrate reducing conditions.

396B Microbial population dynamics in groundwater and surrogate sediments during HRC® biostimulation for Cr(VI)-reduction
Kara Bowen De Leon1, B. D. Ramsay1, D. R. Newcomer2, B. Faybishenko3, T. C. Hazen4, M. W. Fields1
1Montana State University, United States, 2Pacific Northwest National Laboratory, United States, 3Lawrence Berkeley National Laboratory, United States, 4University of Tennessee/Oak Ridge National Laboratory, United States

The Hanford 100-H site is a chromium-contaminated site that has been designated by the Department of Energy Environmental Management as a field study site for in situ chromium reduction. In August 2004, the first injection of hydrogen release compound (HRC®) resulted in an increase of microorganisms and a reduction of soluble chromium(VI) to insoluble chromium(III). Little is understood about the microbial community composition and dynamics during stimulation. The aim of this study is to compare microbial communities of groundwater and soil samples across time and space during a second injection of HRC®. A second injection occurred November 2008 and geochemical data collected throughout the study showed an overall decrease in nitrate, sulfate, and chromium(VI). Spatial and temporal water and soil samples (n=34) were collected pre- and post-injection from four wells at the field site. Soil columns constructed from stainless steel mesh were lined with nylon mesh and filled with Hanford soils from the 100-H site. The soil columns were used to represent not only the microbes flowing through the soil via groundwater, but the microbes that require a matrix in order to grow. DNA was extracted from each of the samples and the V1V2 region of the 16S rRNA gene was sequenced via multiplex pyrosequencing. Soil sample populations differed from the corresponding groundwater (even at the phyla level) and were more diverse (p=0.001). While many of the populations were observed in both groundwater and surrogate sediments, the respective matrices appeared to enrich for particular OTUs. Of 667 total genera, 141 and 69 were unique to groundwater and soil, respectively. Genera observed only in the sediment included Marinomonas while genera observed only in the groundwater included Desulfonauticus, Desulfovibrio, and Syntrophobacter. Pseudomonas, Acidovorax, Clostridium, and Herbaspirillum were dominant regardless of sample type. Results do not indicate a large shift in dominant organisms in soil from pre- to post-injection, and this may be due to the organisms remaining dominant from the first stimulation. Correlation analyses of genera were done for each sample type using SparCC. Metal-reducing organisms such as Geobacter, Desulfovibrio, and Geothrix were correlated in soil while possible fermenting bacteria such as Clostridium, Pelotomaculum, and Pelosinus were correlated in groundwater. For each well, HRC® injection resulted in increased diversity, but the greatest changes during stimulation occurred in the populations of mid-dominance either between wells or across time. These organisms could be important to consider as possible indicator species in future work that includes targeted isolations to better understand the mechanisms of microbial interactions.
**397B Insights into the diversity of iron depositing biofilms in technical water systems**

Burga Braun*, Josephin Schröder, Ulrich Szewzyk  
*Berlin University of Technology, Germany*

Biofilms are matrix-enclosed microbial populations which are ubiquitous at any interface. In technical water systems, biofilms grow on the surfaces of piping, well screens, pumps and pipelines. Iron bacteria play an important role within these biofilms in areas where anaerobic water comes in contact with oxygen. These bacteria oxidize reduced iron and deposit iron (III) minerals in their surroundings. The accumulation of these deposits causes serious clogging problems since well production efficiency drops significantly, potentially causing complete blockage. This leads to cleanup and rehabilitation of the wells and is an important economic concern. While negative effects of the iron deposits are well documented and studied, bacteria responsible for these processes are still little examined due to the difficulties in obtaining pure cultures.

The aim of our project was to determine the phylogenetic diversity of iron-depositing bacteria in different water wells at neutral pH and to prevent and remediate the ochreous accumulations. For this propose, key players involved in precipitation of oxidized iron compounds should be identified and strategies for remediation of water wells will be developed. Ochreous samples from several technical water systems in Germany and Russia were examined to get an overview of the composition of the different iron-mineral-containing biofilms. Iron density, which varies from fluffy to more cemented, will be correlated to the community structure and parameters of the sampling point.

16S rDNA genomic clone libraries of eleven samples derived from ochreous water wells (opencast mine and well reactors) revealed that 60% of the sequences were affiliated to the groups of to Alpha- and Betaproteobacteria and 28% of the clones were related to the Deltaproteobacteria. Iron depositing isolates were grouped into 9 distinct OTU’s as determined by ARDRA. Phylogenetic analysis based on 16S rRNA gene sequencing revealed that most of our strains were unaffiliated to known iron bacteria. These isolates occur in a number of genera like Nocardioides, Marmorica, Arthrobacter, Tetraspheara, Micromonospora, Terracoccus and Rheinheimera. However, some isolates already associated with iron-precipitating bacteria such as Bacillus, Kineosporia and Albidiferax were also detected. Based on data revealed from our clone libraries and new isolates, different specific primers for qPCR were developed to detect and quantify the iron bacteria and make a fast sample screening possible. FISH, in combination with confocal laser scanning microscopy, was applied to get insights in the distribution of iron bacteria within their native habitat.

**398B Similar organic micropollutants fate in anaerobic mesophilic digesters fed with the same contaminated sludge but exhibiting different microbial populations and different metabolisms**

Florence Braun*, Jerome Hamelin, Anais Bonnafous, Jean-philippe Steyer, Dominique Patureau  
*INRA, France*

Due to anthropogenic practices, the sludge of urban origin is contaminated by organic pollutants such as polycyclic aromatic hydrocarbons (PAH) and polychlorobiphenyls (PCB). Efficient biodegradations under mesophilic methanogenic conditions were already reported for these micropollutants (Trably et al 2003; Barret et al 2010). Here, we have determined the influence of the microbial community on the micropollutants removal while physico-chemical conditions are strictly controlled.

We investigated three microbial communities extracted from ecosystems with contrasting pollution histories; PAH contaminated soil, PCB contaminated sediment and moderately contaminated anaerobic sludge. We used an original combination of enzymatic treatments and a cell flotation step to harbour the communities free of their surroundings (Braun et al 2011). Each microbial community served as inoculum for three 400mL anaerobic digesters. A total of 12 mesophilic continuous reactors were operated during 100days with a hydraulic retention time (HRT) of 20days. The substrate was a sterilised activated sludge, spiked with 13 PAH and 7 PCB (5mg.kg\(^{-1}\) DM). The organic matter degradation, the biogas production rate and composition, and the micropollutants removals were monitored. The bacterial and archaeal communities were characterized in abundance (qPCR) and community structure (SSCP fingerprinting). Population shifts were quantified using diversity index and principal component analysis (PCA).
Functional steady states, based on organic matter degradation, were observed after 3-4 HRT. The PAH removals varied from 5 to 30% depending on the molecules but not on the inoculum, and were highly correlated with the organic matter removal (cor=0.4475-0.614, p-value<0.001). By contrast, the dynamics of biogas productions and the biogas compositions differed according the inoculums tested, with increasing CH4/CO2 ratios for PAH soil, PCB sediment, and anaerobic sludge. Greater PCB removal were observed for the reactors inoculated with PCB contaminated sediment.

The bacterial densities reached values of 10^{12} Bacteria.gVS^{-1} at steady state for all reactors. By contrast, the density of Archaea varied from 3.8x10^9 to 3.5x10^{10} Archaea.gVS^{-1} depending on the origin of inoculum (PAH soil < PCB soil < anaerobic sludge). The bacterial and archaeal genetic structures were compared with fingerprinting data using PCA. The inoculum significantly influenced the genetic structure of bacterial (cor=0.321, p-value<0.001) and archaeal (cor=0.420, p-value<0.001) communities. The diversity level was also affected by the inoculum used to seed anaerobic reactors (cor=0.774 for Bacteria and cor=0.310 for Archaea, p-value=0.001). The reactors inoculated with the anaerobic sludge displayed always higher diversities than the others. A diversity-functioning relationship was evidenced, when comparing diversity indices and VFA concentrations (cor=0.669 for Bacteria and cor=0.537 for Archaea, p-value=0.001), but no correlation between microbial communities and micropollutants removal were observed.

As a conclusion, we obtained three functional stable consortia with contrasted macroscopic functioning and microbial dynamics, but similar pollutant removals. The removal of persistent organic pollutants did depend on the molecule type, but did not depend on the metabolic functioning during methanogenesis. The bioavailability of micropollutants in regard with their biodegradation will be discussed.

**399B** Marine biofilm communities colonizing antifouling paints in the Mediterranean Sea
Jean-François Briand*1, Mercedes Camps2, Gérald Gregori3, Agnès Bouchez4, Aude Barani5, Brigitte Le Berre6, Christine Bressy7, Yves Blache8
1MAPIEM - Université Sud-Toulon Var, France, 2Université Sud-Toulon Var, France, 3CNRS, France, 4INRA, France, 5Aix-Marseille University, France

When immersed in sea water, any substrate would be rapidly colonized by micro and then macroorganisms. This complex and sequential natural process called biofouling induces economical and ecological prejudices, especially talking about ship hull or aquaculture nets.

In situ biofilm communities settle on antifouling coatings immersed in Toulon harbour (France, North-Western Mediterranean Sea) were studied. Immersion was performed in July during one month in order to get mature biofilms beyond pioneer stages. Complex biofilm communities were described, in term of both abundance and diversity, using flow cytometry, inverted microscopy and PCR-DGGE on six different coatings in triplicates, including coatings without biocides (Poly(vinyl chloride)) as a reference and a Fouling Release Coating) and with biocides (four Self-Polishing Copolymer (SPC) Coatings including three commercial ones). Globally, picocyanobacteria and nanoeukaryotes showed similar densities, lower than heterotrophic bacteria. Diatoms, the only microphytobentlic class identified, displayed the lowest densities. Licmophora, Navicula and Nitzschia were determined as the dominant diatom genera. Coatings without biocides showed higher densities than biocidal coatings, whatever the group of microorganisms (bacteria, picocyanobacteria, pico- and nanoeukaryotes, diatoms). However, significant variations in both abundance and diversity were observed between the coatings depending on the microorganism group. Despite the SPC antifouling coatings each included a different cocktail of biocides, some specificity of biocide action could be observed. Especially, the SPC copper-free coating failed to prevent diatom settlement whereas the SPC pyrithione-free coating exhibited high picocyanobacteria density.

**400B** Isolation and identification of microbial species native to an oil sands tailings pond and capable of degrading oil sands process-affected water acid-extractable organics
Lisa Brown*, Elena Dlusskaya, Ania Ulrich
University of Alberta, Canada

The oil sands industry has become an integral part of Alberta's and Canada's economy as more than 170 billion barrels of crude oil are currently recoverable from oil sands deposits located in north-
eastern Alberta. Shallow deposits are processed via surface mining and caustic hot water extraction, generating 200 million litres of tailings, a mixture of sand, fines and process-affected water, every day. Oil sands facilities are required to operate under a policy of zero water discharge, resulting in impoundments that cover more than 170 square kilometers of land area to date. A significant challenge facing the oil sands industry is treating toxic organic compounds found in the oil sands process-affected water, historically called "naphthenic acids". Biodegradation is a cost-effective treatment option, but recalcitrance of naphthenic acids to degradation by microorganisms has been described previously. Despite extreme conditions found in oil sands tailings ponds, such as high salinity and pH, acclimated microbial communities are present. Isolation of microorganisms capable of degrading the organic compounds of concern from oil sands tailings ponds presents an opportunity to optimize a biological treatment system for naphthenic acids.

Six species, two fungi and four bacteria, were isolated from oil sands process-affected water sampled from the South Tailings Pond located at Suncor Energy's Millennium mine. The microbial community was selectively enriched on Bushnell Haas medium with increasing concentration of oil sands process-affected water-sourced acid-extractable organics (in which naphthenic acids are included) as the only source of carbon. Pure cultures were isolated after 14 day incubation with 300 mg/L of acid-extractable organics. Preliminary results showed that two of the isolates, fungus *Trichoderma harzianum* and an alpha-proteobacterium, identified as *Brevundimonas* sp., were capable of decreasing concentration of "naphthenic acids", as measured by FTIR, over three months. To our knowledge, this is the first study, in which *T. harzianum* has been shown to use "naphthenic acids" as a carbon source. Isolates were then maintained in Bushnell Haas medium with 300 mg/L of acid-extractable organics from Syncrude West In-Pit tailings pond or 100 mg/L commercially available naphthenic acids from Merichem Chemicals & Refinery Services LLC.

To verify naphthenic acid degradation capability of the isolates, another three month incubation experiment was conducted. Isolates were exposed to 50 mg/L acid-extractable organics from Syncrude West In-Pit in mineral salts medium. Commercial Merichem naphthenic acids were used as positive control in addition to killed and abiotic controls. Naphthenic acid concentrations were measured via a Polar Organic Compound Integrative Sampler, GC-FID, and Microtox. Microbial growth was monitored using optical density and quantitative PCR.

**401B Seasonal variability of microorganisms in different sources and water storage areas in the same town**

M. Angeles Calvo*, Esteban Leonardo Arosemena, Judith Pérez, Laura Corbella, Alex Rodríguez, Gisela Girmé, Eduard Grau, Jana Cantavella, Judit Fuentes, Montserrat Mora

*Universidad Autónoma de Barcelona, Spain*

We report a study of the microbiota present in six water storage points, selected in the same area in the province of Barcelona (Spain). The sampling area is located in the urbanization "La Font del Bosc" in Sant Joan de Mediona in the region of l’Alt Penedès. The main objective of this study was to establish the complete microbiota of water over the twelve months of the year. It has also been conducted evaluating the presence of bacteria capable of altering the pipes and containers in which they were stored.

Samples were collected over twelve months, using sterile containers and proceeded with them to assess the quantitative and qualitative presence of both fungi and bacteria.

From the results can be noted that the presence qualitative and/or quantitative evaluation of the microorganisms in the water did not show any relation to the temperature of the water or the environment. The waters had lower contamination were obtained from the source and distribution of water taps. Unlike those who had a greater degree of pollution have been the pool water and deposits as well as from the rain. The prevalence of filamentous fungi is constant in all waters tested, highlighting the presence of species of the genera: Cladosporium, Penicillium and Aspergillus by the majority. Between bacteria the sporulated forms and those capable of degrading the storage tanks have reached the maximum impact. No significant differences over the samplings carried out in different seasons.
Hydrocarbonoclastic bacteria are important players in bioremediation of hydrocarbon contaminated ecosystems with additional potential for application in biological treatment of industrial wastewaters. Synthesis and accumulation of storage lipids such as triacylglycerols (TAGs) and wax esters (WEs), as well as polyhydroxyalkanoate (PHA), has been reported in this group of bacteria when submitted to growth-limiting conditions (e.g. nitrogen limitation). These compounds are relevant raw materials for biofuels and oleochemicals production. Its biosynthesis in combination with industrial wastewater treatment can contribute to make the process more economic and environmentally sustainable. The aim of this work was to obtain suitable inocula for use in biotechnological processes to produce valuable bacterial products from hydrocarbon-based wastewaters. These carbon-rich, nutrient-poor wastewaters exhibit appropriate conditions to promote the production of bacterial storage compounds, thus being an interesting application target for the proposed combined approach.

Sludge collected at the wastewater treatment plant, from an engine's repairing workshop, was enriched in carbon storage producing bacteria. The enrichments were carried out in 250 ml flasks with a working volume of 50 mL each, at 22°C with shaking (150rpm). Wastewater collected from the same service station, containing lubricant and engine oil waste, was used as carbon source. The selective pressure was applied in the form of alternating periods of presence of the carbon substrate (feast phase) followed by its absence (famine phase). Cells having stored sufficiently amounts of reserve materials can survive the starvation period, whereas non-accumulating bacteria were not able to survive (selection phase). In a first period, the feast and famine phases lasted 7 days each and in a second period, the feast phase was decreased to 3 days. The total process had the duration of 7 months.

Throughout the enrichment, biomass samples were collected and characterized in terms of lipid storage profiles by thin-layer chromatography (TLC) and bacterial diversity by 16S rRNA-based techniques (DGGE, cloning and sequencing).

The obtained TLC profiles showed a decrease in TAG levels with a concomitant increase in WEs levels throughout time. Interestingly, in the final phase of the enrichment a lipidic compound of unknown identity was detected at increased levels.

DGGE profiles revealed a decrease of bacterial diversity throughout time until the establishment of a nearly stable bacterial profile. The majority of bacterial 16S rRNA gene sequences retrieved from the obtained culture belong to the phylum Proteobacteria, being mainly assigned to the orders Pseudomonadales and Burkholderiales. Some DGGE-bands corresponded to sequences with high similarity to those of members of the genus Rhodococcus, Acinetobacter and Pseudomonas (99% similarity) which are known for their ability to produce TAG, WEs and PHAs, respectively. The remaining sequences showed also high levels of similarity (>.> 95%) to members of the genus Caulobacter, Zoogloea and Acidovorax, where some species are described as capable to produce and accumulate polyhydroxybutyrylates (PHBs).

The results obtained in this work show the potential of using feast and famine phases as a selective pressure to obtain a stable hydrocarbonoclastic consortium able to produce several types of lipidic storage compounds from real hydrocarbon-based wastewaters.

**Microbial communities involved in the corrosion of concrete sewer infrastructure**
Barry Cayford*1, Paul Dennis2, Gene Tyson2, Phil Bond1
1Advanced Water Management Centre, The University of Queensland, Australia, 2Australian Centre for Ecogenomics and Advanced Water Management Centre, The University of Queensland, Australia

The corrosion of concrete infrastructure is a major problem in the operation of urban sewer systems. The action of *Acidithiobacillus thiooxidans* in oxidising gaseous hydrogen sulfide to sulfuric acid has...
traditionally been thought to be the mechanism by which sewer concrete corrosion occurs. This dogma was developed through experiments that were primarily culture-dependant. In this work, a culture-independent investigation of the microbial communities within sewer concrete corrosion layers was undertaken, using pyrosequencing of 16S rRNA gene amplicons. We reveal that the level of diversity is much higher than previously reported and we show the presence of two distinct types of community. Furthermore, this study aimed to determine the spatial arrangement of these two communities within the pipe.

This work was undertaken in the Sydney sewer system with a pair of parallel concrete pipes providing near replicate conditions. These pipes measure 2m x 3m (internal dimensions) with a waterline approximately 1m from the ceiling. This system contains active corrosion layers with a pH of 3-4.5 and gaseous hydrogen sulfide level of up to 20ppm. Sample collection occurred at regular intervals from the waterline on one side of the pipe up the wall to the ceiling, along the ceiling, and down to the waterline on the opposite side of the pipe. This provided 11 samples from a particular section of the sewer pipe. This sampling was performed at several points both upstream and downstream of the access points. Additionally, samples of the water and sludge from the bottom of the pipe were taken. DNA was extracted and the microbial community determined by high-throughput 16S rRNA gene amplicon pyrosequencing using universal primers.

Analysis of this data revealed distinct differences between the communities present in the pipe depending on the collection location. The waterline samples were removed as they were determined to be the same as the wastewater community. No difference was seen between pipes therefore the samples were consequently clustered into two groups, wall and ceiling, for further analysis. The organisms that dominate the corrosion layer samples were; Actinomycetales, Rhodospirillales, Acidithiobacillales, Xanthomonadales and Chromatiales. Although found throughout the wall and ceiling samples these five lineages are found at differing levels of dominance, with the ceiling having relatively higher levels of Actinomycetales, Rhodospirillales and Acidithiobacillales, and the wall had relatively higher levels of Xanthomonadales, and Chromatiales. Some understanding of the role the Rhodospirillales, Acidithiobacillales and Chromatiales play in the community function can be inferred from characteristics of affiliated members of the orders being either acidophilic, sulfur oxidisers or both.

The role of the Actinomycetales and Xanthomonadales in such corrosive environments is currently unknown. The Actinomycetales have been reported in several previous studies of this environment and are also well known to exist in other extreme environments. Xanthomonadales have not previously been detected in either this or other acidic environments at such high levels. Further examination of the communities may reveal that rather than a single corrosion process, multiple organisms and pathways are involved, and therefore more advanced corrosion management practices may be required.

404B  Tracking the survival of bacteria exposed to monochloramine disinfection in drinking water treated by a biologically active filter
Tara Clancy*, Tzu-Hsin Chiao, Ameet Pinto, Chuanwu Xi, Lutgarde Raskin  
University of Michigan, United States

Drinking water treatment often includes a final disinfection step to inactivate pathogenic microorganisms and prevent regrowth of bacteria in the distribution system. Inactivation research has focused on specific pathogenic bacteria, without taking into account interspecies interactions and biofilm matrix effects, and has almost exclusively used culture-based methods to enumerate bacteria that survive disinfection. Thus, little is known about the effects of disinfection on the dynamics of the microbial communities present in finished drinking water. The aim of this study is to investigate the effect of disinfection on the complex microbial communities in the effluent of a biologically active filter and evaluate differential survival of the populations present.

Culture based (heterotrophic plate counts) and culture independent (quantitative PCR (qPCR)) techniques were applied to measure the inactivation requirements of effluent from a lab scale biologically active carbon filter. This filter was used to remove arsenic and nitrate from drinking water sources under anaerobic conditions using acetic acid as an electron donor. A complex microbial community containing nitrate-, ferric iron-, sulfate-, and arsenate- reducing bacteria was responsible
for contaminant removal. The dye, propidium monoazide (PMA) was used to selectively remove DNA from membrane compromised bacterial cells (that is, dead cells) and qPCR was conducted to quantify live cells (that is, possessing intact cell membranes). The inactivation kinetics curve constructed using qPCR following PMA treatment was drastically different from a typical inactivation kinetics curve obtained using culture-based methods. For example, culture-based methods indicate a 2.3 log removal of viable bacteria at a disinfection concentration of 6.5 mg Cl₂/L and contact time of 26 min-mg Cl₂/L while PMA-qPCR showed only 0.9 log removal.

Samples exposed to various levels of disinfection with monochloramine (both with and without PMA treatment) were submitted for pyrosequencing targeting the V4-V5 region of the 16S rRNA gene to identify bacteria that are resistant to monochloramine and monitor changes in community structure during disinfection. Results will be analyzed to monitor shifts in community composition and structure along the disinfection curve. We will estimate the decay in community similarity (membership, structure) with increasing exposure to monochloramine and evaluate its correspondence with the disinfection rates obtained through PMA-qPCR. The range of inactivation rates exhibited by different bacterial groups within the mixed community will be quantified. We will compare these rates to culture based inactivation curves collected in the current study and in previous work, which have been used to design disinfection processes in drinking water treatment plants.

This work highlights the importance of understanding the effect of disinfection on the complex microbial communities present in drinking water. Current drinking water standards and operational practices are based primarily on culture based measurements of proxy microorganisms (for example fecal coliform as indicator for pathogens), which do not accurately represent the dynamics of mixed microbial communities. This research explores disinfection within the complex matrix of an effluent from a biological drinking water filter, providing tools and information to more rationally manage microbial communities in drinking water treatment processes.

405B  Response of the bacteria community and the IncP1 mobilome to linuron in on farm biopurification systems treating pesticide contaminated wastewater
Simone Dealtry*, Viola Weichelt, Holger Heuer, Vincent Dunon, Dirk Springael, Kornelia Smalla
Julius Kühn-Institute, Germany, KU Leuven, Belgium

To reduce negative environmental effects of pesticides, on farm biopurification system (BPS), are being increasingly used on farms to treat pesticide contaminated wastewater. BPS acts as a biofilter in which the contaminated waters sprayed onto a solid matrix composed of different materials, for example straw, peat and soil and in which the pesticides are removed by sorption and biodegradation processes. In a microcosm experiment, linuron (25 mg/g⁻¹) was added to the matrix material sampled from a BPS in operation and linuron treated and untreated material was monitored over time. Total community DNA extracted from samples taken at one day, 12 and 25 days after contamination was analyzed by denaturing gradient gel electrophoresis and quantitative real time PCR targeting 16S rRNA gene and korB of the IncP-1 α, β, γ, δ and ε subgroups. In addition, exogenous isolation of plasmids conferring mercury resistance using P. putida as a recipient was done. DGGE analysis of BPS material treated with linuron revealed shifts in Betaproteobacteria and in the Variovorax community groups. Moreover, korB copy numbers showed an increased relative abundance compared to the non-treated material. Conventional PCR coupled with Southern blot hybridization using an IncP-1 mixed probe from the trfA region of five IncP-1 subgroups also revealed an increase in IncP-1 plasmid number after 12 and 25 days in the treated material. A real time PCR targeting the Variovorax 16S rRNA gene showed a remarkable increase of Variovorax compared to the control. In addition, different IncP-1 plasmids belonging to different subgroups (β, δ, ε) were captured from the BPS material by exogenous plasmid isolation. The findings are suggesting involvement of IncP-1 plasmids and Variovorax in the specific response of an existing BPS bacterial community to linuron addition.
Gut microbiota dominated by Enterobacter spp. in a morbidly obese patient
Na Fei*, Shuiming Xiao, Jian Shen, Xiaoyan Pang, Linghua Wang, Menghui Zhang, Xiaojun Zhang, Liping Zhao
Shanghai Jiao Tong University, China

Gut microbiota may play a pivotal role in metabolic syndrome of the host, since germfree mice are essentially resistant to high-fat diet-induced obesity. Endotoxin producers in the gut may be a key contributing factor as a low dose of purified endotoxin induces obesity and insulin resistance when subcutaneously infused into mice. In order to investigate the role of endotoxin producers in human obesity development, we design a dietary intervention to help a morbidly obese human lose weight by modulating the structure of gut microbiota. We showed that the gut microbiota of the morbidly obese human was dominated by endotoxin-producing bacteria - Enterobacter spp. by 16S rRNA gene clone library analysis. The endotoxin-producing Enterobacter reduced from 35% of the volunteer's gut bacteria to non-detectable, when he lost 51.4 kg of 174.8 kg initial weight and recovered from hyperglycemia and hypertension after 23 weeks on a diet of whole grains, traditional Chinese medicinal foods and prebiotics. A diminution of endotoxin biosynthetic genes in his gut based on metagenomic sequencing was correlated with a decrease of serum endotoxin load and alleviation of systemic inflammation during weight loss. We then obtained one clinical isolate (B29) from the volunteer's fecal sample via a "sequence-guided isolation" scheme and identified it as Enterobacter cloacae via biochemical tests and the 16S rRNA gene sequencing. The draft whole genome sequence of the isolate B29 showed Enterobacter cloacae subsp. cloacae ATCC 13047 as its nearest neighbor using CVTree. A limulus amebocyte lysate (LAL) test showed that LPS purified from B29 had strong endotoxin activity and its draft genome sequence revealed LPS biosynthetic genes similar to those in metagenomic sequences of the volunteer's fecal sample before intervention. In conclusion, our design showed that the dietary intervention significantly modified the patient's gut microbiota by diminishing the growth of the endotoxin producers (Enterobacter), so it may alleviate the inflammation of the host, and finally led to weight reduction, the improvement in insulin sensitivity and systemic metabolic homeostasis. In future, we will reproduce the disease in animal models as an ultimate proof of causation of endotoxin-producing bacteria of the gut microbiota in excessive fat deposition and insulin resistance in host, identify more such pathogen-like bacteria for obesity from various human populations, understand molecular mechanisms of their interactions with diet and host for obesity development, and use them as a novel and potentially powerful target to reduce the devastating epidemic of metabolic diseases.

Use of an aerobic selector to overcome filamentous bulking in activated sludge
Vânia Ferreira*, Catarina Martins, Maria Olívia Pereira, Ana Nicolau
University of Minho / IBB, Portugal

Secondary biological treatment of wastewater is a complex process and one of the most important steps to ensure the quality of the final effluent in activated sludge plants. In activated sludge systems, the performance of the process largely depends on the balance between filamentous and floc-forming bacteria. When the normal balance of the community is disturbed, filamentous bacteria tend to proliferate, causing various problems. Bulking and foaming phenomena are the major referred problems usually resulting in poor sedimentation of sludge and low quality final effluents. The application of selector reactor technology has become one of the promoted methods for the control of filamentous proliferation, enhancing sludge settleability in activated sludge systems.

The main objective of this research was the use of an aerobic selector to improve the performance of an urban wastewater treatment plant (WWTP), located in North of Portugal with two different parallel lines of treatment during four months.

This WWTP receive domestic wastewater with irregular industrial discharges and filamentous bulking was caused by Sphaerotilus natans and eventual occurrences of Nocardioforms and Type 1863. An aerobic selector was introduced in both lines in the beginning of the studied period, suppressed in one of the lines during 6 weeks, and then put into operation again until the end of the study.

Monitoring of the WWTP overall performance was accomplished through the determination of chemical oxygen demand (COD), mixed liquor total and volatile suspended solids (MLSS and MLVSS)
and biochemical oxygen demand (BOD₅). Other physico-chemical parameters and operation were also analysed, including pH, dissolved oxygen (DO), sludge volume index (SVI), sludge retention time (SRT) and food to microorganisms ratio (F/M).

A total of 14 filamentous bacteria morphotypes were identified. The results showed that the aerobic biological selector in continuous operation prevented the overgrowth of the filamentous Type 1863, of Nocardioforms and, in particular, of Sphaerotilus natans. Simultaneously, it allowed to lowering the oxygen levels in the aeration tanks without negative consequences in the overall performance of the WWTP. In this way, a significant energy save was allowed, even considering the aeration of the selector. The results are more relevant if one considers the fact that the main cause of the bulking problems in this WWTP was the overgrowth of Sphaerotilus natans, a filamentous bacterium known to be stimulated by low DO levels.

408B Characterising the physical structure and microbial community structure of drinking water biofilms
Katherine Fish*, Isabel Douterelo¹, A. Mark Osborn², Joby Boxall¹
¹The University of Sheffield, United Kingdom, ²The University of Hull, United Kingdom

Drinking water distribution systems (DWDS) harbour microbial life, the majority of which is found in mixed species biofilms attached to the inner pipe surface. Biofilms adhere to the pipe wall via an extracellular polymeric substance (EPS) matrix (primarily composed of carbohydrates and proteins), synthesised by members of the sessile microbial community. If the adhesive forces of the EPS are overcome biofilm will be shed into the water column potentially leading to water quality degradation. Although credited with providing cohesion and stability to biofilms, little is known regarding the physical characteristics of the EPS of DWDS biofilms, or the influence of environmental conditions (for example nutrients, hydraulics) or microbial community structure upon its structure. This research aimed to combine fluorescence microscopic characterisation of drinking water biofilm physical-biochemical structure and molecular analysis of microbial communities to enable characterisation of biofilm formation and determine the influence of microbial ecology upon EPS structure.

Biofilms were developed at 16°C, for 28 days, upon high-density polyethylene coupons inserted within a full-scale DWDS experimental pipeline facility. The facility accurately replicates the DWDS conditions including material, water quality and hydraulic conditions, while facilitating the control of environmental parameters. The coupons comprise a curved (to maintain boundary hydraulic conditions) outer section (used for community structure analysis) and a flat removable insert (for microscopy analysis of physical structure). A compatible triple-fluorophore protocol targeting cells and EPS (carbohydrates and proteins) was developed following analysis of excitation and emission spectra and empirical evaluation of the ability to separate fluorophore signals. Z-stack images of the stained samples were produced and digital image analysis employed to quantify each component.

DNA was extracted from sampled biofilms and PCRs for bacteria, fungi and archaea carried out. Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis of bacterial 16S rRNA genes and Amplified Ribosomal Intergenic Spacer Analysis (ARISA) of the fungal ITS1-ITS4 region were used to analyse microbial community structure.

The newly-developed staining protocol enabled concurrent 3D visualisation of the structure of proteins, carbohydrates and cells within biofilms. Quantitative characterisation of DWDS biofilm will be presented comprising EPS volume and composition, cell abundance, biofilm thickness and distribution. These data will be compared to microbial community composition datasets to determine interrelationships between microbial community structure and biofilm matrix characteristics.

This study provides a novel tool to characterise microbial biofilms within a DWDS which is directly representative of real networks. The data presented provide first evidence of the new information that is being collected to enhance understanding of the interactions between the pipeline environment, microbial community structure and EPS matrix characteristics. Such knowledge is integral to developing our understanding of biofilm behaviour and thus to developing future maintenance strategies to manage their potentially deleterious effects upon water quality within DWDS.
409B  Study of encystment and resistance to disinfection treatments of Hartmannella, a free-living amoebae
Emilie Fouque*1, Marie-Cécile Trouilhé2, Philippe Humeau2, Philippe Hartemann3, Vincent Thomas4, Yann Héchard5
1CSTB, France, 2Centre scientifique et technique du bâtiment, France, 3University of Lorraine, France, 4STERIS R&D, France, 5University of Poitiers, France

Free-living amoebae, widely distributed in the environment, colonize hot water systems and are the reservoir of pathogenic bacteria such as Legionella pneumophilia. They have two stages of development: a vegetative form named trophozoite and a resistance form called cyst. The encystment occurs when environmental conditions become unfavorable providing free-living amoebae and their intracellular bacteria with a high resistance. Therefore, their control in hot water systems is important in the management of Legionella risk and a better understanding of the process of encystment seems essential. Although the free-living amoebae of the genus Hartmannella are often found in hot water systems, few data are available on this genus.

Thus, we conducted a detailed study on the influence of environmental conditions (temperature, osmotic pressure, pH) and cell concentration on encystment of Hartmannella. Two strains of H. vermiformis were chosen: a reference strain (H. vermiformis ATCC 50237) and an environmental strain (H. vermiformis 172A). Each strain culture was encysted under different conditions and the rate of encystment was measured at two different incubation times (6 and 9 hours) using counting chamber. The resistance of Hartmannella cysts to conventional disinfection treatments (chlorine, thermal shock) was also evaluated. The final aim is to identify factors favoring trophozoic form which is more sensitive to disinfection treatments.

Regarding the influence of temperature on encystment, the two tested strains reacted similarly. It appears that encystment is limited by a low temperature (4°C) and not influenced by average temperatures (25°C and 37°C) whereas a high temperature (50°C) is lethal for the trophozoites. Low and high osmotic pressures limited the encystment, which is likely due to cell turgor and cell plasmolysis respectively. It was observed that H. vermiformis 172A was more sensitive to osmotic pressure than H. vermiformis ATCC 50237. It results in a greater rate of encystment. With respect to pH, basic values (8 and 9) seemed respectively to favor and not influence the encystment of H. vermiformis 172A and of H. vermiformis ATCC50237. Neutral or slightly acid pH values (5, 6 and 7) seemed to limit only the encystment of H. vermiformis 172A. At last, low cell concentrations (10^3 and 10^4 amoebae/mL) limited the encystment while high cell concentrations (higher than 10^4 amoebae/mL) promoted it for both strains.

Regarding the resistance of Hartmannella cysts to conventional disinfection treatments, early results showed that cysts are resistant to exposure for one hour at a chlorine concentration of 10 mg/L while they are completely inactivated after exposure for 10 minutes at a chlorine concentration of 15 mg/L.

This study improves the current knowledge of encystment and resistance to disinfection treatments of Hartmannella in order to try to develop innovative processes for disinfecting hot water systems to anticipate health risks associated with amoebae resistant bacteria.

410B  Impact of an inoculation step on the start-up phase of a novel MBBR autotrophic N removal process
Gilberte Gaval*
Veolia Environment Research and Innovation, France

To improve the energy and carbon footprint of wastewater treatment plant, the Veolia Environment group developed a novel autotrophic N-removal MBBR process called ANITA™Mox. This process involves an anaerobic oxidation of ammonium which is particularly relevant for treatment of influent displaying high ammonium concentration such as anaerobic digestor effluent. This biological pathway is performed by very specific microorganisms: Anammox bacteria (AnAOB). These bacteria work together with ammonium oxidizing bacteria (AOB) to convert ammonium and nitrite into nitrogen gas. Partial nitrification and autotrophic N-removal are forced to occur simultaneously within the aerobic and anoxic zones resulting from oxygen mass transfer limitation in the carrier biofilm. From the process concern, using AnAOB allows to significantly reduce aeration cost compared to the
conventional N-removal process. Nevertheless, most of the time, carrier colonization with AnAOB biofilm is a very slow process leading to extended start-up phase. This is due to the AnAOB particularly slow generation time. This AnAOB specificity is one of the main limitation for the widespread industrial application of autotrophic N-removal processes.

The aim of this study was to speed up the AnAOB biofilm growth and thus reduce the process start-up phase. It was decided to inoculate the reactor with carriers previously colonized with AnAOB. Then, to determine the minimum and most efficient inoculation filling ratio necessary to achieve N-removal, experiments were carried out in 4 lab-scale MBBR. Carriers with AnAOB biofilm were used at different concentration to inoculate 4L-MBBR reactors filled with brand-new carriers. Among the reactors, three were inoculate with previously seeded carriers at various filling ratio (0%, 2% and 10%) and the last reactor displayed an inoculation filling ratio of 0% and was used to assess the impact of addition of nitrifying activated sludge on the kinetic of carrier colonization by AnAOB. Beside the process monitoring, microbial analyses were performed. They consisted of: (i) quantification by real-time PCR of the different microbial population involved in the N-removal process (AnAOB, AOB and total bacteria); (ii) monitoring of population dynamics by using Denaturing Gradient Gel Electrophoresis (DGGE). DGGE targeted total bacteria but also AnAOB. Based on the results obtained for these analyses, it appears that the inoculation step significantly speeds up the start-up phase. In addition, the higher is the inoculation filling ratio, the shortest is the start-up phase. The strategy which consisted of adding nitrifying sludge without inoculation with seeded carriers did not provide better results that the reactor with no inoculation. It is important to notice that in the reactors without inoculation, if the AnAOB growth is quite lower compared to that observed in seeded reactors, at the end of the study, AnAOB concentration on the brand-new carriers are equivalent for the four reactors. DGGE analysis showed that the biofilm composition in terms of total bacteria is different for reactors with and without AnAOB inoculation. For AnAOB, all the reactors displayed the same DGGE profiles. Molecular analyses highlight the fact that under optimal operational conditions, AnAOB were able to quickly colonize the brand-new carriers.

411B Chloroflexi in methanogenic crude oil degrading systems
Russell Grant*1, A. Sherry2, C. M. Aitken2, D. M. Jones2, N. D. Gray2, B. F. J Bowler2, S. R. Larter3, I. M. Head2
1University of York, United Kingdom, 2Newcastle University, United Kingdom, 3University of Calgary, Canada

Chloroflexi have frequently been identified as predominant member of deep subsurface sediment communities and in anoxic, predominantly methanogenic oil impacted systems (Dojka et al., 1998; Yamada et al., 2005; Penner and Fought, 2010). As well as the more obvious photosynthetic members, the Chloroflexi contain non-photosynthetic members such as the Anaerolineaceae. During a study on the distribution of methanogenic oil-degrading consortia in pollution impacted sediments in the UK we frequently detected sequences from Chloroflexi in 16S rRNA gene clone libraries.

We therefore investigated enrichment of Chloroflexi during alkane degradation and methane production in anoxic methanogenic microcosms and compared the degree of enrichment to the enrichment of Smithella sp. of the family Syntrophaceae, which are considered the primary alkane degraders in these systems.

Anaerobic oil-amended microcosms containing carbonatebuffered low sulfate medium(Widdel and Bak, 1992) were prepared with anoxic sediments and digester sludges from 11 different sites. Methane was monitored in headspace gas and those showing higher methane production in oil-amended systems compared to no oil controls were considered to harbour methanogenic oil degrading consortia and were analysed for their alkane content. Quantitative PCR was performed to enumerate total bacteria, Syntrophaceae and Chloroflexi.

During incubation we observed an increase in the relative representation of both Smithella sp. (mean Log gene abundance (lga) 5.00 +/- 0.08 on Day 0 to lga 8.22 +/- 0.52 on Day 475 before reducing to lga 7.31 +/- 0.08 by Day 1150) and Chloroflexi (lga 5.70 +/- 0.18 on Day 0 to lga 6.26 +/- 0.07 on Day 1150) when methane was production was observed, with the rapid increase in Chloroflexi abundance occurring over 100 days after the peak in abundance of the Smithella sp.
The increase of *Chloroflexi* (that includes the fermentative bacteria *Anaerolineae* that can produce hydrogen, acetate and propionate) by an order of magnitude as the *Smithella* sp. of *Syntropheaceae* rapidly decrease in number, coupled with continued methane production after exhaustion of the n-alkanes raises the intriguing possibility that the *Chloroflexi* selected in oil degrading methanogenic systems may be responsible for the degradation of more recalcitrant components of crude oil.

412B  Use of microorganisms in sustainable water treatment for horticultural production: the case of constructed wetlands
Nicolas Gruyer¹, Martine Dorais², Gérald J. Zagury³, Beatrix W. Alsanius⁴
¹Agriculture and Agrifood Canada / Laval University, Canada, ²Agriculture and Agrifood, Canada, ³Department of Civil, Geological and Mining Engineering, École Polytechnique de Montréal, Canada, ⁴Swedish University of Agricultural Sciences, Department of Horticulture, Microbial Horticulture Laboratory, Sweden

Due to the high nutrient input of intensive horticulture production such as greenhouse culture, environmental issues have been highlighted. To reduce the pollution of groundwater by leached nutrients such as nitrate and phosphorus, recirculation of the nutrient solution is now unavoidable. Although reuse water growing systems offer several advantages from an environmental standpoint, economical issue and risks of pathogen dissemination are a major concern for growers. Consequently, this study focused on the potential of microorganisms to reduce the nutrient pollutants and remove plant-pathogens such as *Pythium ultimum* and *Fusarium oxysporum* from greenhouse effluent. The experiment was conducted in a greenhouse using 24 horizontal subsurface flow constructed wetlands (0.6 m x 0.4 m x 0.35 m) in a complete randomized block design with 8 replicates, filled with pozzolana, implanted with common cattail (*Typha latifolia*) and supplemented with different C inputs (1- sucrose, 2- compost mixture, 3- without external C source). Wetlands units received a reconstituted greenhouse effluent of 7.25 mM SO₄²- and 4.84 mM NO₃⁻ and were inoculated 5 times at 7 day interval with 10⁶ CFU *Pythium ultimum* propagules per mL and then 5 times with 10⁶ *Fusarium oxysporum* conidies per mL. Chemical and biological parameters were daily or weekly measured. Our results showed that a high SO₄²- (98%) and NO₃⁻ (99%) removal were only observed when the dissolved organic carbon to sulfate ratio (DOC: SO₄²-) was 0.36 for a C:N ratio of 3.4. By using metabolic profile (Biolog®) combined with the evaluation of the phylogenetic diversity, L-valine, L-pyroglutamic acid, L-arginine, γ-hydroxy-butyric acid, L-isoleucine, L-leucine and quinic acid were identified as preferential forms of C used by the microorganisms, and the mixture of compost changed the phylogenetic profile of the wetlands compared to wetlands enriched with sucrose or none enriched with external C where 90% similarity was observed. Regardless of the C input, plants pathogens were reduced to 96.9 to 99.9%. These removals, mediated by microorganisms, were based on different mechanisms, such as release of antimicrobial compounds (exo-enzymes), polysaccharide biofilm formation, and competition for space and nutrients. This study showed that indigenous microflora in constructed wetlands were highly efficient to reduce plant-pathogens and nutrient pollutants when an adequate carbon input is provided to maintain high denitrification and sulfato reduction.

413B  Stability assessment of the microbial community in a wastewater treatment plant via correlation analysis
Susanne Günther¹, Christin Koch¹, Thomas Hübschmann¹, Isolde Röske², Roland Arno Müller¹, Thomas Bley³, Hauke Harms³, Susann Müller³
¹Helmhotz-Centre for Environmental Research, Germany, ²University of Technology, Germany

Wastewater treatment often suffers from instabilities and the failure of specific functions such as biological phosphorus removal by polyphosphate accumulating organisms. Since most of the microorganisms involved in water clarification are unknown it is challenging to operate the process accounting for the permanent varying abiotic parameters and the complex composition and unrevealed metabolic capacity of a wastewater microbial community. Fulfilling the demands for water quality irrespective of substrate inflow conditions may emit severe problems if the limited management resources of municipal wastewater treatment plants are regarded.

Flow cytometric analyses of cellular DNA and polyphosphate were used to create patterns mirroring dynamics in community structure. These patterns were resolved in up to 15 subcommunities, the presence and abundances of which correlated with abiotic data. Biostatistics were applied to
determine the kind and strength of the correlation. Samples investigated were obtained from a primary clarifier and two activated sludge basins.

The stability of microbial community structure was found to be high in the basins and low in the primary clarifier. Despite major abiotic changes certain subcommunities were dominantly present (up to 80% stability), whereas others emerged only sporadically (down to 3% stability, both according to equivalence testing). Additionally, subcommunities of diagnostic value were detected showing positive correlation with substrate influxes. For instance blackwater (rs = 0.5) and brewery inflow (rs = 0.6) were mirrored by increases in cell abundances in 4 subcommunities. Phosphate accumulation was positively correlated with nitrate (rs = 0.4) and the presence of denitrifying organisms (Rhodocyclaceae). Various other correlations between community structure and abiotic parameters were apparent.

In essence, a monitoring tool was developed which is quick, cheap and causal in its interpretation. It promises to make laborious PCR based technique less obligatory as it allows for reliable process monitoring and control in wastewater treatment plants.

414B  Long-term preservation of key players of nitrogen and carbon cycles
Kim Heylen*1, Sven Hoefman1, Eva Spieck2, Boran Kartal3, Andreas Pommerening-Roeser2, Katharina Ettwig1, Bram Vekeman1, Paul De Vos1
1Ghent University, Belgium, 2Hamburg University, Germany, 3Radboud University, Netherlands

Long-term preservation of key players of nitrogen and carbon cycles has proven difficult, making them unavailable for the scientific community. Lack of suitable storage can limit widespread research efforts but also hampers biotechnological advancements and patent applications. Although cryopreservation is considered as generally applicable, its success for these specific microorganisms, if at all, is usually only for short periods. Scarce interest in the preservation aspect of microbial ecological research and historical focus on glycerol as cryoprotective agent are probably the main reasons for the current situation. Here, we present a large-scale study to find suitable, and ideally universal, preservation conditions for representatives of different key players of nitrogen and carbon cycles.

Five different cryoprotective agents, in different concentrations, and the protective effect of carbon were tested in either liquid nitrogen or -80°C. Scoring of viability (live-dead flow cytometry), growth (OD and ATP measurements, most-probable number analysis and plating), or specific activity was used to evaluate preservation success.

Viability and growth recovery experiments of representative of ten different methanotrophic species after cryopreservation for a one-year period consistently demonstrated the best results for dimethyl sulfoxide (DMSO) as cryoprotective agent and its combination with 1% trehalose in tenfold diluted trypticase soy broth. The latter condition even allowed complete recovery of viability and growth, while other conditions clearly induced a viable but non-culturable state. Suitability of this preservation condition was further confirmed for six different species of nitrite-oxidizing bacteria, as well as for several ammonium-oxidizing bacteria strains and (single cell) enrichment cultures of anaerobic ammonium oxidizing bacteria and nitrite-dependent anaerobic methane oxidizing bacteria. In addition, activity and growth recovery after cryopreservation of nitrite-oxidizing bacteria also demonstrated that carbon compounds can successfully be used as sole cryoprotective agents, although choice of carbon compound and ideal percentage of DMSO were strain-dependent.

Long-term preservation of fastidious key players of nitrogen and carbon cycle is possible and should systematically be tested for novel representatives. Our results so far suggest that 5% DMSO (v/v) alone or combined with 1% trehalose in tenfold diluted trypticase soy broth could potentially be universally applicable as cryoprotective agents.
415B Optimisation of total protein extraction and sample preparation for the metaproteomic analysis of complex microbial communities by tandem mass spectrometry
Susan Hove Jensen*, Per Halkjær Nielsen, Allan Stensballe
Aalborg University, Denmark

The different "-omics" techniques enable the analysis of physiological characteristics of microbial communities in situ. With the availability of metagenomic sequences and the increasing number of complete individual genome sequences it is now possible to enter the field of proteogenomics investigating complex microbial communities although it is still in its infancy. A recent metagenomic study of microbial communities from waste water treatment plants enables the investigation of functional gene products using metaproteomics in the activated sludge ecosystem. The aim of this study was to evaluate and optimise the combination of different protein extraction methods prior to a metaproteomic study. The extraction methods chosen for optimisation were previously reported in metaproteomic studies of complex microbial communities. The setup included three different lysis buffers: PBS, B-PER, and 0.1 M NaOH/phenol and three mechanical cell lysis techniques including freeze/thaw, sonication, and BeadBeating. The latter was used in the DNA extraction protocols for the metagenomic work. After the extraction protein precipitation was performed with TCA, ammonium sulphate, and ammonium acetate in methanol. A combination of the different cell lysis techniques and protein precipitation methods were evaluated using three different sample types. Activated sludge from a full-scale plant served as a sample with very high complexity, flocculated biomass from an enriched lab-scale reactor served as a less complex sample, and E. coli was used as a pure culture control. SDS-PAGE was used for the evaluation and comparison of the quality of the different protein extraction and precipitation methods. From the SDS-PAGE it was clear that the more harsh buffer conditions and mechanical cell lysis techniques were necessary in the complex samples. Subsequently, three types of in-solution digestion protocols and an in-gel digestion protocol were evaluated by protein identification using LC-MS/MS. Finally, the identified proteins were used for species identification in order to evaluate the protein extraction efficiency from different types of bacteria.

416B Effect of a hyperthermophilic pre-treatment on nitrous oxide emission - pattern and population dynamics of nitrifying and denitrifying bacteria during composting of swine waste
Wakako Ikeda-Ohtsubo*, Erika Shindo, Keisuke Miyauchi, Ginro Endo
Tohoku Gakuin University, Japan

Hyperthermophilic pre-treatment (HTPRT) prior to traditional windrow composting has been shown to be beneficial to mitigate ammonia odor and to stimulate rapid humification. However, it is still unclear whether and how the treatment influences nitrification, denitrification, and nitrous oxide ($N_2O$) emission during the composting process. In this study, we investigated $N_2O$ emission profile and population dynamics of nitrifying and denitrifying bacteria during composting of swine waste, and compared the results between compost samples prepared with or without HTPRT. At the early stage of composting (0-3 weeks), the temperature of the compost pile prepared with HTPRT reached up to 73°C, while the other without HTPRT was as low as 50°C. We found that the peak of $N_2O$ emission from the compost pile with HTPRT was delayed for ~3 weeks as compared with the other without HTPRT, and that the emission apparently followed a thermal phase transition and ammonium nitrogen (NH$_4$-N) reduction. Besides, ammonia monooxidase gene ($amoA$) was not detected in the early (0-3 weeks old) compost samples prepared with HTPRT, in contrast to that the gene was detected in all samples prepared without HTPRT. These findings collectively indicate that the occurrence of ammonia-oxidizing bacteria, which depends on the temperature decrease to < 50°C, was an important factor for the $N_2O$ emission. Furthermore, phylogenetic analyses of $amoA$ and $N_2O$-reductase gene ($nosZ$) amplified from each sample revealed that the community structure of nitrifying and denitrifying bacteria significantly changed at the early stage of composting. The predominant populations at the later stage of composting with or without HTPRT represented similar phylogenetic profiles, which indicates that indigenous populations and not those in the composting material are responsible for nitrification, denitrification, and $N_2O$ emission during composting processes.
**417B** Managing microbial life by a novel bioreactor equipped with a microbubble generator by an oscillating porous board  
Tsukasa Ito¹, Tomo Kubota¹, Seichin Kinuta², Kenji Amagai¹  
¹Gunma University, Japan, ²Optics Precision Co., Ltd., Japan

Microorganisms in the aeration tank of the biological wastewater treatment plants are under the stress of insufficient oxygen concentration and share stress by water flow. Since the aeration is the most energy consuming process in the biological wastewater treatment, which occupies almost 50% of energy consumption of wastewater treatment plants, aeration generating microscale bubble have been introduced in some wastewater treatment plants. However, it is still largely unknown how such microscale bubbles are effective for microbial life and microbial activity. At present the equipments of microbubble generator are too big to apply for microbial study in the laboratory. Therefore, authors developed a novel microbubble generator by using an oscillating porous board (MiBos), and further developed a novel laboratory-scale bioreactor equipped with the MiBos. Air supplied into the MiBos becomes a large number of microscale bubbles through the porous board being oscillated by an ultrasonic with only a few volts. The MiBos bioreactor enhanced oxygen transfer efficiency and allowed a precise control of dissolved oxygen concentration in the reactor. Cultivation of Escherichia coli by the MiBos bioreactor gave a higher growth yield and higher organic carbon utilization rate. Fluorescent lectin-based staining technique applied for E. coli culture revealed that amount of extracellular polymeric substances (EPS) of E. coli was significantly reduced by the MiBos bioreactor cultivation. The MiBos bioreactor probably provides an efficient cultivation environment for E. coli and may be available for managing the life and interaction of microorganisms.

**418B** Limitation of short solid retention time operation in an inclined plate membrane bioreactor and effect on microbial community and activity  
Suda Ittisupornrat*, Tomohiro Tobino, Kyoungjin An, Kazuo Yamamoto  
The University of Tokyo, Japan

Inclined plate membrane bioreactor (ip-MBR) is a unique MBR process for wastewater treatment in which a sludge thickening process is placed in mainstream of treatment. With inclined plates installed in anoxic tank, sludge returned from aerobic tank settles down and accumulates at bottom of anoxic tank, thus enabling recovery of dense excess sludge without side stream process. Due to the heterogeneity in the system with low strength wastewater under different SRTs, ip-MBR should be clarified to achieve clearly changes of microbial behaviour related to SRT operation. Two identical bench scale ip-MBRs were operated in a municipal wastewater treatment plant in Japan. The ip-MBR consisted of two tanks, 30 L aerobic tank and 22.5 L anoxic tank. Ten hollow fiber membrane modules (polyvinylidene fluoride with nominal pore size and total filtration area of 0.4 μm and 0.5 m²) were submerged in aerobic tank. Underneath membrane modules, 5 fine bubble diffusers were fixed to supply oxygen to mixed liquor and scour the membrane surface. Ten inclined plates at 60° were installed inside anoxic tank. Raw wastewater was fed to the reactors initially through the anoxic tank and then overflowed to the downstream aerobic tank. Both systems were operated at hydraulic retention time of 6 hours and sludge recycle ratio of 300% at 28-30°C. SRT was controlled by discharging sludge from bottom of anoxic tank. Sludge samples were collected along the operations and terminal restriction fragment length polymorphism (TRFLP) analysis of bacterial 16S rRNA gene and bacterial ammonia monoxygenase gene (amoA) was performed whereas enzymatic assay was analysed by using fluorogenic substrates and spectrophotometry. The ip-MBRs were initially operated at SRT 20 days after 1 week acclimation period with initial sludge concentration was 8.6 g/L. After operating 2 weeks, both systems showed that sludge concentration in aerobic tank was sharply declined to 2.1 g/L whereas sludge highly accumulated in anoxic tank. Because of imbalance of sludge concentration between 2 tanks, average removal efficiency of carbon, ammonia and total nitrogen were reduced to be 58, 30 and 34%, respectively. Both systems could not reach to steady state at this SRT 20 days. Subsequently, sludge withdrawal was stopped. After 2 weeks of no sludge discharge period, however, systems could recover the high efficiency of carbon and total nitrogen removal at more than 90% and 70%, respectively. It was revealed that SRT 20 days was shorter than critical SRT for these ip-MBRs. TRF profiles of total bacteria and ammonia oxidizing bacteria showed little change over the operation. On the other hand, enzymatic activities of sludge community showed similar trend between the two reactors where maximum velocities (Vmax) were slightly decreased for aminopeptidase and β-glucosidase whereas by 5-10 fold for esterase after stopping sludge withdrawal 8 days. Moreover, Vmax of aminopeptidase and β-glucosidase in aerobic tanks was higher than anoxic tank but esterase assay gave converse results. These results suggested that sludge community
adapted their catabolic functions in response to changing SRT even though their bacterial structure was unchanged.

**419B  Assessment of methods for measuring biocorrosion of carbon steel and comparison of corrosion capabilities between two species Desulfovibrio**

Crystal Johnson*, Heather Drilling, Bradley Stevenson, Paul Lawson  
Department of Microbiology and Plant Biology and OU Biocorrosion Center, University of Oklahoma, USA

Corrosion related issues affect many industrial processes including petroleum and energy related production and transportation. Though the mechanisms are not completely understood, biocorrosion results from synergistic interactions between bacterial cells, metal surfaces, and abiotic corrosion products, and poses many environmental and economic concerns. Numerous other studies have concentrated on the molecular detection of microbial populations and their metabolic activities associated with biocorrosion. The study presented here, however, used a series of measurements to directly characterize the corrosive capabilities of microbial assemblages. Ultimately, these parameters will be essential for determining the risk of resident bacteria as well as for assessing the efficacy of current corrosion detection and mitigation strategies.

A series of corrosion experiments were performed with Desulfovibrio alaskensis and Desulfovibrio indonensis, two closely related sulfate-reducing bacteria isolated from hydrocarbon-containing environments. These two organisms share many physiological and metabolic characteristics yet noticeably different rates of corrosion. For each organism, an initial inoculum of 10^5 cells per mL was added to anaerobic VMI medium in stoppered tubes containing pre-weighed carbon steel coupons in triplicate and incubated at 37°C. After 1 month, coupons were cleared of biofilm and corrosion products, weighed for mass loss determination, and imaged via SEM on a Zeiss DSM-960A in order to analyze surface topography. For quantitative height reconstruction, stereo image pairs were taken at 20 kV on a +/-5° tilt, stacked, and analyzed with Scandium software. 3-D surface profiles were created of stacked images from which single line scans and height differences were mapped. Roughness measurements of the reconstructed surface were generated from each line scan, averaged from three biological replicates, and reported as the value Rf (roughness factor). Additionally, a single poly-line scan covering a 1700 micron surface distance was performed on one coupon for each organism and roughness values were calculated. The concentration of liberated Fe^{2+} in the culture medium was also determined using a modified ferrozine assay and verified with atomic absorption. As expected, cultures of D. indonensis utilized more iron from the coupon and demonstrated significantly different surface roughness (p<0.05) than the less corrosive D. alaskensis.

Additionally, confocal microscopy of the biofilm cells and EPS matrix combined with profilometry of metal surface topography have been performed to validate microbial activity. In addition to the more traditional ways of measuring corrosion, such as weight loss and Fe^{2+} accumulation, this polyphasic assessment provides much insight into the corrosive capabilities of individual microbes. While SEM affords a complete analysis of pitting corrosion, Scandium generated data suggests that this high technology instrumentation can be used as a screening tool to measure the corrosive contributions of indigenous bacteria to determine their roles in biocorrosion, ultimately resulting in better mitigation initiatives.

**420B  Large-scale production of a mixed culture of sulfate reducing bacteria for bioremediation of acid mine drainage**

Carl Johnston, Douglas Schell*, Daniel Lisko  
Youngstown State University, USA

Acid mine drainage (AMD) is a worldwide problem that has severely impacted aquatic resources, terrestrial plant growth, wetlands, and groundwater. Contaminated water from abandoned coalmines is one of the most challenging contributors to stream pollution in former and current coal-producing areas. A very promising remediation alternative is a semi-passive treatment technology that uses sulfate-reducing bacteria (SRB). SRBs are very genetically diverse and can be isolated from many different anoxic environments containing sulfate such as hot springs, marine sediments and AMD sites. AMD remediation using SRB has been demonstrated in a variety of experimental settings. However reports of large-scale production of SRB and large scale in situ bioremediation using SRB is
not evident in the scientific literature. The goal of this study is to validate the process of large-scale SRB culture production to be used for in situ bioremediation by monitoring total bacteria, SRB, and pH.

SRB mixed cultured was prepared using AMD impacted soil, SRB selective media and a proprietary carbon source. SRB cultures were scaled up in a bioreactor kept anaerobic using N₂. The mixed culture was monitored over time for SRBs, total bacteria, and pH. SRBs were enumerated by most probable number (MPN) assays using a modified Postgate B media. Total bacteria were enumerated by staining with 4',6-diamidino-2-phenylindole (DAPI). A commercial pH probe was used to monitor pH. DNA was also extracted from the mixed cultures bacterial. Polymerase chain reaction reaction was used to amplify bacterial rDNA prior to cloning and sequencing for phylogenetic analyses.

The initial culture (1 liter volume) on day 1 presented SRB-MPNs greater than 10⁶/ml. After 75 days, 1000 liter culture volume (following a scale up production) had total bacteria counts of 3.6x10⁷/ml and SRB-MPNs greater than 10⁵/ml. After 112 days the mixed culture was split, diluted and brought up to 1000 liters. This mixture had 3.0x10⁷/ml total bacteria and >10⁶/ml SRB-MPN. On day 127, the culture was again split, diluted and brought up to 1000 liters. This mixture had increased total bacteria numbers (7.6x10⁷/ml), however the SRB-MPN population did not change. This mixed culture will be sampled one final time prior to field application. Throughout culture production pH ranged between 8.8 and 9.1.

Consistently high numbers of SRB throughout the culturing process was observed. Total bacteria showed fluctuation over time likely due to sampling heterogeneity. Preliminary sequence data of bacterial rDNA revealed the presence of anaerobes and fermentative bacteria. More cloning is underway to characterize SRB communities in this mixed culture. Industrial production of a mixed culture was achieved. Monitoring SRBs and total bacteria appeared to be an adequate approach for large-scale processes in AMD bioremediation.

421B Detection of aquatic Streptomyces by quantitative PCR for monitoring of taste-and-odour episodes in water reservoirs

Niels O. G. Jorgensen¹, Jeanette E. Lylloff¹, Maria H. Mogensen¹, Michele Burford², Louise Schlüter³

¹University of Copenhagen, Department of Agriculture & Ecology, Denmark, ²Australian Riverine Institute, Griffith University, Australia, ³Carbon 14 Agency, DHI Group, Denmark

The Streptomyces genus includes several species that produce taste-and-odour compounds (TOCs) but knowledge on the abundance of these streptomycetes in drinking water reservoirs and other aquatic environments is scarce. In this study, quantitative PCR was applied, for the first time, to determine densities of streptomycetes in a river, at a weir and in two reservoirs in subtropical Australia. The PCR approach was optimized with respect to (a) collection of streptomycetes in water, (b) extraction of DNA, and (c) a procedure to correct for inhibition of PCR amplification by natural substances in the water. Mean densities of Streptomyces cells at the study sites varied from 225 to 46,000 cells l⁻¹. The highest density occurred in bottom water (8.5 m deep) of one of the reservoirs, while densities in the Brisbane River varied between 260 and 8,000 cells l⁻¹. At the weir site, seasonal variation in abundance in winter and spring in surface water (mean densities of 430 to 13,500 cells l⁻¹) did not correlate with total bacterial abundance (0.9 to 3.5 × 10⁹ cells l⁻¹). A similar qPCR approach was tested for enumeration of cyanobacteria of which several species are known to produce TOCs. Cell densities of pure or mixed cultures of cyanobacteria were quantified with high accuracy but unreliable results were obtained for natural water samples containing known densities of cyanobacteria. The qPCR approach shows that quantitation of streptomycetes in freshwater can be successfully achieved and may prove valuable in predicting TOC episodes in aquatic systems used for drinking water supplies.
Physico-chemical and microbiological monitoring of three biotrickling filters dedicated for the treatment of hydrogen sulfide (H$_2$S) and methyl mercaptan (CH$_3$SH)

Marc Jovic*, Nathalie Brack, Cécile Rouillon, Isabelle Charron, Philippe Zozor, Christophe Renner, Anne-Sophie Lepeuple
Veolia Environnement Recherche & Innovation, France

Removal of hydrogen sulfide (H$_2$S) and methyl mercaptan (CH$_3$SH) in gaseous effluent is a major concern in water industry. Indeed, these reduced sulfur compounds are commonly reported odorous compounds in off-gases of waste water treatment plants and are characterized by very low odor thresholds.

A wide range of options is available to an operator to control H$_2$S and CH$_3$SH emissions and thereby to reduce the impact on sensitive areas. One of them consists to use end-of-pipe abatement options. The principal behind of end-of-pipe abatement techniques is to remove odorous compounds using a treatment system. Among available treatment technologies, biofiltration (biofilter or biotrickling filter) is increasingly used. Indeed, this technique has proved to be economical and environmentally viable.

A biofilter consists in a column packed with a mineral biomass carrier material through which is passed an off-gas charged with H$_2$S and CH$_3$SH. During biofiltration process, H$_2$S and CH$_3$SH are, in a first time, transferred from the gas phase to the water phase and to the biofilm and, in a second time, oxidized into sulfuric acid (H$_2$SO$_4$) by Sulfur Oxidizing Bacteria (SOB) present in the biofilm.

In biofiltration, packing material is an essential parameter to insure good biofilter performances. Nowadays biofilm carrier media selection is only based on physical and chemical parameters and little attention has been paid so far for microbiological aspects. However it appears that a better knowledge of microbiological ecology of biofilters treating reduced sulfur compounds could pave the way for a new approach to select appropriate packing materials and to optimize their performances.

In this study we monitored, during one year, three biotrickling filters pilots (BF1, BF2 and BF3) packed with three different three biofilm carrier media (expended schist, lava rock and waste residue) using physico chemical and microbiological parameters. Physico chemical monitoring included Removal Efficiency (RE), Empty Bed Retention Time (EBRT), pollutants loads, flow watering, pH and temperature. Microbiological parameters consisted in quantitative data (qPCR on rrn gene for total biomass quantification and qPCR on the functional soxB gene for SOB quantification) and qualitative data (diversity and population dynamics of SOB populations using High Resolution Melting Analyses (HRMA) on the functional soxB gene).

Results indicate that the 3 biotrickling filters were able to treat H$_2$S and CH$_3$SH. Even if, high and stable purification performances were obtained for H$_2$S, on the other hand, elimination of CH$_3$SH is more problematic. Indeed, this activity seems to be more difficult to maintain in the biotrickling filters. Finally, it appears that for the CH$_3$SH oxidation, biofilters BF1, BF2 BF3 have different levels of robustness (biotrickling filters BF2 and BF3 are more robust that the biotrickling filter BF1) and the stability in the treatment of this reduced sulfur compound seems to be rely with the biomass robustness.

Role of Hyphomicrobium-like bacteria associated with granular activated carbon filter in drinking water purification process in the removal of assimilable organic carbon

Ikuro Kasuga*, Suwat Soonglerdsongpha, Yukihiro Osaka, Futoshi Kurisu, Hiroaki Furumai
The University of Tokyo, Japan

Biologically stable drinking water is of great concern for water quality management. Bacterial regrowth in drinking water distribution system leads to deterioration of water quality and potential risk of microbial diseases. Assimilable organic carbon (AOC) is considered as a major factor supporting growth of microorganisms. In advanced water purification process, ozonation followed by granular activated carbon (GAC) filter is widely used. Ozonation can degrade large organic matter and generate AOC mainly consisting of carboxylic acids such as formate, acetate, and oxalate. Subsequent treatment of GAC filter can efficiently remove the generated AOC thorough the function of bacterial community associated with GAC. However, key players in the removal of AOC remain
unknown. In this study, we applied stable isotope probing (SIP) to identify them to further elucidate biological function of GAC.

GAC samples were collected from two drinking water purification plants. These two plants receive raw water from the same river and have the same purification process. Formate, acetate, and oxalate were tested as model AOC substances. $^{13}$C-labeled and non-labeled substrates were continuously fed to GAC. In order to find bacteria which had affinity to low concentration of substrates, substrate concentration was set at 0.5 mg C/L. After fractionation of DNA based on buoyant density, terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes was applied to find candidates capable of utilizing these substrates.

T-RFLP analysis of DNA in different buoyant density revealed several candidates responsible for the removal of carboxylic acids. In particular, bacteria corresponding to 330 bp of restriction fragment were commonly found from both GAC samples for all substrates. Phylogenetic analysis demonstrated that this group was closely related to Hyphomicrobium spp. Hyphomicrobium group is known as facultative methylotroph utilizing C1 compounds as well as multi-carbon compounds. Thus, the result of DNA-SIP is consistent with physiology of Hyphomicrobium group. Specific primer set was designed for the target 16S rRNA genes in this study. Distribution of 16S rRNA genes of Hyphomicrobium-like bacteria in different buoyant density fractions was evaluated. Except for one case, 16S rRNA genes of Hyphomicrobium-like bacteria were indeed enriched in heavier fractions for $^{13}$C-cases. Settlement of Hyphomicrobium-like bacteria in GAC was monitored at a new drinking water purification plant from its start-up. Total bacterial 16S rRNA genes rapidly increased from below detection limit to $2.0 \times 10^8$ gene copies/g-dry after six months of operation. On the other hand, Hyphomicrobium-like bacteria started to settle in GAC after two months and jumped to $2.0 \times 10^6$ gene copies/g-dry after three months. They maintained the level thereafter. The maximum ratio of 16S rRNA genes of total bacteria to Hyphomicrobium-like bacteria was 9.6%.

In this study, DNA-SIP revealed that Hyphomicrobium-like bacteria were involved in the removal of carboxylic acids in GAC filter used for drinking water purification process. In addition, we confirmed the settlement process of this group in the full-scale GAC filter.

424B  Hydrolytic community development in low temperature anaerobic bioreactors treating synthetic sewage
Ciara Keating*, Denise Cysneiros, Dermot Hughes, Therese Mahony, Vincent O'Flaherty
National University of Ireland, Galway, Ireland

Anaerobic digestion is an established technology for the treatment of wastes and wastewaters, which exploits methanogenic consortia to achieve the biodegradation of organic matter to biogas (CH$_4$ and CO$_2$). Full-scale anaerobic digesters are generally operated at mesophilic (>25°C) or thermophilic (>45°C) temperatures. AD would be more economically and practically favourable, especially for treatment of dilute wastewater streams, if it was applicable under ambient/low temperatures (LTAD). Such applications present significant challenges, however, including limitations with respect to the rate and extent of the initial biological hydrolysis step of methanoogenesis of complex organic compounds, including proteinaceous residues. The current research explores the viability of applying LTAD for the treatment of sewage wastewater with a previously acclimatised biomass, with particular emphasis on hydrolysis. An expanded granular sludge bed anaerobic filter (EGSB-AF) bioreactor, was employed to treat a synthetic sewage wastewater with a concentration of 500 mg COD l$^{-1}$ at 12 ºC for ~700 days. Chemical oxygen demand removal efficiency (CODRE) was used to evaluate bioreactor performance. CODRE was consistently high throughout the trial, with values in the range 70-90%. The specific methanogenic activity (SMA) profiles of the biomass were assessed throughout the trial. SMA values indicated a shift toward predominantly hydrogenotrophic methanogenic activity. Protein degradation tests illustrated the mesophilic hydrolytic nature of the seed sludge, with hydrolysis constants of 0.048 h$^{-1}$ at 37 ºC and 0.017 h$^{-1}$ at 12 ºC. An increase in the hydrolysis constant over time at 12 ºC, however, provided evidence of successful biomass adaptation to low temperature operation. Molecular fingerprinting techniques were carried out in parallel to elucidate the functional groups responsible for hydrolysis within the granular sludge bed and filter zones of the bioreactor. Clone libraries and qPCR analysis identified the major bacterial and archaeal groups present in the system, including those likely to be responsible for protein hydrolysis during low-temperature anaerobic sewage treatment.
PS13 – Managing Microbial Communities

425B  **Quantitative proteomic analysis of ibuprofen-degrading *Patulibacter* sp. strain I11**
Henrik Kjeldal*,1, Barbara Almeida2, Ihab Lolas1, Anders Dahl Knudsen1, Gilda Carvalho2, Kåre Lehmann Nielsen1, Maria Teresa Barreto Crespo2, Jeppe Lund Nielsen1

1Aalborg University, Denmark, 2Instituto de Biologia Experimental e Tecnológica, Portugal

The increase in diversity and quantity of Pharmaceuticals Active Compounds (PhACs) detected in waste water treatment plants (WWTPs) effluents is an issue of rising concern due to the potential negative impact of the PhACs on the surrounding environment.

Of PhACs, Ibuprofen, a non-steroidal anti-inflammatory drug, is considered one of the frequently occurring contaminants in the influent of WWTPs, typically being found in the range of 10-400 μg/L. Concentrations of Ibuprofen in the effluent wastewater is considerably lower indicating a partial removal, ascribed primarily to biodegradation of Ibuprofen.

However, if biodegradation is to be used as a solution for removing ibuprofen from wastewater, figuring out which bacteria are responsible for degrading the compound and understanding the processes by which it is being degraded are the first steps on the way.

In the current study, the pathway of degradation of ibuprofen of the ibuprofen degrading strain *Patulibacter* sp. strain I11 was characterised using quantitative proteomics. The genome of *Patulibacter* sp. strain I11 was sequenced and annotated in order to improve the efficiency of the subsequent mass spectrometry-based protein identification. The proteomic data analysis revealed several proteins which were up-regulated in response to ibuprofen by *Patulibacter* sp. strain I11. These proteins might be involved in the degradation of ibuprofen.

426B  **Bacterial biofilm communities enhance the degradation of polychlorinated biphenyls (PCBs) in sediment**
Birthe Venoe Kjellerup*,1, Sarah Edwards1, Chiara Draghi1, Emily Balbier1, Kevin Sowers2

1Goucher College, United States, 2University of Maryland Baltimore County, United States

Polychlorinated biphenyls (PCBs) are toxic and persistent organic pollutants that have remained in the environment despite a commercial ban almost 40 years ago. Due to their hydrophobicity, PCBs adsorb to surfaces in the aquatic environment, where they are ingested by animals and accumulate in the food chain. To minimize the concentration of PCBs in the water phase granular activated carbon (GAC) has successfully been applied as a remediation solution to sediments for sequestration of PCBs. However, this is only part of a bioremediation solution unless complete microbial degradation of PCBs adsorbed to GAC can occur. The overall aim of this study was to apply microbial communities capable of PCB degradation based on biofilm covered activated carbon aggregates. Specifically, the effects of the biofilm communities on anaerobic dechlorination activity combined with aerobic mineralization of the PCBs was evaluated to examine if this applied biofilm strategy could be used as an enhanced bioremediation solution for PCB contaminated sediments.

Micro- and mesocosms were set up using enrichment cultures of PCB degrading bacteria as well as sediment slurries spiked with Aroclor 1248 (commercial product consisting of different PCB congeners) with and without GAC (3%). Biofilm communities were formed on the GAC surfaces by growing *Dehalobium chlorocoercia* DF1 (anaerobic), *Burkholderia xenovorans* LB400 (aerobic) as well as enrichment cultures (aerobic and anaerobic) originating from sediment and activated sludge. PCB concentrations were determined by Gas Chromatography. DNA was extracted with MoBio PowerSoil DNA extraction kit, amplified by PCR with specific 16S rDNA primers (348F/884R) and identified by separation using DHPLC (denaturing high-performance liquid chromatography) and sequencing. Microscopic analyses were performed using DAPI, fluorescence in situ hybridization with PNA-FISH probes, and microscopic examination with Confocal Laser Scanning Microscopy (CLSM) and Scanning Electron Microscopy (SEM).

Biofilms were formed on the GAC surfaces in microcosms after seven days under aerobic conditions and 35 days under anaerobic conditions, respectively. The number of bacteria (DAPI stain) in the slurries that contained GAC increased significantly (more than 10-fold to 10^7 cells per ml) compared to slurries without GAC. Evaluation with fluorescent stains (DAPI, PNA-FISH) and microscopy (CLSM, SEM) showed that the biofilm covered the entirety of the GAC surfaces and consisted of thick layers of...
both aerobic and anaerobic bacterial biofilms. Aerobic degradation as well as anaerobic dechlorination activity was enhanced in the presence of GAC covered biofilm aggregates resulting in significant degradation of Aroclor 1248 in the sediment slurries.

Biofilms formed on GAC surfaces and enhanced aerobic and anaerobic PCB transformation activity in slurries. This increased activity might likely be due to two processes: PCB adsorption onto the GAC and subsequent formation of PCB degrading biofilms that are directly in contact with the adsorbed and bioavailable PCBs. These results show that dechlorination by anaerobes coupled with biphenyl degradation by aerobic bacteria can take place in biofilms located on GAC surfaces. Application of microbial communities in the form of biofilm covered GAC aggregates may be a suitable and enhanced bioremediation strategy for PCB contaminated sediments.

427B Impact of temperature variations on bacterial population involved in MBBR autotrophic N-removal process

Sébastien Lacroix1, Gilberte Gaval1, Régis Gagneux1, Mickael Samspön1, Estelle Gonidec1, Romain LEMAIRE2, Juan-Carlos Ochoa1, Anne-Sophie LEPEUPLE1

1Veolia Environnement Research and Innovation, France, 2Veolia Water, France

Since it has been identified, Anammox bacteria (AnAOB), offer a cost- and energy-effective alternative for development of new N-removal processes. Indeed, using anaerobic ammonia-oxidation offers many advantages: reduction of O2 demand (60%), no COD requirement and less sludge production. In this context, a one-stage MBBR deammonification process, called ANITA™ Mox was developed. In this process, partial nitrification occurs simultaneously with anaerobic ammonia-oxidation within the biofilm. Ammonia-oxidizing bacteria (AOB) oxidize NH4 to NO2 in the aerobic zone. Then NO2 diffuse in the inner part of the biofilm and is consumed together with the excess of NH4 by AnAOB. ANITA™ Mox process was initially defined for the treatment of NH4-rich effluents such as anaerobic sludge digester centrate. In this case, the process operates at 30°C. The aim of this study was to assess the impact of a significant temperature change on the biofilm microbial population (AOB and AnAOB).

Experiments were performed in 4L lab-scale MBBR filled with BiofilmChip™ M carriers. The carriers came from an ANITA™Mox fullscale-plant and possessed an active AnAOB biofilm. In one reactor, temperature was lowered gradually from 30°C to 13°C, then suddenly rose to 30°C, while in the second the temperature was maintained at 30°C and then lowered at 15°C. Beside the chemical monitoring (Load, %N-reduction, %N-NH4 reduction), molecular analysis were performed to characterized microbial population in the biofilm. qPCR were run all along the study to quantify the total bacterial population as well as species involved in the N-removal process (AOB and AnAOB). Denaturating Gradient Gel Electrophoresis (DGGE) targeting total bacterial and Anammox population were also performed for monitoring population dynamic and diversity. RT-qPCR targeting hzo and amoA genes were also carried out to assess the AOB and AnAOB activity in function of temperature.

The molecular analysis combined with process monitoring of the MBBR showed that a decrease of temperature from 30°C to 13°C does not impact significantly the AnAOB and AOB density in the biofilm. Nevertheless, this change impacts the proportion of AOB and AnAOB by inducing the growth of heterotrophic bacteria in the biofilm. In contrast, after 3 months operation at low temperature, a sudden increase from 13°C to 30°C leads to a decrease of AnAOB density in the biofilm and a shift in the composition of the AOB population. Regarding the AnAOB activity, even if a fall in temperature does not impact highly the AnAOB density, N-NH4 removal monitoring together with RT-qPCR results shows that AnAOB activity decrease with temperature. A sudden rise to 30°C can restore a part of the AnAOB activity, but it still lower than the initial N-NH4 removal capacity.

Although their activity is affected, AnAOB support a decrease in temperature but are very affected by a sudden increase. Variations in temperatures induced changes in the composition of the AOB population but did not affect their global activity.
A global industrial shift from a fossil carbon based economy toward a closed loop life cycle economy is under way, starting from renewable raw materials for the production of energy and materials and optimizing resource management (recovering all the intrinsic value of biomass). Wastewater treatment (WWT) processes are evolving in phase with this change: sludge valorization through PHA production leads the way for a broader paradigm shift from treatment to valorization targeting higher-value products. PHA production by microbial consortia used in biological WWT relies on microbial culture selection enhancing the PHA storage function. The culture selection is based on microbial competition in open biological systems targeting a given function for the production of biopolymers or other added value chemicals has been designated as ecosystems biotechnology. Subjecting these systems to alternate availability of carbon supply, designated as Feast and Famine (FF), creates a competitive advantage for microorganisms capable of storing PHA. The effectiveness of FF operated processes is assessed by the evolution of the PHA storage performance determined in a subsequent PHA accumulation stage. However, this indirect measure is also a function of the operating parameters influencing the accumulation stage itself. The development of tools able to characterize microbial ecology on the enrichment stage will contribute to deepening the knowledge on population dynamics. The goal is to deepen perspective of community structure influence on the function of PHA storage potential of different wastewater consortiums experiencing similar or distinct FF. In full-scale applications influent variations will naturally occur for which the coupling or independence of the structure and function become of important practical engineering interest.

PHA-storing microorganisms contain in their genome a gene cluster (between 5 and 8) involved in PHA synthesis. Based on the cluster composition, PHA producers are classified in four classes. Among these genes, \( \text{pha}C \) is the only one present in all PHA-producing microorganisms. Two couples of PCR primers targeting \( \text{pha}C \) were defined to target on one hand classes I and II, and, on the other hand, class IV PHA-producers. As class III is largely a minority, it has not been followed.

These primers were used to monitor the evolution of PHA-storing microorganisms during the enrichment step (qPCR), but they also allowed determining PHA synthesis activity during the accumulation step (RT-qPCR).

Two sequencing batch reactors (SBRs) were inoculated with activated sludge and operated under FF conditions (operating parameters consistent with the current state of the art for mixed culture PHA production). Two distinct feeding regimes were imposed. The impact of the distinct operating conditions on the microbial population dynamics were followed assessing microbial diversity (DGGE) and function specialization-enrichment in PHA storing organisms (qPCR) and activity of the PHA storage function (RT-qPCR).

The developed molecular tools allowed describing a dynamic of the bacterial population specific to the feeding strategies imposed. A simplification of the bacterial populations toward dominant PHA producers was more distinctly observed for one of the two cases. Moreover, these populations seem to have a specific activity response depending on the feeding rate.

Pesticides are detected in an increasing number of aquifers all over the world resulting in closure of many water abstraction wells. The natural redox conditions of aquifers are considered to be an important factor governing the pesticide degradation in ground water. These observations emphasize the importance of the only possible process mineralization as a result of the biological processes to complete removal of pesticides from the environment. Microbial degradation of organic pollutants in groundwater takes place naturally, sustained by electron acceptors, electron donors and nutrients or
using enhanced bioremediation strategies. One relevant approach for the remediation of pesticides in contaminated aquifers is to stimulate indigenous microbial populations with addition of electron acceptors such as oxygen. In this research we investigate the ability and effects of oxygen addition at different concentrations to stimulate biodegradation of (RS)-2-(4-chloro-2-methylphenoxy) propanoic acid (mecoprop), (R)-2-(2,4-dichlorophenoxy) propanoic acid (dichlorprop) and 3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide (bentazone) in an anaerobic sediment and groundwater.

Anaerobic sediment and groundwater samples were collected from 2.3 to 7.1 mbs from a Danish aquifer. Experiments were conducted with groundwater and sediment in laboratory batch systems amended with oxygen concentrations 0.0, 0.1, 0.5, 1, 2, 4, 5, 8, 9 and 11 mg L\(^{-1}\). The microcosms were incubated in the dark at 10°C for 200 days. \(^{14}\)C labeled mecoprop, dichlorprop and bentazone mineralization were monitored as produced \(^{14}\)CO\(_2\). Oxygen concentrations were measured from outside of the microcosms by a fiber optical oxygen meter using oxygen sensitive luminescent sensor foil mounted inside the microcosms at the beginning of set up and during the sampling periods. More oxygen was added during the experiment to achieve the desired oxygen concentration level in each bottle.

The results demonstrate that addition of oxygen has a positive effect on herbicides degradation. The highest mineralization potential was found in the microcosms amended with high oxygen concentrations. Here, 14-27 % of mecoprop, 3-9 % of dichlorprop and 15-20 % of bentazone were degraded over 200 incubation days. There was a clear increase in mecoprop and bentazone degradation in highly oxygenated microcosms. Furthermore, addition of low oxygen concentration (1 mg L\(^{-1}\)) would be adequate to initiate and have significant impact on aerobic degradation. We have observed no mineralization in the control microcosms that were inactivated microbiologically or microcosms that were not amended with oxygen, indicating an occurrence of the microbial degradation and that need of oxygen for biodegradation of the herbicides. To our knowledge, this is one of the first reports showing a link between the oxygen addition and bentazone degradation in aquifer systems.

Our findings suggest that shifting anaerobic conditions to aerobic conditions by increasing oxygen concentration would provide a potential and promising remediation technology for enhancement of microbial degradation of pesticides, although high oxygen consumption by organic matter and reduced species may limit the application of enhanced bioremediation.

430B T-RFLP and Pyrosequencing Approach to Clarify Bacterial Groups that Affects Dewaterability of Activated Sludge  
Ning Li*, Hiroyasu Satoh, Takashi Mino  
Department of Socio-cultural and Environmental Studies, Graduate School of Frontier Sciences, The University of Tokyo, Japan

Bacteria which affect dewaterability of activated sludge were investigated in this study. In Japan, waste biomass generated from biological wastewater treatment is often dewatered and then incinerated. The performance of dewatering affects the energy efficiency of incineration process, and thus the control of dewaterability is the key in waste biomass treatment. However, factors that affect dewaterability of waste biomass are not well clarified yet. In the present study, we hypothesized that certain group of bacteria is positively or negatively affecting dewaterability of waste biomass. We operated a laboratory activated sludge reactor for 214 days with synthetic wastewater, and monitored dewaterability of activated sludge biomass and microbial population at 73 sampling days. Dewaterability was monitored by water content of dewatered (WCDS) testing method. Microbial population was analyzed by molecular methods targeted at a partial 16S rRNA amplified by a 27f/519r primer set: Terminal Restriction Fragment Length Polymorphism (T-RFLP) was conducted with a 27f primer labeled with FAM and RsaI restriction enzyme, and pyrosequencing was done with barcoded 27f and 519r primers. PCR products were sequenced from forward or reverse primer ends by a Roche 454 FLX+ pyrosequencer. Obtained sequences were assigned to their original samples, restriction sites in reads with forward primer region were identified, and reads count for different "virtual" terminal restriction fragment sizes were calculated. In T-RFLP analysis, three of the terminal restriction fragments (T-RFs) and four T-RFs were found to have positive and negative correlations with dewaterability, respectively. Multiple regression analysis of these 7 T-RFs with dewaterability showed a high correlation with \(R^2=0.84\). By pyrosequencing, a total of 78761 reads were obtained, or per
sample, 1079 reads in average ranging from a minimum of 325 (Day 65) to a maximum of 8217 (Day 163) reads. The number of reads which had forward primer region and effectively used for virtual T-RFLP was 69343. Each T-RF size contained fragments from plural OTUs. Yet, major OTUs related to each T-RF were identified. Following bacterial groups were suggested to be associated with the 3 T-RFs that had positive correlation with dewaterability: Myxococcales (closest to Haliangiaceae with 49% confidence), Planctomycetales, Thiothrix at genus level respectively. The 4 T-RFs negatively related with dewaterability were associated with following groups: Bacteroidetes (closest to class Ohtaekwangia with 92% confidence.), two groups in Alphaproteobacteria both closest to Rhizobiales at confidence levels of 97% and 40% respectively, Haliscomenobacter and Propionivibrio at genus levels.

431B Strategies for the management of surface microbial communities based on volatile compounds and visible light
Stefan Liebminger\textsuperscript{1}, Tomislav Cernava\textsuperscript{1}, Lisa Oberauner\textsuperscript{2}, Michael Fürnkranz\textsuperscript{3}, Massimiliano Cardinale\textsuperscript{3}, Gabriele Berg\textsuperscript{3}
\textsuperscript{1}RCPE GmbH, Austria, \textsuperscript{2}ACIB GmbH, Austria, \textsuperscript{3}Graz, University of Technology / Institute of Environmental Biotechnology, Austria

In our everyday life we constantly stay in contact with surfaces. These surfaces can be compared with ecosystems, which contain microbial communities. Especially surfaces in indoor environments are directly influenced by humans and their microbes. To date disinfection techniques were applied with the objective to kill the whole microbial community including beneficial bacteria. Bacterial antagonists, who belong to this group, suppress the growth of other organisms, including pathogens, by competition and secretion of antimicrobial/lytic substances. In this context, volatile organic compounds (VOCs) show a novel mechanism for bacterial antagonism and biocontrol of pathogenic organisms. Beyond that, photobiology and the influence of light on living organisms could also be used to control microbial communities.

We analyzed endophytic bacteria isolated from mistletoe (Viscum album) and Styrian oil pumpkin (Cucurbita pepo var. styriaca) in dual culture assays combined with GC-MS headspace analysis for the identification and characterization of bioactive VOCs. A high proportion of antagonists towards human associated pathogens (for example methicillin-resistant Staphylococcus aureus, Stenotrophomonas maltophilia and Pseudomonas aeruginosa) were determined. VOCs, which were produced by Pseudomonas and Paenibacillus strains were identified by GC-MS headspace analysis. Volatiles of Pseudomonas chlororaphis are characterized by different unspecific toxic sulfuric compounds like methanethiol and dimethyldisulfide as well as 1-undecene. In contrast, Paenibacillus polymyxa isolates secreted distinctive amounts of different, highly specific pyrazines into the gas phase. Additionally, the action of certain types of photosensitisers with photodynamic antibacterial properties was successfully incorporated in synthetic polymers and will serve for the production of self-cleaning antimicrobial textiles and surfaces.

The combination of naturally occurring antagonists with photobiological control is a new approach for managing indoor microbial communities.

432B Long-term microbial community dynamics in a full scale biogas plant
Rico Lucas*, Daniel Beyer, Anne Brandt
Helmholtz Centre for Environmental Research GmbH - UFZ, Germany

To mitigate the ongoing climate change and the progressive scarcity of fossil fuels it is essential to establish a sustainable "low carbon" energy industry. The utilization of wind and solar power as fluctuating renewable energy sources can be complemented by biogas that represents a continuously available energy source. Biogas is produced by anaerobic digestion of biomass such as energy crops and organic wastes of different origin. However, various factors can disturb the complex microbial ecosystem in a biogas reactor; thereby drastically reducing the process efficiency. Current methods for process control are mostly based on trial and error. Therefore a detailed understanding of the microbial community functioning and the respective physicochemical parameters governing microbial activities is necessary to prevent reactor failures especially at high organic loading rates.
To approach this problem, a long-term monitoring of three full scale biogas reactors of identical construction and operation was conducted. The reactors were fed with maize silage and full plant silage of rye as a co-substrate including trace elements. Fresh fermenter content of the reactors was sampled weekly over a whole year. In parallel, a wide range of physicochemical parameters reflecting reactor performance and process stability was determined by several analytical methods. The microbial community composition was monitored based on genomic DNA extracted from the fermenter samples. For PCR amplification bacterial 16S rRNA genes as well as the genes encoding the alpha-subunit of the methyl coenzyme M reductase (mcrA) were targeted. Molecular fingerprints of the bacterial and methanogenic communities were generated based on terminal restriction fragment length polymorphism. Samples were also analysed as technical replicates to ensure the reproducibility of the experimental approach. To assign the major terminal restriction fragments to the respective microbial groups, corresponding clone libraries were established and representative clones were sequenced. Multivariate statistics were applied to correlate community composition with reactor parameters and environmental variables.

The results showed a high stability of the bacterial communities over time and a high similarity between the three parallel reactors. This matched with the overall stable performance of the reactors. However, minor differences between the community profiles at different sampling times and between the parallel reactors were partly correlated e.g. with changes of the reactor temperature and total organic solid content. The methanogenic communities were also stable over time and highly similar between the parallel reactors. The methanogenic communities were dominated by members of the *Methanosarcinales*, *Methanomicrobiales* and *Methanobacteriales*. Minor fluctuations within the mcrA community profiles showed no correlation with any of the physicochemical parameters.

Our study shows for the first time the long-term dynamics of bacterial and methanogenic communities in a full scale biogas plant fed with energy crops. Further investigations will include amplicon pyrosequencing for a more complete analysis of the bacterial communities and the use of further functional markers for specific microbial functions such as hydrogenase genes.

**433B  The bacterial and archaeal demographics of farm wastes bioreactors**

Terence Marsh\(^1\), F. Yang\(^1\), R. Rossi\(^1\), Z. Yue\(^2\), R. Chen\(^1\), J. M. MacLellan\(^1\), W. Liao\(^1\)

\(^1\)Michigan State University, USA, \(^2\)Hefei University of Technology, China

A growing human population has led to increasing farm production and associated farm wastes. How we handle the wastes will determine, in part, to what extent our food system is sustainable. Large animal feeding operations generate substantial quantities of animal excreta that require processing prior to disposal. Anaerobic digestion (AD) is a biological conversion process that has been proven effective at converting organic wastes into methane gas capable of producing relatively clean electricity while also alleviating many of the environmental concerns associated with the wastes. Recently, an increasing number of animal farms are using AD as part of their wastes management strategy to collectively produce methane as a renewable energy source. Due to the diversity of waste streams available on animal farms, better understanding of microbial responses on different waste streams would significantly improve the efficiency of anaerobic digestion and extend its applicability.

Six anaerobic digestions under three different hydraulic retention times (HRT) and two different feed regimens of dairy cow waste and corn stover were tested to derive the relationship between microbial community and digestion performance. The phylogenetic composition of individual digestions was determined along with methane production and fiber degradation. For the bacterial community we used pyrosequencing of 16S rRNA genes and have generated 5000-6000 sequences for each bioreactor. Based on comparative sequence analysis we have identified *Bacteriodetes*, *E. coli*, *Petrimonas*, *Clostridiales* and *Pseudomonas* as major populations that vary in relative size depending on HRT and the presence of corn stover in the dairy waste. The reactor with highest methane productivity had a bacterial community dominated by a unique uncultivated *Bacteriodetes* population and had the fewest *E. coli* sequences. Unclassified *Clostridiales* were also abundant in this reactor. Clone libraries of archaeal 16S rRNA genes revealed that *Methanosarcina* and *Methanosaeta* dominated at low and high HRTs respectively, suggesting that acetate concentration diminished as the retention times increased. *Methanobacterium* populations appeared variable ranging from 4% to 33% with the higher values correlated with feed supplemented with corn stover. In the high methane producing bioreactor the *Methanosaeta* and *Methanobacterium* accounted for 88% of the archaeal
sequences. Because hydrogen is an important commodity in these reactors we have determined the phylogenetic distribution of [FeFe]-hydrogenase genes using previously described primers. The phylogenetic distribution of the hydrogenase genes appeared sensitive to both HRT and feed composition. The dominant phylotypes were phylogenetically closest to Aminomonas paucivorans, Moorella thermoacetica, Bacteroides, Desulfotomaculum kuznetsovi, Syntrophus aciditrophicus, Clostridium thermocellum, Desulfovibrio fructosovorans and Spirochaeta smaragdinae. A. paucivorans and D. kuznetsovi sequences were consistently abundant and more numerous in the corn stover supplemented samples whereas the Bacteroides sp. affiliates were more abundant in the unsupplemented reactors. The reactor with the highest methane production was dominated by sequences affiliated with A. paucivorans, M. thermoacetica and Bacteroides sp., accounting for approximately 68% of the [FeFe]-hydrogenase sequences. In general the diversity of hydrogenases was greater in the two reactors with low HRTs. These data indicate that by adjusting abiotic features and feed composition, anaerobic digestion can be optimized.

434B Synthetic ecosystem engineering for improved microbial consortia design
Michael Mee1, Harris Wang2, George Church1
1Harvard Medical School, United States, 2Wyss Institute for Biologically Inspired Engineering, USA

A natural extension of the synthetic biology framework is the combination of different cells into groups and artificial consortia of increasing complexity. This approach is important since heterogeneous populations can often outperform homogenous populations of genetically identical individuals in many tasks that require more sophisticated divisions of labor. Microbial consortia for example are able to degrade complex substrates more efficiently than any single member, are more robust to environmental variations and can potentially be reprogrammed in modular ways. However, building higher-order biological systems rely on improving our understanding of these dynamic communities, both natural and synthetic. We have probed such systems by studying consortia of E. coli strains with engineered single or double (91 strains) auxotrophies of 14 amino acids. Grown together, cocultures replicate metabolic exchange networks of natural microbial consortia where each member is only able to propagate in the presence of another consortia member that supplies the necessary metabolites. Amino acids are prevalent in natural metabolite exchange networks as seen in diverse symbiotic relationships: between higher metazoans and their associated endosymbionts (for example Aphids and Buchnera); between endosymbionts (for example Sulcia and Baumannia); and between organisms used in diverse bioprocesses (for example Streptococcus and Lactobacillus). We analyzed the growth dynamics of all 91 pairs of single auxotrophs and all 364 triplets of double auxotrophs as well as determined how coculture dynamics change as mutations develop. Lag period, growth rate, and maximum cell density of each coculture is dependent on the shared metabolites. The amino acids resulting in the highest cell densities were determined and selected for subsequent investigation of the benefits of aggregation in these cooperative communities. Aggregation strengthens metabolic interaction between cooperating strains. How it enables engineered consortia to resist invading cheater strains was studied. Again, the metabolites connecting consortia members were shown to modulate the relative fitness of aggregating strains over cheater strains. Long-term coculture growth experiments allowed mutations that strengthen or weaken the interdependencies between strains to develop. High-throughput sequencing of 60 isolates after 400 generations identified many mutations that could potentially be the cause of the observed shifts in population structure and dynamics. We hope that these findings will serve as building blocks to improve the forward engineering of microbial consortia. Future developments in this area have the potential to transform fields of medicine, bioprocessing and environmental engineering. Precise manipulation and control of community composition, capabilities, and dynamics will generate a suite of reconfigurable cellular modules that can help confront the health and environmental challenges of our day.

435B Disturbances as lever for managing biofilm morphology and community structure
Kim Milferstedt*, Gaëlle Santa-Catalina, Renaud Escudié, Jean-Jacques Godon, Nicolas Bernet
INRA-LBE, France

The management of microbial communities implies the availability of biotic or abiotic levers that can be used to navigate the development of the ecosystem. In our work, we tested how the frequency of chemical disturbances, that is the application of monochloramine pulse injections, can be used to maintain a biofilm community between two developmental states.
We exposed matured mixed-culture biofilms in bubble column reactors to several monochloramine pulses at daily or weekly intervals. One additional bubble column reactor served as untreated control. The development of biofilm community structure and biofilm morphology was monitored in all reactors. Molecular fingerprints of the bacterial communities were obtained from single-strand conformation polymorphisms (SSCP). Texture on stereomicroscopic biofilm images was analyzed with Spatial Gray Level Dependence Matrices (SGLDM) (Milferstedt et al. 2008). In SGLDM, the pairwise comparisons of graylevels in pixel pairs are used to extract textural features on the images. These are then related to biofilm morphology.

The undisturbed biofilm developed a steady morphology after two weeks into the experiment. In the same biofilm, the bacterial community continued to develop towards a more complex community structure with increasing –log Simpson diversity without reaching a steady state. A correlation between morphology and community development was not observed.

Weekly disturbances simplified biofilm morphology as well as the bacterial community. During the week after the disturbance, however, a resilient biofilm recovered its initial complex morphology and community structure. During the recovery of the biofilm, biofilm morphology and community structure were significantly correlated.

In contrast to the observed recovery between weekly disturbances, the intervals between daily pulse injections of monochloramine did not allow a recovery of the entire biofilm community. A high disturbance frequency selected for a strain from the genus Aquabacterium that gained dominance over the community. Biofilm morphology and community structure approached a steady state. We suggest that the dominance of this member of Aquabacterium is based on quicker regrowth than the remainder of the community once the biocide was washed out of the system. However, even the highly disturbed biofilm showed a resilient development of morphology and community structure within days after the treatment phase was terminated.

We demonstrated in our experiments that abiotic disturbances like carefully dosed biocide pulses can be used to actively manage the ecology of a biofilm ecosystem. This kind of control may allow us to produce and maintain for example a biofilm that has desired properties regarding mass transfer or community composition.

**436B How to choose the best inoculum for starting an anaerobic digester?**
Kim Milferstedt*, Aline Sandré, Jérôme Hamelin, Jean-Jacques Godon
INRA-LBE, France

The anaerobic degradation of complex organic substrates involves a network of coupled steps catalyzed by Bacteria and Archaea. Complete degradation to methane and carbon dioxide largely depends on the structure and composition of the microbial communities. The complete anaerobic foodweb is known in the engineering world under the term anaerobic digestion and is currently facing renewed attention in the field of bioenergy production. Anaerobic digesters are most frequently empirically inoculated with a microbial community that has proven to produce methane under the desired operating conditions. Whether this microbial community compared to communities from other natural or engineered ecosystems produces the highest methane yield or possesses otherwise more beneficial properties has rarely been demonstrated. It may be that a more educated selection of the inocula for anaerobic digesters can further improve process performance.

In our experiment, we therefore screen the metabolic potential of a wide range of inocula in a systematic and standardized procedure. All inocula are incubated with the same radiation-sterilized complex substrate containing cellulosic fibers and polysaccharides (20%), proteins (15%) and lipids (3.5%) suspended in a phosphate buffer at pH 7.5. We adapt the initial community to the complex substrate in semi-batch reactors over a period of at least 60 days at a dilution rate of 0.05/day. Several pulses of substrate at a sufficiently high substrate to microorganism ratio of 3 g substrate/g biomass (as volatile suspended solids) allow the development of an active adapted community towards the end of the incubations. The comparison of the initial and adapted communities by high-throughput sequencing will reveal on the molecular level the changes in the community during the adaptation phase. The adapted community structure is then related to ecosystem function (that is methane yield.
PS13 – Managing Microbial Communities

and production kinetics, volatile fatty acid composition) and serves as criterion to classify the microbial communities between highly productive and non-performing ecosystems.

By correlating properties of the microbial communities (for example distributions of rank-abundance or pairwise genetic distances) with the performance of ecosystem functions, we attempt to identify generally applicable molecular indicators that will be useful in the assessment of communities in managed microbial ecosystems but also when evaluating stability of ecosystem function in natural systems.

437B  Biopolymers productions by mixed aerobic cultures using crude glycerol as feedstock
Rita Moita Fidalgo*, Paulo Costa Lemos
Requimte, FCT/UNL, Portugal

Biodiesel is one of the most promising alternatives to petroleum-derived fuels. This fuel is produced from renewable source like vegetable oils or animal fats and can be used in conventional diesel engines with little to no major modifications to the engine. However, one of the problems regarding biodiesel production has to do with its major by-product, glycerol. Biodiesel production generates 10% (w/w) crude glycerol and since it cannot be disposed into the environment, the growing demand of biodiesel worldwide can cause the excess glycerol to become an environmental problem. Hence, the development of processes to convert low-priced glycerol into higher value products is therefore an excellent opportunity to add value to biodiesel production. Nowadays, glycerol is present in many applications in the cosmetic, paint, automotive, food, tobacco, pharmaceutical, pulp and paper, leather and textile industries. However it has also been considered as a feedstock for new industrial fermentations.

Polyhydroxyalkanoates (PHAs) are polyesters with similar properties to polypropylene but biodegradable, biocompatible and able to be produced from renewable resources. These biopolymers are stored inside the cells under stress conditions caused by limitation of a nutrient, electron donor or accepter, in the presence of carbon excess. Despite the effort for the development of less costly processes with pure culture fermentation, commercialization of PHA is mainly limited to added value applications. One strategy to develop more cost effective processes for PHA production involves the use of microbial mixed cultures combined with the utilization of low-value substrates, as agro-industrial waste and by-products.

The aim of this study was to evaluate the possibility to valorize crude glycerol into PHAs using mixed microbial cultures. The selection of PHA accumulating microorganisms was performed in a sequencing batch reactor operated under aerobic dynamic feed conditions. The reactor was inoculated with activated sludge from a wastewater treatment plant and fed initially with 30 C-mmol/day of glycerol. The crude glycerol used contains methanol (25% C mol/C mol) and glycerol (75% C mol/C mol). Initial condition led to a selection of different populations that accumulated PHA and glycogen with substrate yields of 0.60 and 0.30 respectively. Frequently monitoring of the population with Nile Blue A staining revealed two main populations, one able to accumulate PHAs and another one without this ability. Along the acclimatization time an increase in PHA biomass content was coincident with an increase of the populations presenting fluorescence. Glycerol consumption seems to be responsible for both accumulations since methanol is only lightly consumed when glycerol decreases to limiting concentration. An increase of the substrate concentration to 45 C-mmol/day of glycerol appears to increase the selective pressure of the system favoring the PHA accumulating in detriment of glycogen. High value products, such as PHAs, can result from the valorization of glycerol through microbiological fermentation.
Distinct and complex microbial communities in water-phase and biofilms of a wastewater bioreactor as revealed by barcoded 16S rRNA gene pyrosequencing

Jose A. Morillo-Perez1, Desiree Villahermosa2, Juan M. González2, Maximino Piñeiro-Vidal3, Emilio García-Robledo2, Sokratis Papaspyrou1, Waleed A. Al-Soud5, Søren J. Sørensen5, Jesus González-López1, Hauke Smidt6, Alfonso Corzo2
1Water Research Institute, Department of Microbiology, University of Granada, Spain, 2Department of Biology, Faculty of Marine and Environmental Sciences, University of Cadiz, Spain, 3Institute for Natural Resources and Agrobiology, IRNAS-CSIC, Spain, 4Institute for Marine Science of Andalusia, ICMAN-CSIC, Spain, 5Department of Biology, University of Copenhagen, Denmark, 6Laboratory of Microbiology, Wageningen University, Netherlands

Microbial sewage communities are a combination of human fecal and environmental microbes. However, the interactions between the water-phase microbial communities and those forming biofilms on the inner surfaces of the bioreactors are largely unknown. These biofilms are key components for the majority of wastewater treatment processes and their associated biological activities are important to achieve a good treatment performance. On the other hand, they can lead to the well known biofouling and corrosion problems. Thus, an extensive investigation of these key complex microbial communities is essential.

In this study, barcoded 16S rRNA gene 454 pyrosequencing of V3-V4 regions was used to profile simultaneously the water-phase and biofilm microbial communities in an experimental bioreactor. A continuous stirred-tank bioreactor (175 liters net volume) was fed with domestic wastewater during 4 cycles of wastewater treatment. Biofilms covered all submerged surfaces. Pyrosequencing of PCR-amplified bacterial and archaeal 16S rRNA gene amplicons obtained from 12 water-phase and 12 biofilm samples taken during reactor operation over a period of one year generated 291964 raw sequences. The analysis of the sequence data using Qiime 1.4.0 pipeline revealed highly diverse microbial communities (29 bacterial and one archaeal phyla detected), with the generation of in total 13817 OTUs (97% sequence identity cutoff). Principal coordinate analysis of generated OTUs and Unifrac showed a clear segregation between the water-phase and biofilm samples. Biofilm community was relatively unaffected by environmental changes and showed a higher microbial diversity as indicated by rarefaction analysis. Synergistetes (in average, 22.76% in biofilms vs 2.43% in water samples) and δ-Proteobacteria (20.74% vs 2.54%, mostly putative sulfate reducers including Desulfomicrobium and Desulfobulbus) were two groups clearly associated to biofilms. Synergistetes (strictly anaerobic Gram-negative rods that ferment amino acids) is a relatively rare and unexplored microbial phylum with an unknown and intriguing role in wastewater biofilms. Water samples were dominated by γ-Proteobacteria (30%), ε-Proteobacteria (22.87%), and β-Proteobacteria (11.98%). ε-Proteobacteria, probably of fecal origin, were almost undetectable in biofilms (<0.1%). The most abundant genus in water samples was Arcobacter, recently suggested as a good indicator of fecal contamination.

Overall, this study showed that wastewater bioreactors host rich microbial communities, that can differ substantially between different phases of the system, and whose composition and dynamics investigated using next-generation sequencing technologies will help for better understanding and optimization of waste water treatment plants.
lead to competition for substrates as well space within the single reactor system, that could result in imbalance regarding target community and structure. Many theories are proposed to explain the act of aggregation by microorganisms (aerobic or anaerobic), but the factors controlling it, are still not clear. Our aim is to impose certain operational actions to the single-reactor system based on changing substrate loading rates in order to control the biomass architecture and monitor aggregate evolution, bringing insight into aggregation and differences in architecture of these aggregates. The biomass architecture throughout operation are monitored with stereomicroscopic imaging, quantitative FISH coupled with CLSM, qPCR and laser diffractometry in order the combine physical descriptors such as size, fractal dimension, color with spatial distribution and abundance of different guilds within the biomass. The results combining qFISH, particle size distribution and stereomicroscope images upon increasing substrate loading suggest that the floccular light brown biomass with mixed composition has turned to large red compact aggregates of AnAOB covered by a diffuse layer of AOB, and a separate group of smaller white aggregates formed comprising mainly AOB and little NOB. Presence of DNB as implied by qFISH can be supported by qPCR analysis. Additionally, EPS composition analysis indicates that upon compaction soluble protein content has decreased while the soluble carbohydrate content remained on similar levels. Generic staining of EPS components implies proteins are majorly tightly-bounded within aggregate structure, and polysaccharides are loosely-bound around aggregates. Further changes in operational conditions to impose stratification will be applied which will help us evaluate the mechanisms behind aggregation and spatial segregation of different microbial guilds.

440B Monitoring the changes in marine aquaculture environment associated with the emergence of antibiotic resistance genes

Windu Muziasari1*, Antti Karkman1, Manu Tamminen2, Satoshi Managaki3, Ogo Mitsuko4, Satoru Suzuki5, Marko Virta6
1University of Helsinki, Finland, 2MIT, Massachusetts Institute of Technology, USA, 3Yokohama National University, Japan, 4Ehime University, Japan

Marine aquaculture production is increasing rapidly as a source of fish supply for human consumption. Consequently it introduces microbes, nutrients, and other chemicals such as antibiotics to the marine environment. The effect of aquaculture on microbial community had been reported. Also the effect of antibiotics use to be associated with the persistent of antibiotic resistances genes in sediment below aquaculture had been reported. The research goals are to monitor the changes in marine aquaculture environment and the emergence of resistant bacteria in fish supply. In order to reach the goals, bioavailability and concentration of oxytetracycline were measured using biosensor cells and HPLC respectively; sulfodiazine and trimethoprim concentrations were measured using LC/MS-MS; the quantification of the resistance genes was quantified using QPCR; and the expression of the resistance genes was monitored using Q-RT-PCR.

Sediment samples were collected from two medium sized of marine aquacultures and pristine areas in the northern Baltic Sea during summer time for six successive year. Bioavailability was low in every sampling time. Antibiotic concentrations were low in all sampling times except for oxytetracycline concentration in one farm sample of year 2011. However, the number of their antibiotic resistance genes was elevated in all farm samples but not in pristine samples taken from an area nearby. We concluded that the resistance genes are persistent in the sediments below marine aquaculture environment without selection pressures.

In future studies, we will track the source of the persistent genes by monitoring the expression of the resistance genes from fish guts and fish mucus using Q-RT-PCR. The results are to be used to improve the management of marine aquaculture environment.

441B Optimization of the attachment of bacteria on inert or biodegradable supports for environmental applications

Vizma Nikolajeva*, Zaiga Petrina, Tatjana Griba, Katrina Potapova, Andrejs Berzins, Olga Muter
University of Latvia, Latvia

Immobilization of microorganisms on inert or biodegradable supports allows to increase the stability of microbial communities. The main advantages of the use of immobilized cells in comparison with
suspended ones include the retention of higher concentration of microorganisms and protection of cells against toxic substances. However, alterations in cell growth and metabolism are induced by cell immobilization and biofilm formation. Moreover, addition of nutrients and combination of different types of carriers provide a broad spectrum of conditions for microbial activity regulation.

The aim of our study was to find out the most favourable conditions for bacterial attachment on the appropriate carrier for further development of different types of environmental biotechnologies, e.g., air and water biofiltration, soil remediation etc.

The model experiments with microorganisms and different types of carriers were done. Among the methods used in this study, the main approaches were as follows: (i) experiments with single microbial culture (Pseudomonas putida) and consortium (5 strains of Stenotrophomonas maltophilia and 2 strains of Pseudomonas spp.); (ii) short-term incubation or 2-3 days cultivation of bacteria in the presence of carrier; (iii) comparative study on inert and organic carriers for bacterial immobilization; (iv) performance of the model systems with biofiltration columns.

Bacterial attachment, viability and activity on the carrier were tested by microbiological plating techniques, biochemical (enzymatic activity) and chemical (total nitrogen, carbon) methods, as well as by microscopy (light, SEM, fluorescence). During the degradation experiments, a decrease of the contaminant concentration was measured. Adhesion of bacteria was carried out in the controlled batch and column experiments. The number of free-living and attached bacteria was monitored throughout incubation.

Experimental data showed the bacterial adhesion on the surface of all clay granules tested. The number of viable adhered and detached P. putida reached $10^3$-$10^5$ CFU per gram of Quaternary clay granules and about of $10^6$ CFU per gram of Devonian clay granules. However, the kind of granules markedly influenced the both, bacterial adhesion and viability. In biofiltration experiments with diesel vapours as a model contaminant, the performance of five biofiltration columns packed with rape straw and ceramic granules in different composition was compared. The hydrocarbon-degrading bacterial consortium MDK-EKO-7 was immobilized onto a packing material. During 44 days experiment, the removal efficiency for volatile hydrocarbons, saturation capacity time, compaction of the packing material as well as enzymatic activity of attached cells was estimated.

442B Evaluation of the microbial diversity in the São Paulo´s Zoo Park Foundation to obtain polyhydroxyalkanoates
Bruna Nunes Buscariollo*1, Vitor Ferrari1, Leonardo Jun Otuyama1, João Batista Cruz2, José G. C. Gomez3, Suzan P. Vasconcellos1, Patrícia L. Ramos1, Luiz Juliano Neto1, Rafael C. S. Rocha1
1Federal University of São Paulo - UNIFESP, Brazil, 2São Paulo’s Zoo Park Foundation, Brazil, 3University of São Paulo - USP, Brazil

Polyhydroxyalkanoates (PHA) are classes of polyesters produced by several groups of bacteria under unbalanced growth conditions as a mechanism to store excess carbon and energy. These polymers are thermoplastics with biodegradable and biocompatible properties and can be synthesized from renewable resources. The carbon source utilized by the bacteria has influence on the generated class polyhydroxyalkanoates: short chain length and medium chain length, each one presenting different thermoplastic properties.

São Paulo’s Zoo Park Foundation operates a Composting System - a self-sustaining, aerobic solid-phase biodegradative process of organic materials under controlled conditions, where organic residue from dead animals, native Brazilian Atlantic Forest (material with vast microbial diversity but only 1% of existing species formally identified), animal food and excrements, generate humus, which is later used as fertilizer on the foundation’s farm. A sustainable cycle is completed when the food produced on the farm returns to the zoo. As composting is rich in microbial diversity and still little explored in Brazil, it was decided to investigate the presence of polyhydroxyalkanoates producing bacteria in this material.

The material was harvested from the three composting phases and from that it was obtained 540 isolates. All the isolates were screened with Sudan Black B staining for their polyester accumulating
capacity in nitrogen-limiting medium using glucose as the single carbon source. The selected strains were cultivated in shaking flasks (erlenmeyer flasks of 250 mL at 30°C and 150 rpm) for posterior determination of total biomass, glucose consumption, PHA content and identification of isolates based on 16S rRNA sequences. 540 isolates bacterial were evaluated and 147 were selected to be studied in shake flasks. The identification by 16S rRNA gene sequences of these isolates showed that the most strains belong to genera: Bacillus sp., Staphylococcus sp. and Acinetobacter sp. Values were obtained around 40% (PHA content) for the most promising isolates. The medium chain lengths are the most abundant biopolymers detected using glucose as sole source carbon. The strains FPZSP514 and FPZSP336 (Pseudomonas sp.) showed until now better performance to obtain high concentrations of biomass and PHA content. The data presented here, indicates that the Sao Paulo's Zoo composting system is self-sustainable and represents an important site for bio-prospecting microorganisms with biotechnological potential to produce polyhydroxyalkanoates.

443B Comparative community genomics of San Juan basin coal beds
Antoine Pagé*1, Young Song1, Charles Howes1, Eugene Kuatsjah1, Nicole Sukdeo1, Brad Huizinga2, Dariusz Strapoc3, Steven Hallam1

1University of British Columbia, Canada, 2ConocoPhillips Corporation, USA, 3Dariusz BioGeoChem LLC, USA

Long considered an unconventional form of natural gas, coal bed methane (CBM) is becoming an increasingly important global energy resource. Although many biochemical processes and microbial players responsible for methane formation from biodegraded coal have been identified, little is known about the agents and processes responsible for organic matter degradation within coal seams. These initial degradation processes likely impose a rate-limiting step in CBM formation, and therefore constrain the productivity and sustainability of proven reserves. Here, we present a comparative community genomic survey of San Juan basin coal bed microbial communities aimed at better constraining the assemblages that are involved in CBM formation. Microbial biomass was obtained by filtering production waters from eleven wells with differing environmental parameters. Filtered biomass was used to extract genomic DNA for use in high-resolution V6 pyrotag sequencing targeting the small subunit ribosomal RNA gene. Multivariate analysis of V6 pyrotags identified three major clusters associated with differing regimes of temperature, metal or salt concentration. Representative samples from each cluster were selected for metagenomic sequencing, and resulting sequence datasets were annotated and compared to identify common and unique metabolic potential. PFAM-directed searches recovered biomarkers of pathways for coal solubilization and depolymerization, aromatic and aliphatic hydrocarbons anaerobic degradation, acetogenesis, and methanogenesis. These markers were not uniformly distributed across samples, correlating with overlapping but not identical community composition profiles. This study opens a functional genomic window into the metabolic potential of deep subsurface environments and provides an ecological blueprint for enhancing methane recovery from coal seams.

444B Proteolytic screening of isolated microorganisms from the composting process adopted at São Paulo Zoo Park Foundation
Suzan Pantaroto de Vasconcellos*1, Marghuel A. Silveira2, Rafael Costa Santos Rocha2, Débora Okamoto2, João Batista Cruz2, Maria Helena S. Cezari2, Luiz Juliano Neto2

1Federal University of São Paulo (UNIFESP), Brazil, 2UNIFESP, Brazil, 3São Paulo Zoo Park Foundation, Brazil

Microorganisms represent the largest source of genetic diversity in our planet. The prestige of the microorganisms is attributed to their great metabolic versatility, which allows inferring about their potential biotechnological applications, including the production of enzymes for environmental and industrial interests. The residue composting process performed at São Paulo Zoo can be considered as an important source of microbial diversity for biocatalytic uses. It is composed by animal and vegetable residues from the zoo, including material originated from Brazilian Atlantic rainforest. In this context, this study aims the investigation of the proteolytic ability of bacterial cultures isolated from the composting process of the São Paulo Zoo.

The action of proteases present in the isolated bacteria were detected by casein hydrolysis method, according methodology described by Rossi et al. (2007). Negative results were evaluated using methodology described by Oliveira et al. (2012), based on the cleaving of the fluorescent peptide
probe Abz-GXXXXQ-EDDnp. As results, it was observed that among 270 isolates, 91 strains showed proteolytic activity under casein agar. Four randomly selected microorganisms that showed negative results on solid medium, showed proteolytic ability under the fluorescent peptide. It is important to clarify that more assays based on fluorescence is running as a form to obtain more bacteria with proteolytic ability. For the moment, it is possible to confirm about the proteolytic potential of the microbiota originated from the São Paulo Zoo, which can be now described as a new source for bioprospection of new biocatalysts.

445B In-situ microbial activity in membrane-aerated biofilms performing autotrophic nitrogen conversion
Carles Pellicer*, Carlos Domingo, Barth F. Smets
DTU Environment, Technical University of Denmark, Denmark

Aerobic and strictly anaerobic ammonium oxidizing bacteria can grow symbiotically in biofilms as long as oxygen is limiting and does not completely penetrate the biofilm. Such biofilms can be used in bioreactors to treat wastewaters with high ammonium concentrations. The establishment of well-defined steep redox gradients allows then for completely autotrophic removal of nitrogen in a single reactor. Autotrophic nitrogen removal has gained attention over the last decade because of its low operating costs compared to traditional nitrogen removal technologies.

We have previously demonstrated that this process is also feasible in membrane-aerated biofilm reactors. Here, oxygen penetrates the biofilm through the membrane-biofilm interface while ammonium diffuses into the biofilm from the biofilm-liquid interface, creating unique counter-diffusion gradients. Given the superior oxygen transfer efficiency, we speculate that biofilms developed in MABRs can remove more pollutants per biofilm unit volume than conventional co-diffusion biofilms while accounting for lower energy, environmental and spatial footprints.

Our interest is on the technical performance of these biofilms reactors, but also on the ecophysiology and ecology of the stratified biofilm communities that develop on the membranes. Using lineage-specific oligonucleotide probes, we have shown that the communities on such biofilms are strongly stratified with aerobic ammonium oxidizing bacteria populating the biofilm base and anaerobic ammonium oxidizing bacteria dominating in the outer edges of the biofilm. Nitrite oxidizing bacteria are successfully outcompeted, and a layer of apparently inactive biomass forms a buffer between aerobic and anaerobic zones. Oxygen transfer can actually be enhanced due to biofilm covering of the membrane. In addition, we have seen that the gaseous N₂O intermediate is transiently produced and consumed within the same biofilm.

The present work involved the start-up, operation and microbial investigation of two membrane-aerated biofilm reactors to treat concentrated synthetic wastewater in complete autotrophic fashion. Two 800 mL reactor were equipped with 2 hollow fibre membranes each capable of supplying oxygen at rates up to 47 g/m²/day (air at 0.5 bar used as oxygen source). The medium in both systems was recirculated at 2 l/min in order to assure completely mixed conditions. pH and DO were continuously monitored with macroelectrodes placed in the recirculation line. Both reactors were inoculated with a previously enriched bacterial culture containing aerobic and anaerobic ammonium oxidizers. The reactors were fed continuously with synthetic wastewater (15 g-N/m²/day), and subject to periodic aeration (6h on/6h off) to minimize the competitiveness of nitrite oxidizing bacteria. Micro-electrochemical sensors capable of measuring relevant nitrogen species in wastewater environments (NH₄⁺, NO₂⁻, NO₃⁻, O₂ and N₂O) are used to assess (in-situ and in a non-destructive way) the ecological interactions and microbial activity within the biofilm. Ultimately, we will conclude on the successful practices to engineer spatially stratified microbial communities of N cycling bacteria.

446B Response of pig slurry sulphate-reducing community to additions of sulphate and volatile fatty acids
Pascal Peu*, Anne-Marie Pourcher, Patrick Dabert
IRSTEA, France

Changes in farming practices for pig livestock has paradoxically contributed to both the improvement of farmer working conditions (less manure management) and the increased risk of acute and chronic exposure to hydrogen sulfide. At present this risk is underestimated and uncontrolled by the profession.
despite the fatalities and long-term pathologies described in the literature. Hydrogen sulfide produced in these farms is the result of the anaerobic activity of sulfate-reducing microorganisms that degrade organic and mineral matter contained in manure. Currently few studies have been conducted to describe the microbial community in this environment.

The aim of this work was to study the abundance and diversity of these microorganisms in simulated storages of pig slurry supplemented or not with sulfate and volatile fatty acids.

Storage experiments were conducted from a pig slurry, collected from a storage pit on a farming unit. To simulate conditions of storage pit, various tests with or without addition of sulphate ($S$-$SO_4^{2-}$) and/or volatile fatty acids (6.5 g / L) were performed. Pig slurries were incubated in a water bath for 45 J at 20°C. For all these experiments, the slurry was sampled at the beginning and at the end of trials.

Sulphates, sulphides and volatile fatty acids were analyzed using liquid or gas chromatography.

Total bacteria were quantified by real time PCR technique with Sybergreen chemistry targeting the V3 region of 16S rDNA. The community of sulfate-reducers microorganisms was quantified using dsrA gene. Sulphato-reducers biodiversity analysis was produced by cloning-sequencing coupled with ARB sequence analysis software.

The main results of this study show that sulphate-reducing bacteria have a low abundance ($10^6$ to $10^8$ bacteria / g dry matter) compared to all the bacteria in the pig slurry ($10^9$-$10^{10}$ bacteria / g dry matter). Despite this low abundance, added sulphates are reduced by dissimilatory reduction and this reaction is coupled to the presence of volatile fatty acids. For all tests, the diversity of sulphate-reducing, analyzed through gene dsrAB, is low. For all pig slurries studied, eight operational taxonomic units were created. Three families are mainly present: the Desulfobacteriacea the Desulfobulbacea, and the Desulfovibrionacea. Much of the microorganisms identified are genetically distant from known sulphate-reducers bacteria to date. Finally, this study highlights the versatility of the community that adapts to the environment requires it.

447B  Enhanced natural attenuation of contaminated aquifers with iron oxide nanoparticles

Giovanni Pilloni*1, Carolin Meyer1, Armin Meyer2, Julian Bosch1, Sebastian Höss3, Heinrich Eisenmann5, Rainer Meckenstock1, Tillmann Lueders1

1Helmholtz Zentrum Munich, Germany, 2Isodetect GmbH, Germany, 3Ecossa, Germany

Microbially mediated degradation of hydrocarbons in aquifers mostly relies on electron acceptors other than oxygen. Amongst them, iron oxide is one of the most favourable, but often poorly available to microorganisms in situ. In the NanoSan project (http://www.isodetect.de/nanosan.html), we aim to establish a bioremediation strategy based on iron oxide in nanoparticulate form. These are applied to enhance the microbial oxidation of petroleum hydrocarbons in groundwater, via a stimulation of intrinsic iron reduction and a sustainable mobilization of bulk iron phases normally abundant, but accessible only at low rates to microbes in the subsurface.

Here, in a first phase, we investigate the stimulatory effects of iron oxide nanoparticles on iron-reducing BTEX-degradation in laboratory cultures and model systems, as well as the ecotoxicology of selected iron oxide nanoparticles on microbes and higher organisms (nematodes) in the subsurface. We combined quantitative balancing of iron species, microbial ATP production and contaminant stable isotopes with cutting-edge molecular tools to unravel the effects of iron oxide nanoparticles on a model organism (Geobacter toluenoxidans) and on sedimentary microbes in close-to-in situ mesocosms. Our results revealed that iron oxide nanoparticles can strongly enhance iron reduction and hydrocarbon degradation in batch cultures, and at the same time stimulate rearrangements of the microbial communities in model systems. While the total diversity and abundance of sedimentary bacteria remained unchanged with increasing concentrations of nanoparticles application, we observed a linear increase in pyrotag abundance of microbial populations well known for coping with metal stress. At the same time, we observed that the tested nanoparticles caused an increasing of general bacterial activity, determined as variation of ATP, for concentrations below 5 mM nanoparticles, and an inhibition above 16 mM. These thresholds for activity stimulation and inhibition from lab simulations are currently implemented in a field trial to demonstrate the use of iron oxide nanoparticle as remediation technology in situ.
**448B** Rpf protein from *Tomitella biformata* AHU 1821 increase the number of cultivable bacteria from permafrost ice wedge

Indun Dewi Puspita¹, Moe Uehara¹, Taiki Katayama², Michiko Tanaka¹, Wataru Kitagawa¹, Cindy H. Nakatsu³, Yoichi Kamagata¹, Kozo Asano¹

¹Hokkaido University, Japan, ²National Institute of Advanced Industrial Science and Technology, Japan, ³Purdue University, United States

Resuscitation promoting factor (Rpf) is a protein that promotes growth of active cells and resuscitation of non-dividing cells. The rpf gene homolog has been found in a number of *Actinobacteria* but the biological activity of the Rpf protein from only *Micrococcus luteus*, *Mycobacterium tuberculosis*, and *Corynebacterium glutamicum* has been confirmed. Our laboratory has previously reported the presence of an Rpf protein in *Tomitella biformata* AHU 1821, a novel *Actinobacteria* from permafrost ice wedge, and confirmed its biological activity in promoting growth of non-dividing *T. biformata* cells. We hypothesized that the Rpf protein from *T. biformata* can promote growth of bacteria from its original habitat. The objective of this study was to determine if addition of recombinant Rpf from *T. biformata* can increase the number of cultivable bacteria from permafrost ice wedge samples. An ice wedge sample was surface sterilized, melted, and serially diluted using 0.85% NaCl. The cloned rpf-gene from *T. biformata* was expressed and the recombinant Rpf (rRpf) protein was purified using nickel affinity chromatography, filter sterilized, and diluted in 0.85% NaCl prior to use. Melted ice and an rRpf fraction were mixed then spread onto agar medium in triplicate. Plates were incubated at 15°C for 2 weeks and colony growth was monitored. The number of CFUs was approximately 2-3 times greater (p<0.05) on plates inoculated with melted ice samples with the addition of rRpf than on control plates without rRpf. Colonies on plates amended with rRpf also appeared earlier forming many small colonies. These results indicate that the addition of exogenous Rpf promotes growth and resuscitation of cells in melted ice wedge samples. To our knowledge, this is the first report demonstrating an increase in colony formation from an environmental sample amended with rRpf.

**449B** Evaluation of the lipolytic activity of isolated microorganisms arising from composting process of the São Paulo Zoo

Patricia Locosque Ramos¹, Bruna Carolina Borim², Marghuel A. Silveira², Rafael Costa Santos Rocha², Bruna Nynes Buscariol³, João Batista Cruz², Luiz Juliano Neto², Suzan Pantaroto de Vasconcellos³

¹Universidade Federal de São Paulo, Brazil, ²Unifesp, Brazil, ³SPZFP, Brazil

São Paulo Zoo Park Foundation (SPZPF) processes by composting technique around 4 ton/day of organic waste comprising plant materials from the Atlantic Rain Forest, organic residues from animal feces, their carcasses, as well as waste from zoo water recycling process. The microbial biodiversity from this material is being explored by our research group, aiming the investigation of its metabolic activity under different substrates. In the present study, lipases were selected as target, due to their potential benefits as in industrial processes as in the environmental ones and, furthermore, the possibility of developing future technologies. Recent examples of its application are the treatment of fatty effluents and bioremediation, biocatalytic processes to biodiesel production, manufacturing of pharmaceuticals, food and supplies. In this sense, 11 different culture media, 2 temperatures (28 and 45 °C) and 3 dilutions (10⁻¹, 10⁻³ and 10⁻⁵) were adopted aiming the isolation of different bacteria genus from the composting process of the organic residues from SPZPF. For these inoculations, it was developed three sampling at the different phases of the process: (day 0) construction of the pile composting, (60 days) mixing of the pile, (90 days) end of the process. Assays for determination of the lipolytic activity of bacterial isolates from the composting process at SPZPF, were performed in Petri dishes containing tributyrin agar, according methodology described by Lee et al. (1998). The lipase activity was detected by the formation of a hydrolysis halo of the substrate around the bacterial colony, after the incubation of the assays at 30 °C during 7 days.

It was obtained a total of 597 bacterial isolates which were preliminarily characterized macroscopic and microscopically. All of these cultures were evaluated about their lipolytic ability and 564 strains were able to grow in the quoted substrate. Among these ones, 275 bacteria promoted the hydrolysis of tributyrin through halos between 0.5 to 5 mm, which proved the enzymatic activity of theirs. According to the obtained results, it was possible to verify the potential of the composting process developed at the São Paulo Zoo as a source of microbial communities with ability to lipase production for future biotechnological applications.
450B  Functional microbial communities involved in glucose and xylose fermentation: influence of pH
Charlotte Richard*1, Thomas Labatut1, Angeline Guenne1, Celine Madigou1, Laurent Mazeas1, Marielle Bouix2, Theodore Bouchez1
1Irstea, France, 2AgroParisTech, France

The regulatory and political context in Europe gives strong incentives for the development of biofuels transportation during the ten coming years. The environmental benefit of using first biofuels generation (agro-fuels) is however questionable. In this framework, the development of bioethanol from other organic resources such as household waste has been reported to be more economically and environmentally attractive. We are therefore working on the coupling of an ethanol production reactor to existing anaerobic digestion processes. Household waste being a complex and heterogeneous matrix, yeast fermentation would require energy intensive pretreatments. We have consequently focussed on ethanolic fermentation by complex anaerobic microbial communities which are able to cope with a wider range of substrates without pretreatment. In this study, we focus on the influence of pH, being reported in the litterature as a key element for ethanol production by mixed anaerobic cultures.

Experiments were carried out in batch with glucose or xylose under anaerobic conditions. A concentration of 4 g/L was used for the two sugars. We have tested six different pH, in triplicate (ranging from 4.5 to 7). Furthermore, 13C-labelled sugars were used: at pH 5 and 7 for glucose and at pH 7 for xylose in order to clearly identify chemical intermediates and microorganisms implicated in ethanol production. Isotopic analysis of biogas were performed using a GC-C-IRMS and isotopic analysis of VFAs and ethanol using a GC-MS. Stable-isotope probing (SIP) method, which relies on the incorporation of a heavy isotope into nucleic acids, coupled with ARISA (Automated Ribosomal Intergenic Spacer Analysis) was used to monitor microbes potentially involved in the process. In order to clearly identify microorganisms we have made pyrosequencing on DNA samples obtained thanks to the SIP method.

We have shown that the production of ethanol was possible from glucose and xylose. For each sugar, the greatest ethanol concentration was obtained at pH 7 at day 2: around 500 mg/L which represents 12.5% of the initial carbon. From day 3, ethanol is degraded and at day 7 no ethanol can be quantified anymore. We have also observed that ethanol degradation is slower for pH under 6. Moreover, we have observed three different profiles depending on pH for glucose fermentation and for xylose fermentation the bacterial populations seem to be selected not by the pH but by the substrate. Pyrosequencing of heavy DNA fractions recovered after SIP enabled the identification of microorganisms involved in ethanol production and degradation. This knowledge could be very useful to adjust parameters to optimize ethanol production and to inhibit its degradation.

Ethanol production from simple sugar is one step of the development of biofuels from household waste. Experiments are in progress to study optimal pH operation strategies, evolution of microbial populations and their functionalities.

451B  Methanotrophic bacteria in oilsands tailings ponds of northern Alberta
Ali Saidi-Mehrabad, Zhiguo He, Ivica Tamas, Christine Sharp*, Allyson Brady, Peter Dunfield
University of Calgary, Canada

Canadian oilsands, primarily located in northern Alberta, represent one of the world’s largest petroleum reserves, estimated at 170 billion barrels. Due to the highly viscous nature of the oil, mining requires extraction with chemical solvents and 15-20 volumes of hot liquid water per volume of oil extracted. This process produces large amounts of tailings comprised of water, silt, clay, residual bitumen, and solvents. This waste water is pumped into large ponds (up to 10 km²), which are often strongly methanogenic. One pond can produce as much as 100 million L of CH₄ per day. As the particulate matter settles, a surface aerobic water layer forms on tailings ponds. We hypothesized that aerobic methanotrophic bacteria should be present in this layer and may reduce potential methane emissions.

We investigated methanotrophic bacteria in surface water of two oilsands tailings ponds. The water is slightly alkaline (pH 7.5-8.8) due to the use of caustic soda in the extraction process. Aerobic methane
oxidation activity was measured over two years, at rates up to 300 nmol CH₄ ml⁻¹ water d⁻¹. Extrapolation over the entire surface of the lake suggests that the methane oxidation rate is similar to the methane efflux rate. Microbial diversity of the surface tailings water was investigated via pyrotag sequencing of amplified 16S rRNA genes as well as of methanotroph-specific pmoA genes. The predominantly detected methanotroph in all tailings ponds at all sampling times was an uncultured species belonging to the Methylocaldum/Methylococcus group of gammaproteobacteria, although a few other methanotrophs were also detected, including Methylophilus. In contrast, the primary methanotrophs detected in natural oilsands outcrops of the region were related to Methyllobacter and Crenothrix. Active species in the tailings were identified via ¹³C₄ stable isotope probing (SIP) of DNA, combined with pyrotag sequencing and complete shotgun metagenomic sequencing of the heavy ¹³C-DNA fractions. The SIP results demonstrated that the Methylococcus/Methylocaldum and Methylomonas strains were primarily responsible for the consumption of methane. Metagenomic analysis of DNA from the heavy fractions verified the PCR-based results and identified additional pmoA genes not detected via PCR. The metagenome suggested that the active community may possess only particulate methane monoxygenase, not soluble methane monoxygenase.

The results demonstrate that the tailings ponds select for a particular methanotrophic community that is not predominant in surrounding natural ecosystems. Methanotrophs may have a large ameliorating effect on methane emissions from these sites, and their activity could probably be stimulated by proper management strategies.

452B Metaproteogenomic insights beyond bacterial response to naphthalene exposure and bio-stimulation

¹UFZ-Helmholtz Center for Environmental Research, Germany, ²CSIC, Institute of Catalysis, Spain, ³Facultad de Medicina, Universidad de Oviedo, Spain, ⁴CSIC, Centro Nacional de Biotecnología, Spain, ⁵Área de Prospección e Investigación Minera, Universidad de Oviedo, Spain, ⁶HZI-Helmholtz Center for Infection Disease, Germany, ⁷CSIC-UIB, Spain

Polyaromatic hydrocarbons (PAH) have a global impact to microbial metabolism due to their occurrence in natural and anthropogenic processes. Thus understanding of the PAH degradation and metabolism remains a major goal. We performed a thorough and holistic (or eco-systems biology approach) phylogenetic, functional and proteomic analysis of the key players in two samples of strongly anthropogenic influenced, PAH-contaminated soil with (Nbs) or without (N) bio-stimulation. In addition, two naphthalene-enriched communities were investigated: one derived from soil N (CN1) and one derived from soil Nbs (CN2). Phylogenetic analysis was performed by denaturing gradient gel electrophoresis (DGGE) and 16S rRNA gene sequencing, and metagenome sequences were obtained by 454 pyrosequencing of each sample. Metaproteomic analyses based on GeLC-MS/MS measurements and label-free quantification were done for sample CN1 and CN2 using the respective metagenome sequences as data base.

The present OMIC investigation provided evidence that the two enrichment cultures maintained an overall stable function and metabolic potential, whereas their phylogenetic compositions fluctuated. Moreover, the results also indicated distinct biodegradation capacities for the utilisation of potential growth-supporting aromatic compounds, which results in the communities being extremely fit to naphthalene exposure and bio-stimulation. Based on comparing protein expression profiles, inter alia interactions among members of the communities were revealed, that demonstrated bacterial communities may exhibit greater biodegradation plasticity than previously predicted. To the best of our knowledge, no degradation network for a complex microbial community has been constructed to date, mainly due to the lack of appropriate databases containing functional information. This problem will be discussed here by reconstructing the metagenomic-derived potential aerobic degradation networks of aromatics via di- and trihydroxylated intermediates in all the investigated communities by using curated databases containing protein sequences with biochemical functions shown to be involved in biodegradation.
Tuesday 21 August

This study clarified the genomic and proteomic basis for the purpose of understanding microbial biodiversity, ecology and function in response to both PAHs (represented by naphthalene) and bio-stimulation.

453B  How immigration might alleviate the effects of an influenza pandemic: a freshwater microbiology story
Andrew Singer1, Katja Lehmann2, Thomas Bell3, Michael Bowes1, David Bass4, Dawn Field1
1NERC - Centre for Ecology & Hydrology, United Kingdom, 2University of Oxford, United Kingdom, 3Imperial College London, United Kingdom, 4Natural History Museum, United Kingdom

Exposure to pharmaceutical pollution originating from sewage effluent is an unavoidable component of freshwater microbial life. Despite this ‘home truth’, the extent to which these pollutants influence the composition and function of freshwater microbial systems remains unclear. Tamiflu is an antiviral with unprecedented projected use patterns during an influenza pandemic. The impact this novel bioactive drug will have on the resilience of freshwater microbial communities remains unexplored. Novel in-river mesocosms that allow for the replication and manipulation of river conditions were used to investigate the effect of Tamiflu on periphyton biofilm in a relatively pristine UK chalk river. Biofilm DNA from Tamiflu exposed and unexposed communities were amplified and sequenced on the 454 titanium sequencing platform in triplicate. The biofilm communities, as defined by the 16S rDNA, were resistant to change in the presence of this bioactive pharmaceutical from the perspective of overall community composition and diversity. However, a component of the microbial community, Gamma- and Beta-proteobacteria, did exhibit a shift in composition and diversity. Analysis of the metagenome by 454 provided insight into the potential interactions between the antiviral, bacteriophage and bacterial community composition. Unlike the field mesocosm study, a laboratory microcosm study showed significant changes between Tamiflu-exposed freshwater bacterial communities and the unexposed controls. The results suggest that an open freshwater system might appear to be more resilient to change as compared with the closed laboratory microcosm environment owing to the masking effect of immigration, whereas any ‘antiviral-induced’ change in the microbial community is made apparent in the closed, laboratory microcosm system. Hence, despite the realism provided by the in-river mesocosms, the batch microcosm studies might be more instructive in identifying acute ecotoxicity, whereas the field mesocosms are more insightful into any chronic effects from pollutant exposure. In conclusion, in the absence of a ‘healthy’ seeding microbial community, sewage-impacted rivers might be negatively impacted by the medical response to an influenza pandemic.

454B  Bioremediation of petroleum contaminated soil
Dr. Padma Singh*, Richa Saini, Shreyasri Dutta
Gurukul Kangri University, India

The ability to isolate high number of certain oil-degrading microorganisms from petroleum-contaminated environment is commonly taken as evidence that these microorganisms are the active degraders of that environment. For Oil spilled soil being the most probable source for hydrocarbon utilizer, was collected from a place where the surface soil contaminated by petroleum. Bioremediation of petroleum and other hydrocarbons in the environment is a complex process, whose quantitative and qualitative aspect, depends on the nature and amount of the oil or hydrocarbons conditions and microbial community. After collecting petroleum contaminated soil from garages, the total population of bacteria Actinomycetes and fungi were isolated by growing on NAM (bacteria), GYE (actinomycetes) and CDA (fungus) by serial dilution method. The result of present study indicated that total population of Bacteria, Actinomycetes, and Fungi. After purification, distinct colonies were observed and identified colonies resembling strains of Bacillus, Micrococcus, Streptomycies, Actinomycetes, Nocardia, Fusarium, Trichthecium, and Aspergillus. Evidences from the other scientists work upon these strains, indicate that Bacillus sp. Isolated from petroleum contaminated soil was potent degrader and capable of utilizing different fraction of hydrocarbons but actinomycetes also comprises a large and diverse group of largely mycelial bacteria, many of which are important ecologically and are exploited commercially for bioremediation. Despite these fungi isolated from soil and their capability was assessed to degrade petroleum hydrocarbon, so their potential use could be done in terms of bioremediation. Thus out of the eight species, five species were isolated and identified as hydrocarbon degrading species. These are- Bacillus, Micrococcus, Fusarium, Aspergillus, and Nocardia are proved from past research findings and can be exploited in hydrocarbon bioremediation to check the pollution caused by oil spilled in garages. Hence bioremediation could be used to attack specific contaminants.
such as petroleum products etc which are degraded by bacteria by using multiple technique to save the environment.

455B  **Microbiomics towards improved intestinal health and function in monogastric farm animals**
Hauke Smidt*
*Wageningen University, Netherlands*

Soon after birth the gastrointestinal tract (GI) of humans and other monogastric animals is colonized by a myriad of microbes, which are collectively called the GI tract microbiota. This microbiota plays an important role in the host’s health and nutrition and is characterized by its wide diversity. Despite all the efforts in improving the cultivation of novel GI tract microbes, the use of culture-independent is crucial to get a comprehensive picture of the GI tract microbiota. Since the introduction of culture-independent approaches, mainly those based on 16S ribosomal RNA (rRNA) and its encoding gene, GI tract ecology has experienced a revival. These culture-independent approaches gave insight into the temporal, spatial and inter-individual microbial diversity in the GI tract of humans and animals. The past years major developments have been made in high throughput methodologies to characterize microbial communities. Novel technologies, such as barcoded pyrosequencing of 16S rRNA genes, as well as phylogenetic fingerprinting using DNA microarrays such as the Human, Pig and Poultry Intestinal Tract Chips have recently been described. Since multiple samples can be analyzed in detail in a rather short time, these approaches offer great potential in finding significant correlations between the GI tract microbiota composition and the health status of the host. In addition, we have applied metatranscriptome analysis of intestinal digesta samples in order to provide detailed insight in the functional properties of the GI tract microbiota at a given point in time and space in the animal’s intestinal tract. The application of these approaches to understanding the interplay of intestinal microbiota with intestinal functioning and immune system, and production animal health, in response to the production environment as well as dietary additives, can provide the necessary knowledge for the development of innovative nutritional strategies towards more sustainable animal production. Examples will be provided from current research on various production animals, including the pig, and an overview of the current state of the art of microbiomics research will be given, integrating datasets with those focusing on assessing the developing immune system as well as intestinal physiology, using advanced multivariate statistical approaches.

456B  **Variance calculations for quantitative real-time polymerase chain reaction experiments with multiple levels of replication**
Susana Soto Rojo*,1, Gary Glonek1, Cecilia Demergasso2, Patricia Solomon1
1The University of Adelaide, Australia, 2Universidad Catolica del Norte, Chile

Heap bioleaching is an established technology for recovering copper from low-grade sulfide ores. However, only recently genetics-based approaches have been employed to characterize mineral-processing bacteria. In these approaches data analysis is a key issue. Consequently, it is of fundamental importance to provide adequate mathematical models and statistical tools needed to draw reliable conclusions.

The present work relates to current metagenomic studies of the consortium of organisms inhabiting the bioleaching heap of Escondida mine in Northern Chile. These studies aim to describe and understand the relationship between the dynamics of the community and the industrial process. We consider a series of quantitative real-time PCR experiments performed to quantify nine different microorganisms at various stages of the bioleaching cycle. A relevant question that arises when establishing the reliability of the data obtained from these experiments, is: will the use of PCR and/or extraction replicates result in a significantly smaller error variance than not having replicates at all? Answering this question requires careful modelling and estimation of the error variance at several different levels. Three different sets of data and multiple linear regression analysis per microorganism were used to estimate the pertinent components of variance. These values were needed to calculate an estimate for the proportional reduction in residual standard deviation from the use of extraction replicates. The estimates determined for the proportional reduction in residual standard deviation range from 1% to 12% across the different microorganisms. It was concluded that extraction replicates would produce only a modest reduction in the error variance.
Culture independent techniques have become important tools to understand environmental microbial ecology. Quantification of rRNA and rRNA genes is one of the big concerns. Many researchers use quantitative (reverse transcription) real-time PCR; nevertheless, one unfavorable point of this technique is known as PCR biases. Therefore, a method enabling direct quantitative detection of rRNA molecules without PCR amplification is desired. Here, we developed a novel rRNA direct quantitative detection method, which uses molecular weight cut-off membrane (MWCOM). MWCOM is used to separate rRNA (large molecules) and fluorescent-labeled oligonucleotide probes (small molecules).

We first confirmed the proof of concept, and then quantitative performance of this method was evaluated. Furthermore, applicability of the method to extracted RNA from complex microbial communities was demonstrated using anaerobic sludge samples.

The concept of the novel method is as follows: it employs fluorescent-labeled DNA oligonucleotide probes and MWCOM, which cuts off 100 kDa. Large molecules of rRNAs (for example 500 kDa for 16S) are trapped onto the membrane, whereas small molecules of probes (usually less than 8 kDa) pass through the membrane. When probes are hybridized with rRNA, apparent molecular weight of the probes becomes larger, and then, the probes hybridized with rRNA remain on the membrane. The molecules trapped on the membrane were recovered as solution, and then finally fluorescent signals derived from probes were measured by fluorospectrometer. The relative abundance of the specific rRNA can be estimated by calculation of each fluorescent dyes intensity ratio.

At first, a membrane passage rate of probes and RNA recovery rate from a membrane were evaluated. More than 99.5% of probes passed the membrane whereas more than 70% of the RNA was recovered to a solution, indicating the separation of rRNA and probes can be done. Subsequently, specific probes were mixed and hybridized with synthesized RNA, and specifically detected. This result proved our concept. High quantitativity of the method is also demonstrated by high linearity ($R^2=0.99$) between the experimentally obtained values and the theoretical values. Furthermore, this method can detect only 5 ng of targeted rRNA. This sensitivity is comparable to other direct RNA detection methods. Quantitative detection of 16S rRNA of sulfate reducing bacteria (SRB) in biological wastewater treatment processes was carried out. The relative abundances of the SRB’s 16S rRNA were 25.9±1.7% (n=3) in an anaerobic digester sample and 10.0±0.6% (n=3) in an up-flow anaerobic sludge blanket sludge sample. These results indicate high reproducibility of the method. Advantages of this method are as follows; (i) direct rRNA quantitative detection, (ii) simple and quick (within 3 hours including RNA extraction) and (iii) no need of external RNA standard. Furthermore, multiplex detection will be applicable by using plural probes at once.
structure. In order to visually display the community evenness, Lorenz evenness distribution curves were used.

The application of three selective stressors (that is oxygen (O), cell retention (R) and pressure (P)) drove significant variation of microbial populations and species distribution in biofilms. Intriguingly, the degree of such distribution was highly relevant to biofilms growing rate. For the evenly distributed biofilms, e.g. the ones under the selective stressor of O1 and P1 (Gini coefficient $f·d^{-1}$). By contrast, two most uneven communities (under the selective stressor of R2 and R1) had the slowest pressure changing rate (less than 0.6 $P·d^{-1}$). This result strongly demonstrates that an evenly distributed community favors the formation of biofilms. In other words, the biofilms with more even distribution of species can gain a stronger expanding capacity. A community that is highly functionally organized is usually vulnerable to environmental changes and unevenness makes a microbial community behave lower functionality, and such effect is more significant when under environmental stresses. Since biofilm growing closely links to microbial functionality, it is understandable that biofilm formation is also governed by community evenness.

Our study reminds us of not ignoring the egalitarianism of microbes if to study on biofilm. For examples, for some biofilm-based wastewater treatment technologies, we need to maintain the system had high evenness of microbial community. However, for a membrane bioreactor (MBR), which is one of the most promising technique of the future, is stumbled by membrane fouling, which is highly relevant to unwanted biofilm formation (or biofouling), we need to construct an unevenness community in MBR to slow down biofilm-forming process. Hence, from an ecology perspective, biofilm formation could be controlled based on the evenness of microbial community.

459B  A novel method to characterize biodegradable organic matter in reclaimed water using bacterial growth fingerprint
Parinda Thayanukul*, Futoshi Kurisu, Ikuro Kasuga, Hiroaki Furumai
The University of Tokyo, Japan

Microbial regrowth in reclaimed water distribution system and storage is one of the important concerns for wastewater reuse as it causes aesthetic quality deterioration and occurrence of opportunistic pathogen. Biodegradable organic matter (BOM) in reclaimed water that supports microbial regrowth must be controlled together with disinfection. Thus, it is necessary to elucidate content and composition of BOM to improve the removal of the compound in water reclamation treatment. We developed a novel method to characterize BOM in reclaimed water by using bacterial isolates obtained from wastewater.

Reclaimed water samples were collected from six water reclamation facilities in Japan. Almost 200 colonies were obtained from the samples incubated with R2A agar. Terminal restriction fragment length polymorphism (T-RFLP) was introduced to eliminate repetitive strains. Partial 16S rRNA gene sequences of 98 isolates were analyzed, and bacterial isolates were categorized into 35 operational taxonomy units (OTUs, < 3% P-distance pairwise comparison). Substrate utilization patterns of 35 isolates were assayed with BIOLOG GN2 MicroPlate™. Then, growth profiles of representative isolates were evaluated in three kinds of reclaimed water with the addition of inorganic nutrients. Initial concentrations of the isolates were approximately 1000 cells/mL and cell numbers were enumerated with a flow cytometer. These reclaimed water samples were produced by the three water reclamation plants with different processes: (A) coagulation followed by sand filtration, ozonation, and chlorination, (B) pre-chlorination followed by sand filtration, (C) biofiltration followed by ozonation, coagulation, microfiltration, and chlorination.

Substrate utilization of the 35 bacterial isolates were classified into 6 categories comprising of bacteria that preferentially utilized (1) polymers, (2) esters and amides, (3) carbohydrates and alcohols, (4) polymers and amino acids, (5) amino acids and carboxylic acids, (6) carbohydrates and amino acids. On the other hand, the 35 bacteria were categorized into 7 types by growth profiles in the three reclaimed water samples. One-third of bacterial isolates for example; Pseudomonas sp. and Methylobacter sp., grew well in all reclaimed water samples. These bacteria might utilize organic compounds commonly present in reclaimed water. Another one-third of isolates grew to the highest level in reclaimed water from process B in which only sand filtration was used as tertiary treatment. The bacteria in this group, such as Sphingomonas sp. and Riemerella sp., might utilize organic
compounds which could be removed by other treatments such as ozonation. Bacterial isolates grew differently in different samples. The results indicated that the amounts of substrates used for the growth of a particular strain were different among the samples, probably due to the different composition of organic matter in the samples. The difference in composition could be mainly derived from different treatment processes. Bacterial growth fingerprint analysis has potential to characterize BOM in reclaimed water.

**460B  Seasonal variation of fecal indicators in a drinking water reservoir**

Hsin-hsin Tung\(^1\), Chia-Chen Wu\(^1\), Hao-Yu Lo\(^1\), Yung-Hao Ching\(^2\), Pei-Te Chiu\(^1\)

\(^1\)National Taiwan University / Inst Environmental Engineering, Taiwan, \(^2\)Tzu Chi University, Taiwan

The Feitsui water reservoir located in northern Taiwan is the raw water supply for Taipei metropolitan area which has a population of 5 million. High coliform concentrations were frequently detected in the reservoir or its influents. From previous studies, it was found that fecal pollutions might be different in each season due to different farming/tourism activities. The objective of this study was to monitor the fecal indicators in the Feitsui water reservoir by both traditional cultivation methods (Coliform, fecal coliform, *Escherichia coli* and fecal streptococcus) and host-specific PCRs. The project used different host specific PCR primers which identified fecal sources such as chicken, ruminant, swine, deer, and human. To assess the water quality of the river basin and reservoir, water samples from the region were collected and analyzed seasonally. The results showed that high total coliform concentrations were detected in some samples of Beishi River basin and Feitsui Reservoir during the course of study even without any fecal sources detected by host-specific primers tested. The results from host specific PCRs showed that human fecal pollution was constantly detected in the downstream of highly populated area. Chicken and deer fecal were also detected in some sites of Beishi River and Feitsui Reservoir. In the fall season, high concentrations of total coliforms were detected in Feitsui reservoir after heavy rainfall and agricultural activities within the watershed. However, the water parameters such as total organic content, nitrogen and phosphorous concentrations remained similar during the coliform surge. This high total coliform concentration surge occurred almost every fall season in Feitsui Reservoir which requires further investigation.

**461B  Impacts of Oxytetracycline on Performance of Thermophilic Anaerobic Manure Digesters and Active Microbial Population**

Gokhan Turker\(^1\), Orhan Incé\(^2\), Fulya Suzen\(^2\), Emine Ertekin\(^1\), Bahar Incé\(^1\)

\(^1\)Bogazici University, Turkey, \(^2\)Istanbul Technical University, Turkey

Antibiotics used in veterinary practice are poorly metabolized and excreted within manure; hence posing a threat to biogas production from substrates as such, for they are possibly detrimental to microorganisms. In this study, effects of a commonly used veterinary antibiotic, oxytetracycline, on biogas production in thermophilic anaerobic digestion of cattle manure was investigated. Effects of changing operational parameters such as mixing rate and solid content on biogas yield, active microbial population and elimination of oxytetracycline was also studied. In this study, two sets (Set 1 and Set 2) each containing four digesters (D1, D3, D4 and D5) were set with both blank (as control) and medicated cow manure. For both sets thermophilic conditions (55°C ±1) were maintained, with 90rpm mixing rate and two different total solid content (5-5.5% and 7.5-8% TS content) for Set 1. Set 2 was copy of Set 1 except mixing rate which was 120rpm. Seed from a lab-scale manure digester was added at a ratio of 1:10. Hydraulic Retention Time was set to 20 days for both Sets and samples were taken for analytical, molecular and physicochemical analysis for every 5 days. Digesters were monitored for biogas production, total solid reduction, biogas and volatile fatty acid concentrations. In this study, maximum biogas yields of 132-134 L/kg TVS were found in control digesters of both Sets. Results showed that mixing rate did not affect biogas production performance significantly. Inhibitions in cumulative biogas production after 20 days were between 10-18% for the digesters containing oxytetracycline concentrations of 1.5-4.7 mg/L. Maximum total solid reduction reached to 30% at the end of 20 days digestion period. Although acetic and propionic acids were dominantly detected in slurries; their concentrations were not at inhibitory level at the end of 20 days. Oxytetracycline concentration showed a decreasing trend during operational time and half life of OTC was evaluated to be 14 days. Dynamics of active populations were investigated by FISH and RNA based Q-PCR. According to FISH results, *Methanobacteriales* was most abundant methanogen in all digesters as reaching 48%. According to Q-PCR results, bacterial, *Methanomicrobiales* and *Methanoseta* gene copy numbers decreased with operational time in all digesters. *Methanobacteriales* and
**Methanosarcinales** gene copy numbers decreased in Set 1 digester (90rpm) and increased in Set 2 digesters (120rpm). Both activity and gene copy number of *Methanoseta* spp. was low in all digesters suggesting methanogenesis was performed mainly by *Methanosarcinales* spp. and hydrogenotrophic archaea like *Methanobacteriales* and *Methanomicrobiales*.

**462B  Biofilm based bioaugmentation strategies for the treatment of pesticide waste streams**

Pieter Verhagen*, Leen De Gelder, Nico Boon

*University College Ghent, Belgium*

Bad management of pesticides in agriculture during the past decades has given rise to pesticide residues in surface and groundwater. 40 to 90% of the surface water contamination by pesticides can be attributed to direct losses such as spillages resulting from the filling operation, leakages of the spray equipment and technical rest volumes in the tank, pump and booms. Unlike diffuse contaminations, these point source contaminations can be easily collected for treatment. Biological treatment in on farm bioremediation systems could be a cheaper and equally efficient alternative to physico-chemical treatment options. Pesticide biodegradation can be started up or enhanced in these systems, by inoculating them with a suitable pesticide degrading bacterial culture. The performance of these bioaugmented reactors depends on the survival, maintained activity and retention of the inoculated microorganisms inside the reactor. Biofilms could be suitable mediators of bioaugmentation due to the retention of immobilized cells and the protection offered by the biofilm matrix.

In the first part of our study, we obtained chloropropham degrading cultures from sludge and soil samples through two different enrichment techniques i.e. (i) planktonic enrichments in shaken liquid medium and (ii) biofilm enrichments on two types of solid matrixes (plastic chips and gravel). Although the planktonic and biofilm cultures were derived from the same source, community fingerprinting showed that their microbial community composition had a different composition. The presence as well as the type of the added solid matrix during enrichment seemed to affect the enrichments microbial diversities. This was reflected in the unique chloropropham degrading species that could be isolated from these different cultures. Chloropropham degrading activity was also affected by the presence of a solid matrix during enrichment. Biofilm enrichment cultures removed chloropropham more slowly compared to their free suspension counterparts, but less build-up of the toxic intermediate 3-chloroaniline was observed. Disruption of the biofilm architecture resulted in degradation characteristics shifting towards those of the free suspensions, indicating the importance of a good biofilm structure for good performance. These results show that biofilm mediated enrichment techniques can be used to select for pollutant degrading microorganisms that like to proliferate in a biofilm and that cannot be isolated using the classic shaken liquid procedures. Furthermore, the influence of the biofilm architecture on pesticide degradation characteristics suggests that for bioaugmentation the use of biofilm catabolic communities might be a proficient alternative to using planktonic freely suspended cultures.

In a second part of our study, chloropropham degrading biofilm cultures were used to inoculate on farm bioremediation systems. Spatial differences as well as differences between inoculated and non-inoculated columns in chloropropham removal rate and in chloropropham and 3-chloroaniline community composition were investigated. Our results demonstrate the spatial heterogeneity in degradation characteristics and microbial community composition in on farm bioremediation systems and show that the use of biofilm bioaugmentation can significantly shorten the start up period needed to obtain efficient degradation.

**463B  Changes of gut microbiota associated with obesity, insulin resistance and inflammation in high fat diet-fed mice**

Jingjing Wang*, Hang Tang, Chenhong Zhang, Yufeng Zhao, Liping Zhao, Jian Shen

1State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, China, 2Key Laboratory of MOE for Microbial metabolism and School of Life Science & Biotechnology, Shanghai Jiao Tong University, China, 3Shanghai Centre for Systems Biomedicine, Shanghai Jiao Tong University, China

Alterations in the gut microbiota are linked to obesity and insulin resistance, and lipopolysaccharides of gut bacteria provoked inflammation. Previous studies showed high fat diet could change the gut microbiota composition, modify host inflammatory tone, and lead to metabolic syndrome, but the HFD-
induced bacterial changes that are closely associated with obesity, insulin resistance and inflammation remain unclear. Here, C57BL6 mice were respectively fed on high fat diet and normal chow diet for 12 weeks. High fat diet significantly induced obesity, insulin resistance, systemic inflammation and local inflammation in epididymis adipose tissue, liver and jejunum. Principal component analysis and weighted UniFrac analysis based on the 454 pyrosequencing data of fecal bacteria 16S rRNA genes showed that high fat diet changed the overall structure of gut microbiota. Redundancy analysis revealed high fat diet decreased the abundance of lactate and short chain fatty acid (SCFA)-producing bacteria, including Allobaculum, Coprococcus, Bifidobacterium, Olsenella and Porphyromonadaceae, and increased Desulfovibrio, Alistipes, Oscillibacter and Lachnospiraceae_incertae_sedi. According to the spearman correlation analysis, the bacteria reduced and enriched by high fat diet were negatively and positively, respectively, correlated with obesity, insulin resistance and inflammation. Our results suggest that high fat diet may cause obesity, insulin resistance and inflammation at least partially by altering gut microbiota, especially by reducing some lactate and short chain fatty acid (SCFA)-producing bacteria.

464B A gut microbiota-targeted, dietary intervention for amelioration of chronic inflammation underlying metabolic syndrome
Shuiming Xiao*, Na Fei, Xiaoyan Pang, Jian Shen, Linghua Wang, Baorang Zhang, Menghui Zhang, Xiaojun Zhang, Chenhong Zhang, Min Li, Zhengsheng Xue, Jingjing Wang, Jie Feng, Jiaqi Liu, Wenmin Long, Liping Zhao
Shanghai Jiao Tong University, China

Accumulating evidences support that low-grade chronic inflammation induced by diet-disrupted gut microbiota contributes to the development of obesity and related metabolic disorders. A dietary scheme based on whole grains, prebiotics and traditional Chinese medicinal (TCM) foods was designed to meet human nutritional needs as well as balance gut microbiota. 123 central obese volunteers (BMI>28 kg/m2) were recruited and 93 completed a self-controlled, clinical trial of 23 weeks to test the efficacy of this dietary scheme for managing body weight and ameliorating metabolic syndrome. At the completion of the trial, the average weight loss reached 5.79 ± 4.64 kg (6.62 ± 4.94% of initial body weight), coupled with significant improvement of insulin sensitivity, lipid profiles and blood pressure. 454 pyrosequencing of gut microbiota showed that phylotypes related with opportunistic pathogens in Enterobacteriaceae and Desulfovibrionaceae, were reduced significantly, while those related with gut barrier protecting bacteria in Bifidobacteriaceae increased. The intestinal permeability marker, lactulose/mannitol (L/M) ratio, was decreased compared to the baseline. Plasma lipopolysaccharide binding protein (LBP), a biomarker linking gut derived antigen load and inflammation, was also significantly reduced, with associated decrease of TNF-α and IL-6 and increase of adiponectin, indicating an improvement of the inflammatory tone. The results implied that the diet based on whole-grains/prebiotics/TCM foods may alleviate the metabolic syndrome, at least partially, via modulation of the gut microbiota for enhancing the intestinal barrier integrity, reducing antigen load, ultimately ameliorating the inflammatory state underlying the high risk of metabolic diseases.

465B Elucidating nitrous oxide formation pathways in a nitrifying bioreactor towards nitrification: the effect of dissolved oxygen
Tomoko Yamamoto*, Keisuke Hojo, Micil Bellucci, Megumi Kuroiwa, Kazuo Isobe, Chie Katsuyama, Sheng Zhou, Masaaki Hosomi, Yuichi Suwa, Keisuke Koba, Akihiko Terada
1Tokyo University of Agriculture and Technology, Japan, 2Chuo University, Japan

Development of a wastewater technology towards low energy and greenhouse gas emission is of importance. Partial nitrification, that is nitrite (NO₂⁻) accumulation with nitrate production suppressed, attracts tremendous attention as a low cost and energy-saving nitrogen removal process for wastewater treatment since it requires less oxygen and external electron donor demands for nitrification and denitrification, and less sludge production. Partial nitrification can be achieved by mainly controlling oxygen supply; however, it has reportedly amplified nitrous oxide (N₂O) production in comparison with conventional nitrogen removal via nitrate. Thus, N₂O production mechanism and the effect of dissolved oxygen (DO) on N₂O production pathways in a nitrifying bioreactor were investigated by means of ¹⁵N stable isotope probing. To reveal the effect of DO on N₂O production pathway, batch experiments with different DO concentrations (DO: 7, 1.5, 1 and 0.3 mg/l) were performed using two biomasses with ammonia-oxidizing bacteria (AOB) highly enriched with sequencing batch (SBR) and continuous feeding modes (CSTR). MLSS concentration was set at
approximately 2000 mg/L. Water temperature and pH were controlled at 30 ± 1°C and 7, respectively. Each experiment was commenced at an initial NH₄⁺-N concentration of 320 mg-N/L. After NH₄⁺ depletion, ¹⁵N-labeled nitrite (¹⁵NO₂⁻) was added to ensure ¹⁵NO₂⁻-N of 20 mg-¹⁵N/L. Subsequently, ¹⁵N-labeled hydroxylamine (¹⁵NH₂OH) was added to ensure ¹⁵NH₂OH-N of 17 mg-N/L, confirming N₂O production by tracing N₂O concentration and ¹⁵N weight ratio in N₂O for N₂O production pathway. Molar weight of ¹⁵N in total N₂O was measured by a quadrupole GC/MS system.

In the tested DO concentrations, approximately 90% of NH₄⁺-N was converted into NO₂⁻-N (but not NO₃⁻-N) in both reactors fed with two distinct inocula. During this period, N₂O production peak was observed, indicating N₂O production was likely to occur. To better understand N₂O production pathway, ¹⁵NO₂⁻ was added after NH₄⁺ depletion. N₂O production was not observed in the tested DO concentrations, yielding that N₂O production from denitrification pathway (2NO₂⁻ → N₂O) was negligible. When ¹⁵NH₂OH was subsequently added, N₂O dramatically increased in accordance with NH₂OH consumption in the tested DO concentrations. The result clearly indicates that N₂O was produced in the presence of NH₂OH. ¹⁵N percentage in N₂O right after NH₂OH addition leaped from 0% to 59% in the biomass from the SBR (DO 1 mg/L). Given the fact that ¹⁵N percentages in NH₂OH and NO₂⁻ were 100% and 11.6%, respectively, one molar of NH₂OH and NO₂⁻ was plausibly converted into N₂O, showing that hybrid N₂O formation via NH₂OH oxidation is a primary pathway for N₂O production (NH₂OH + NO₂⁻ → N₂O).

N₂O was abruptly produced when NH₂OH confronted with NO₂⁻, which was observed irrespective of the tested two inocula with two distinct AOB compositions and the tested DO concentrations. These results clearly indicate that hybrid N₂O production by confronting NH₂OH with NO₂⁻ is a predominant N₂O production pathway in a nitrifying bioreactor towards partial nitrification. Quantification of functional gene expression involved in nitrification and denitrification by AOB will be presented at the conference.

466B Convergent development of anodic bacterial communities in microbial fuel cells
Matthew Yates¹, Patrick Kiely¹, Douglas Call¹, Hamid Rismani-Yadzi², Kyle Bibby², Jordan Peccia², John Regan¹, Bruce Logan¹
¹Pennsylvania State University, United States, ²Yale University, United States

Microbial fuel cells (MFCs) are often inoculated from a single wastewater source. The extent that the inoculum affects community development or power production is unknown. The stable anodic microbial communities in MFCs were examined using three inocula: a wastewater treatment plant sample known to produce consistent power densities; a second wastewater treatment plant sample; and an anaerobic bog sediment. Each inocula was tested in triplicate reactors. Four different molecular analysis techniques (16S rRNA gene-targeted DGGE, clone libraries, and pyrosequencing; and FISH) were used to study the anodic communities that developed in MFCs. The bog-inoculated MFCs initially produced higher power densities than the wastewater-inoculated MFCs, but after 20 cycles all MFCs on average converged to similar voltages (470±20 mV) and maximum power densities (590±170 mW/m²). The power output from replicate bog-inoculated MFCs was not significantly different, but one wastewater-inoculated MFC (UAJA3) produced substantially less power. Denaturing gradient gel electrophoresis (DGGE) profiling showed a stable exoelectrogenic biofilm community in all samples after 11 cycles. After 16 cycles the predominance of Geobacter spp. in anode communities was identified using 16S rRNA gene clone libraries (56±10%), fluorescent in-situ hybridization (FISH) (63±6%), and pyrosequencing (81±4%). While the clone library analysis for the underperforming UAJA3 had a significantly lower percentage of Geobacter spp. sequences (36%), suggesting that a predominance of this microbe was needed for convergent power densities, the lower percentage of this species was not verified by FISH or pyrosequencing analyses. These results show that the predominance of Geobacter spp. in acetate-fed systems was consistent with good MFC performance and independent of the inoculum source. Based on these results, it is also recommended that a non-PCR based community analysis technique is used to corroborate findings.
**467B** Enhanced rate of fungal biodegradation of polyurethane and a study of fungal colonial succession under composting conditions

Urooj Zafar*, Alan Heyworth¹, Geoff Robson¹

¹University of Manchester, United Kingdom, ²The TEG Group plc, Westmarch House, 42 Eaton Avenue, Buckshaw Village, Chorley, United Kingdom

Composting is a natural process involving the aerobic decomposition of organic waste by a mixed microbial population that goes through a range of temperature changes (~25°C to 75°C then back to 25°C) due to the heat generated by the growth of microorganisms. Historically, a major portion of municipal solid waste has been directed towards landfill, resulting in the scarcity of available sites with plastics representing a major component. A promising alternative for some plastics might be through municipal composting rather directing them to landfill sites. In this study, we examined the rate of biodegradation of the polyurethane (PU), which has a wide variety of applications and is an important component of plastic waste, in collaboration with the TEG Group Plc, based at Todmorden, UK. The PU sheets after 4 weeks of burial under compost were analysed for the physical and chemical degradation by tensile strength, FTIR respectively. Tensile strength (stress that plastic withstands before necking while being stretched) decreased >75% and Scanning Electron Microscopy showed extensive pits and holes in the sheet whereas controls showed only a approx. 20% loss in tensile strength after 12 weeks. Culture dependent and independent techniques were used to study the diversity of fungal population taking part in the biodegradation process and revealed a significant increase in the fungal population colonising the PU surface after 4 weeks with Aspergillus fumigatus, Thermomyces lanuginosus, Leichthiemia ramosa and Emericalla nidulans identified as the principal fungi. The culture independent techniques showed temperature driven change in the population over the surface of the PU sheet. Future studies aim to attempt to further enhance degradation by adding fungi to the compost. In addition, the enzymes secreted and causing degradation of PU will be identified. It is hoped that in the future, waste PU may be diverted for composting rather than disposal via conventional landfill.

**468B** Structural changes of gut microbiota during berberine-mediated prevention of obesity and insulin resistance in high-fat diet-fed rats

Xu Zhang*, Yufeng Zhao, Menghui Zhang, Xiaoyan Pang, Jia Xu, Chaoying Kang, Meng Li, Chenhong Zhang, Zhiguo Zhang, Yifei Zhang, Xiaoying Li, Guang Ning, Liping Zhao

¹State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, China, ²Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, China, ³Shanghai Clinical Center for Endocrine and Metabolic Diseases and Division of Endocrine and Metabolic Diseases, Rui Jin Hospital, Shanghai Jiao Tong University, China

Berberine, a major pharmacological component of the Chinese herb Coptis chinensis which was originally used to treat bacterial diarrhea, has recently been demonstrated to be clinically effective in alleviating type 2 diabetes and dyslipidemia. Although various metabolic pathways have been proposed to be regulated by berberine, the primary target by which berberine exhibited these functions remains largely unknown due to its extremely poor oral bioavailability and low concentration in bloodstream. Modulation of gut microbiota has been hypothesized as one of the mechanisms of its anti-diabetic and anti-obesity effects since berberine is poorly absorbed into the bloodstream from the gut, however, the concrete evidence is still lacking.

In this study, we employed a high-fat diet-induced obesity and insulin resistant rat model and microbiome-wide association study (MiWAS) strategy to resolve the phylotype-level structural changes of gut microbiota induced by berberine and its associations with host phenotypes. We revealed that oral administration of berberine at a dose of 100 mg/kg bodyweight effectively prevented the development of obesity and insulin resistance in rats fed a high-fat diet. Increases in the levels of serum lipopolysaccharide-binding protein, monocyte chemotactractant protein-1, and leptin and decrease in the serum level of adiponectin corrected for body fat induced by high-fat feeding were also significantly retarded by berberine. Bar-coded pyrosequencing of the V3 region of 16S rRNA genes revealed a significant reduction in the gut microbiota diversity of berberine-treated rats. Marked shift of the gut microbiota structure in berberine-treated rats away from that of the controls was observed by using a phylogenetic method, UniFrac principal coordinates analysis. Redundancy analysis identified 268 berberine-responding operational taxonomic units (OTUs), most of which were essentially eliminated, whereas a few putative short-chain fatty acid (SCFA)-producing bacteria, including Blautia...
and Allobaculum, were selectively enriched, along with elevations of fecal SCFA concentrations as measured by gas chromatography. Partial least square regression models based on these 268 OTUs were established ($Q^2 > 0.6$) for predicting the adiposity index, body weight, leptin and adiponectin corrected for body fat, indicating that these discrete phylotypes might have a close association with the host metabolic phenotypes.

Thus, our findings suggest that marked modulation of gut microbiota by berberine, namely inhibition of a wide range of intestinal microbes and enrichment of some SCFA producers, helps to alleviate systemic inflammation, at least in part, by reducing the antigen load to the host and elevating SCFA levels in the intestine and contributes to the beneficial effects of berberine against insulin resistance, obesity, diabetes, and other metabolic disorders. This perspective work opens a new window and provides a MiWAS strategy for studying the efficacy and mechanisms of traditional medicine drugs or regimes. This study also suggests that pharmacological or nutritional modulation of gut microbiota is an effective approach for preventive healthcare.