

# ISMOS<sup>8</sup>

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VIRTUAL SYMPOSIUM

8<sup>TH</sup> INTERNATIONAL SYMPOSIUM ON APPLIED  
MICROBIOLOGY AND MOLECULAR BIOLOGY IN OIL SYSTEMS

June 8-11<sup>th</sup>, 2021



## ABSTRACT BOOK

**Editors:** Sean Caffrey, Annie Biwen An, Torben Lund Skovhus and Corinne Whitby.

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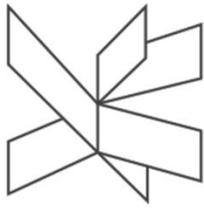
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# Welcome

Dear Delegate,

Welcome to ISMOS-8, our first online symposium!

This is the 8<sup>th</sup> meeting of the International Symposium on Applied Molecular Microbiology in Oil Systems (ISMOS).

ISMOS is the largest event discussing microbiology and molecular biology in the oil and gas industry. This conference explores the application of emerging microbial and molecular tools to help resolve challenges faced by the industry.

The aims of this symposium are to present the latest research on the applications of molecular tools to identify and quantify oil-reservoir microbes in order to resolve potential challenges (e.g. souring, biocorrosion) and encourage beneficial activities (e.g. hydrocarbon biodegradation for bioremediation).

The meeting is multidisciplinary, linking biogeochemists, engineers, molecular biologists and microbiologists, and will include a mixture of high-profile international speakers from industry and academia. We will have three workshops, which focus on Career Building, Failure Analysis and Nanopore Sequencing that are relevant to the oil and gas industry.

We are very grateful to the Technical & Scientific Committee (TSC). We also thank the sponsors for their support to ISMOS-8.

We hope you have an interesting and enjoyable meeting!

Yours,

Torben Lund Skovhus, VIA University College (TSC Chair)  
Corinne Whitby, Essex University (TSC Vice Chair)  
Sean Caffrey, University of Toronto (ISMOS Webmaster)  
Annie An, BAM (ISMOS Web Support)

# Symposium Program

(all times CEST)

**Monday, June 7<sup>th</sup> 2021**

**Workshops:**

**14:30-14.40 Welcome**

14.40-16.10 Workshop 1: Career Building  
(Chairs: Nicole Dopffel, Marko Stipanicev, Annie An)

**16.10-16.35 Break**

16.35-18.05 Workshop 2: Failure Analysis  
(Chairs: Torben Lund Skovhus, Richard Eckert)

**18.05-18.30 Break**

18.30-20.00 Workshop 3: Nanopore Sequencing  
(Chairs: Sean Caffrey, Renato de Paula)

**20.00-20.05 Workshop Wrap Up**

**Tuesday, 8<sup>th</sup> June 2021**

**14:30-14.40 Welcome**

**Session 01: A Green Future- becoming net zero**

Chairs: Ian Head, Alexander Grigoryan

**Keynote**

**14:45-15.15** Jeremy Shears (Shell Research Limited) *The role of biology in the energy transition*

**Offered Papers**

**15:15-15.35** Andrea Koerdt (Federal Institute for Materials Research and Testing (BAM))  
*#20 The impact and potential of halophilic microorganisms on alternative fuels*

**15.35-15.45 Break**

15:45-16.05 Elisabete Silva (University of Lisboa) #21 *Eco-friendly non-biocide release coating inspired in a multifunctional strategy to fight against antifouling resistance bio-foulants*

16:05-16.25 Zach Broussard (Cemvita Factory) #36 *Enhancing Biological-Mediated Conversion of CO<sub>2</sub> to Hydrocarbons in the Subsurface*

**End of Session**

## **Session 02a: MIC and Souring**

Chairs: Tony Mitchell, Annie An

### ***Invited Speaker***

16:30-17.00 Joerg Deutzmann, (Stanford University) *Electron transfer from solid surfaces to microbes - mechanisms, implications, and applications.*

**17.00-17.25 Break**

**End of Session**

### ***Offered Papers***

17.25-17.45 Tanmay Chaturvedi (Aalborg University) #31 *Clean Biocide Project: Halophilic plant extracts for prevention of microbiologically influenced corrosion (MIC)*

17.45-18.05 Alexander Grigoryan (Saudi Arabian Oil Company) #74 *Survey of an oil reservoir indicates that engineers must act to mitigate bacterial souring*

18.05-18.25 Nora Ebergen (DuPont Microbial Control) #44 *Novel Glutaraldehyde-based Formulations for Remediation and Control of Reservoir Souring*

18.25-18.45 Eric Deland (Federal Institute for Materials Research and Testing (BAM)) #18 *Environmental conditions affect the corrosion product composition of Methanogen induced microbiologically influenced corrosion (Mi-MIC)*

**18.45-19.00 Break**

**19.00- 19.35 Flash Presentations** (Chairs: Annie An, Ken Wunch)

**19.35-19.55 Q&A Flash Presentations (Breakout Rooms)**

**End of Session**

## Wednesday, 9<sup>th</sup> June 2021

### Session 02b: MIC and Souring

Chairs: Torben Lund Skovhus, Marko Stipanicev

#### **Offered papers**

14.35-14.55 Mohammed Sindi (Newcastle University) #64 *Effects of Extreme Physicochemical Parameters of injected seawater - produced water (ISW-PW) on sulfidogenesis and Microbially-Influenced Corrosion (MIC)*

14.55-15.15 Gunhild Bødtker (NORCE) #17 *Biofilm Injectivity During Produced Water Re-Injection (PWRI)*

15.15-15.35 Moein Jahanbani Veshareh (Denmark Technical University) #29 *An integrated methodology to study reservoir souring at the lab- and field-scale*

#### **15.35-15.45 Break**

15.45-16.05 Xiang Shi (Heriot-Watt University) #65 *Unmasking the hidden responses of a souring community to repeated glutaraldehyde treatments in sand-packed flow-through bioreactors*

16.05-16.25 Sven Lahme (Exxon Mobil Upstream Research Company) #11 *Severely corrosive sulfate-reducing biofilms contain a diverse multi-heme cytochrome gene cluster*

16.25-16.45 Andre Abilio (University of Alberta) #6 *Review of Current Gaps in Microbiologically Influenced Corrosion (MIC) Failure Investigations in Alberta's Oil and Gas Sector*

#### **End of Session**

#### **16.45-17.15 Break**

## **Session 03a: Hydrocarbon Biodegradation**

Chairs: Corinne Whitby, Paul Evans

### ***Invited Speaker***

17.15-17.45 Yoichi Kamagata, (National Institute of Advanced and Industrial Science (AIST)) *Deep subsurface microbes involved in the degradation of complex organic materials*

### ***Offered papers***

17.45-18.05 Nicolas Tsesmetzis (Shell International Exploration and Production Inc.)  
*#63 Syntrophic Hydrocarbon Degradation in a Decommissioned Off-Shore Subsea Oil Storage Structure*

18.05-18.25 Courtney Toth (University of Toronto) *#38 Field Application of Anaerobic BTEX Bioremediation Technologies in Groundwater*

18.25-18.45 Ibrahim Farag (University of Delaware) *#5 Niche partitioning and high replication rates of aerobic microbes promote biogenic methanogenesis in petroleum reservoirs*

**18.45-19.00 Break**

**19.00- 19.40 Flash Presentations** (Chairs: Ken Wunch, Dennis Enning)

**19.40-20.00 Q&A Flash Presentations (Breakout Rooms)**

**End of Session**

**Thursday, 10<sup>th</sup> June 2021**

**Session 03b: Hydrocarbon Biodegradation**

Chairs: Corinne Whitby, Paul Evans

***Offered papers***

14.35-14.55 Angela Sherry (Northumbria University) #42 *Fibre Highways: translocation of the microbiome for hydrocarbon bioremediation*

14.55-15.15 Shen Guo (University of Toronto) #40 *Increasing the rate of anaerobic benzene degradation in enrichment cultures*

15.15-15.35 Osman Radwan (University of Dayton Research Institute) #48 *Genome Sequencing and Hydrocarbon Degradation Profiling Reveal Metabolic Role of Fungi in Fuel Degradation and Bioremediation*

**15.35-15.45 Break**

15.45-16.05 Meng Ji (University of Calgary) #59 *Hydrocarbon-degrading microbial communities in Arctic sea ice, seawater, and sediment along shipping routes in Canada's Kivalliq region*

16.05-16.25 Xu Chen (University of Toronto) #26 *Characterization of a predicted necromass- recycling bacterium in a methanogenic benzene-degrading enrichment culture*

16.25-16.45 Susmitha Kotu (DNV GL) #61 *Importance of investigating the effect of hydrocarbon bioremediation on corrosion*

**End of Session**

**16.45-17.15 Break**

## **Session 04a: Oil and gas microbiome: Problems, control and opportunities**

Chairs: Nicolas Tsesmetzis, Renato dePaula

### ***Invited Speaker***

17.15-17.45 Turid Liengen, (Equinor, Norway) *MIC monitoring in Equinor; a historic journey*

### ***Offered papers***

17.45-18.05 Ali Mahmoodi (Danish Hydrocarbon Research and Technology Centre) #33 *On the necessity of multi-phase, field scale, and long term simulations in reservoir souring studies*

18.05-18.25 Jaspreet Mand - ExxonMobil Upstream Research Company #12 *Application of novel technologies for the detection and monitoring of corrosive microbiomes in oilfields*

18.25-18.45 Damon Brown (University of Calgary) #14 *Metagenome mining hydrocarbon environments for multidrug (biocide) resistance gene sources*

**End of Session**

**18.45-19.00 Break**

**19.00- 19.30 Flash Presentations** (Chairs: Torben Lund Skovhus, Dennis Enning)

**19.30-19.50 Q&A Flash Presentations (Breakout Rooms)**

**End of Session**

**Friday, 11<sup>th</sup> June 2021**

**Session 04b: Oil and gas microbiome: Problems, control and opportunities**

Chairs: Nicolas Tsesmetzis, Renato dePaula

***Offered papers***

14.35-14.55 Amela Keserovic (Schlumberger) #3 *Field Optimization of Biocide Treatment Based on a Novel Sessile Bacteria Monitoring Program*

14.55-15.15 Verena Brauer (University of Duisburg-Essen) #66 *Selection in microbial islands creates a large core community with variable relative abundances*

15.15-15.35 James Floyd (University of Oklahoma) #73 *Microbial Communities in Biodiesel Storage Tanks Correlate with Fuel Composition*

**15.35-15.45 Break**

15.45-16.05 Alexey Ershov (Research Centre of Biotechnology, RAS) #55 *Sulfidogenic microbial communities of the Uzen oil field and their resistance to biocides*

16.05-16.25 Daniel Gittins (University of Calgary) #72 *Geofluids facilitate a microbial dispersal cycle in the subsurface biosphere*

16.25-16.45 Jose Miguel Seoane (Repsol) #62 *Exploring the use of DNA-based monitoring tools in the biological monitoring of a gas pipeline located in the Peruvian Amazon.*

**End of Session**

**16.45-17.15 Break**

## **Session 05: Alternative fuels to oil and gas**

Chairs: Alexander Grigoryan, Paul Evans

### ***Invited Speaker***

17.15-17.45 Korneel Rabaey (Ghent University) *From CO<sub>2</sub> to novel fuels – and beyond*

### ***Offered papers***

17.45-18.05 Ruth Barnes (University of Sheffield/ Conidia Bioscience Ltd) *#15 Microbial communities in alternative aviation fuels*

18.05-18.25 Pierangela Cristiani (Ricerca sul Sistema Energetico -RSE) *#58 Geological and microbiological characterization of rocks collected from deep and superficial sites for the study of potential underground hydrogen storage sites*

**End of Session**

**18.25-18.30 Break**

**18.30- 18.50 Flash Presentations** (Chairs: Ian Head, Alexander Grigoryan)

**18.50-19.10 Q&A Flash Presentations and Posters (Breakout Rooms)**

**19.10-19.30 Closing Remarks and news on ISMOS-9**

**End of Session**

# ISMOS-8 ABSTRACTS

## Oral program

### Session 01: A Green Future—becoming net zero

#### Plenary:

The role of biology in the energy transition

Jeremy Shears, *Shell Research Limited, UK*

Shell's target is to become a net-zero emissions energy business by 2050, in step with society's progress in achieving the goal of the UN Paris Agreement on climate change. With this target, we will contribute to a net-zero world, where society stops adding to the total amount of greenhouse gas emissions in the atmosphere. This supports the more ambitious goal to tackle climate change laid out in the Paris Agreement: to limit the rise in average global temperature to 1.5°Celsius. Becoming a net-zero emissions energy business means that we are reducing emissions from our operations, and from the fuels and other energy products we sell to our customers. It also means capturing and storing any remaining emissions using technology or balancing them with offsets. The biosphere takes on an increasingly important role in stabilising the climate through the 21st century, both from its carbon storage potential and from its role in providing renewable feedstock options for fuels, chemicals and materials. Photosynthesis not only provides a mechanism to capture solar energy, but also generates molecular building blocks for emerging bio-manufacturing industries. I will provide examples of how Shell is working with biology in a number of areas from biofuels to nature-based solutions and how the latest advances in bioscience such as rapid DNA sequencing and engineering biology can play a role in the energy transition.

#### Offered Talks:

##### #20 The impact and potential of halophilic microorganisms on alternative fuels

1. **Annie An** - *Bundesanstalt für Materialforschung und Prüfung (BAM)*
2. **Eric Deland** - *Bundesanstalt für Materialforschung und Prüfung (BAM)*
3. **Jizheng Yao** - *Northwestern Polytechnical University, Xi'an, Shaanxi*
4. **Oded Sobol** - *Federal Institute for Materials Research and Testing (BAM)*
5. **Leonardo Agudo** - *Bundesanstalt für Materialforschung und Prüfung (BAM)*
6. **Hans-Joerg Kunte** - *Bundesanstalt für Materialforschung und Prüfung (BAM)*
7. **Andrea Koerdt** - *Federal Institute for Materials Research and Testing (BAM)*

As more industrial interests focusing on using salt caverns and repurposed gas or petroleum reservoirs for alternative fuel storage, i.e. CO<sub>2</sub>/H<sub>2</sub>, the question raises whether microorganisms may impact the infrastructure, gas purity and storage condition over time. Environments with high salinity (> 1.5 M<sub>eq</sub> of NaCl) are resided by halophiles (salt-loving microorganisms). To compensate for the intensive osmotic stress, they have resorted to two main adaptation strategies: 1) production of compatible solutes and 2) accumulation of intracellular KCl. Microbial community analysis of several high salinity environments revealed a number of recurring genera, including *Halomonas* and *Halanaerobium*. However, the impact of halophiles on the overall integrity and stability of the storage facilities remain largely unknown. To evaluate the suitability and stability of saline storage facilities, several model halophilic microorganisms, such as

members of *Halomonas*, will be selected as testing subjects. First, the impact of halophiles on the infrastructure will be determined using an integrative approach by combining a number of techniques, including electrochemistry, TOF-SIMS, SEM/FIB/EDS and FIB-TEM. Second, the abilities of halophiles to alter the fuel composition (i.e. increase/decrease the fractions of H<sub>2</sub>) will be monitored using gas chromatography by growing them under high pressure. As a result of climate change and the accompanying mandatory shift to renewable energy resources, microorganisms will continue to play an important role in the energy sector, both to their benefit and detriment. Thus, it is important to achieve a certain level of understanding regarding the activities and mechanisms of halophiles prior to large-scaled excursions.

### **#21 Eco-friendly non-biocide release coating inspired in a multifunctional strategy to fight against antifouling resistance bio-foulants**

1. **Olga Ferreira** - *University of Lisboa*
2. **Patrícia Rijo** - *University of Lusófona de Humanidades e Tecnologia*
3. **Elisabete R. Silva** - *University of Lisboa*

The coexistence of nature with industrial activities, vital for the growth and sustainability of our society, is threatened by the continued release of pollutants used mainly for economic or decontamination reasons. This threat is expressive in aquatic systems such as maritime transport, oil/wind-turbine platforms, desalination units, among others. These systems suffer from biofouling burden, which is allied to costly maintenance, retrofitting measures and the potential spread of diseases through soil/waterborne micro-organisms. To minimise it in these systems, toxic and persistent biocides are continuously employed and eventually accumulate in the environment. Hence, stricter environmental legislation has been issued, compromising their current use, challenging the control of biofouling, and recalling for alternative eco-friendly antifouling solutions. In this study, functional reactive E-conea biocide, able of being grafted into a foul-release polydimethylsiloxane-based coating matrix, has been developed. The generated non-release biocide coatings showed synergistic antifouling effects at simulated and real conditions, leading to a considerable reduction in the biocide release and providing long-lasting antifouling efficacies for more than two years in real scenarios<sup>1,2</sup>. Moreover, they evidenced antimicrobial properties against resistant bacteria, such as the Methicillin-resistant *Staphylococcus aureus*, allowing a log CFU reduction up to 5 orders of magnitude<sup>2</sup>. These promising findings can be a key to future research of new materials, towards prospective benign functionalities against bio-threads, embracing a new generation of non-toxic strategies. <sup>1</sup> Silva E. R. et al., *Science of the Total Environment*, **2019**, 650, 2499-2511. <sup>2</sup> Ferreira O. et al., *ACS Sustainable Chemistry & Engineering*, **2020**, 8, 12-17.

### **#36 Enhancing Biological-Mediated Conversion of CO<sub>2</sub> to Hydrocarbons in the Subsurface**

1. **Zach Broussard** *Cemvita Factory*
2. **Renata Goncalves** *Cemvita Factory*
3. **Marcio Da Silva** *Cemvita Factory*

Currently there are no established methods for in situ utilization of CO<sub>2</sub> used in enhanced oil recovery or carbon capture and storage. It is known the subsurface could work as a bioreactor for CO<sub>2</sub> utilization, but information on required microbial composition and metabolic needs are poorly understood. Concentrations of CO<sub>2</sub> in reservoirs undergoing such practices could serve as electron donor for hydrogenotrophic methanogens whereas hydrogen is not a limiting factor. The goal of this work was to demonstrate biological strategies could be useful in conversion of CO<sub>2</sub> present in reservoirs into valuable compounds, using methane as a precursor. Under controlled

conditions and/or adjustment of indigenous microbiology, it seems possible to accelerate biodegradation of recalcitrant and entrapped crude oil, leading to H<sub>2</sub> and volatile fatty acids for enhanced methanogenesis. To explore further, reactors were prepared using a consortium of model bacteria encountered in petroleum reservoirs (e.g., *Bacillus* and *Clostridia*) and methanogens. Addition of molasses simulating industrial strategies of MEOR demonstrated that CO<sub>2</sub> conversion can be accelerated if fermentative anaerobic bacteria are present. Because methanogens cannot directly utilize glucose, their activity is dependent on bacterial breakdown of glucose under fermentative anoxic conditions to by-products, namely acetate and H<sub>2</sub>. Results show bacteria broke down 1mM glucose to metabolites (e.g. acetate) that were further utilized by methanogens to produce 27 mL methane. Results indicate economically feasible nutrients could be introduced to the reservoir to promote conversion of existing or injected CO<sub>2</sub> from CCUS to produce valuable by-products in situ from CO<sub>2</sub> conversion and utilization.

## **Session 02: Microbiologically Influenced Corrosion (MIC) and Reservoir Souring**

### **Invited Talk:**

**Electron transfer from solid surfaces to microbes - mechanisms, implications, and applications.**

**Joerg Deutzmann, Stanford University, USA**

Microbial electron uptake from solid substrates is a physiological process of great economic importance and academic interest, especially in the fields of microbially influenced corrosion (MIC) and microbial electrosynthesis. This talk aims to bridge our knowledge about mechanisms of microbial electron transfer and the physico-chemical implications of this process and their impact on microbiology. Further, this talk will emphasize the synergies of MIC and electrosynthesis research with the goal to facilitate the application of novel insights across fields. Different mechanisms to facilitate electron uptake have been described in diverse microorganisms, but in many cases, the exact electron uptake pathway remains elusive. Our studies alone have revealed the presence of three distinct electron uptake mechanisms in strictly anaerobic, hydrogenotrophic microorganisms. The methanogen *M. maripaludis* and the acetogen *S. sphaeroides* facilitate electron uptake using extracellular electroactive enzymes that produce hydrogen or formate as substrates for their metabolism. The sulfate reducing strains *D. corrodens* IS4 and *D. ferrophilus* IS5, on the other hand, attach to the cathodic surface and take up electrons directly, but via different metabolic pathways. IS4 catalyzes reversible hydrogen production on an electrode in absence of sulfate as electron acceptor, while strain IS5 only takes up electrons when actively metabolizing sulfate. While increasing electron uptake from solid electron donors, microbes influence their environment by increasing local pH, decreasing the redox potential, and generally promoting gradient development. In turn, microbes with fast electron transfer rates also have to adapt to these characteristic environmental changes. Identifying and characterizing these adaptations or physiological limitations of the adapted microbes could benefit both research fields: 1) to increase the performance of microbial electrosynthesis, or 2) to develop mitigation strategies for microbially influenced corrosion.

## Offered Talks:

### #31 Clean Biocide Project: Halophilic plant extracts for prevention of microbiologically influenced corrosion (MIC)

1. **Jakob Stein** - Aalborg University
2. **Tanmay Chaturvedi** - Aalborg University
3. **Mette Thomsen** - Aalborg University
4. **Torben Lund Skovhus** - VIA University College

Offshore oil production is subjectable to internal corrosion, which can occur through microbiologically influenced corrosion (MIC). In pipelines, sulfur-reducing bacteria (SRB) such as *Desulfovibrio*, *Desulfobacterium*, *Dethiosulfovibrio* can thrive in the sulfate-rich produced waters, forming a MIC promoting biofilm. To mitigate MIC, the oil and gas industry relies primarily on biocides and mechanical cleaning. Halophytes (salt-tolerant plants), produce a variety of bioactive compounds that ensures their survival in an environment low in nutrition and high in free radicals and some of these compounds have antimicrobial activity. MIC was studied on carbon steel coupons inoculated with anaerobic sediment from the Wadden Sea (Denmark) to mimic MIC from oil production facilities in the North Sea. The coupons were treated with extracts from selected halophytes. Using H<sub>2</sub>S as activity indicator for SRBs and ATP for general microbial activity in the liquid phase, initial trials have shown a significant reduction in H<sub>2</sub>S and unchanged ATP concentrations in experiments treated with extracts compared to untreated controls, indicating a reduction of SRB species. Biofilm formation on carbon steel coupons from a bioreactor was reduced by two-thirds with the addition of extracts. Furthermore, next generation 16S rRNA amplicon sequencing of DNA from Bacteria and Archaea, proved a significant shift in the microbial composition when compared to samples not treated with extracts. Lastly, visual and measurable reduction in corrosion was observed with 3D surface scanning. Long-term solutions to prevent MIC using natural antimicrobial compounds from halophyte plants are discussed and proposed in this study.

### #74 Survey of an oil reservoir indicates that engineers must act to mitigate bacterial souring

1. **Grigoryan A**- Saudi Arabian Oil Company
2. **AlOtaibi M**- Saudi Arabian Oil Company
3. **AlWadei A**- Saudi Arabian Oil Company
4. **Humaid G**- Saudi Arabian Oil Company
5. **AlSaleh M**- Saudi Arabian Oil Company
6. **Zhu X**- Saudi Arabian Oil Company

Waterflooding for hydrocarbon recovery stimulates sulfate-reducing bacteria (SRB) that reduce sulfates to sulfide via oxidation of organics in the formation water leading to sulfide accumulation in oil reservoirs. This process, known as reservoir souring, causes multiple safety- and corrosion-related operational issues. Saudi Aramco launched a comprehensive investigation to prevent potential souring in a Saudi Arabian oil field. The survey revealed a broad distribution of bacteria, including SRB, in the topside water injection facilities. Next-generation sequencing of 16S rDNA from injection water confirmed presence of bacterial families *Desulfobacteraceae*, *Desulfomicrobiaceae*, *Desulfovibrionaceae* and *Desulfuromonadaceae* that may mediate reductive sulfur reactions including sulfide production. Though these findings suggest that injection water infrastructure is well-populated by sulfidogens that potentially can be introduced into reservoir, the number of bacteria in produced water from the oil field was relatively low. In sum, a thorough antibacterial measures need to be implemented at the oil field to control the

ongoing bacterial contamination then extend the protection throughout the entire topside system and reservoir.

#### **#44 Novel Glutaraldehyde-based Formulations for Remediation and Control of Reservoir Souring**

1. **Nora Ebergen** - *DuPont Microbial Control*
2. **Joseph Ferrar** - *DuPont Microbial Control*
3. **Jana Rajan** - *DuPont Microbial Control*
4. **Ken Wunch** - *DuPont Microbial Control*
5. **James Donovan** - *DuPont Microbial Control*
6. **Amber Stephenson** - *DuPont Microbial Control*
7. **Rishi Trivedi** - *DuPont Microbial Control*
8. **Geert van der Kraan** - *DuPont Microbial Control*

Water injection is the most prevalent method for secondary petroleum recovery. However, water injection has been associated with biotic reservoir souring leading to the production of increased concentrations of hydrogen sulfide (H<sub>2</sub>S) in produced fluids. H<sub>2</sub>S results in hydrocarbon devaluation, increased EH&S risks, and higher operating and capital expenses. The remediation of H<sub>2</sub>S generated by sulfate reducing prokaryotes (SRP) is one of the top unresolved challenges in the Oil & Gas industry. In this presentation, we'll discuss the methodology for modelling soured reservoirs in the laboratory under field relevant conditions (pressure, temperature, and with field microorganisms) and the development of novel glutaraldehyde-based formulations that demonstrate a rapid reduction & control of H<sub>2</sub>S in simulated sour-field environments under near-wellbore conditions.

#### **#18 Environmental conditions affect the corrosion product composition of Methanogen induced microbiologically influenced corrosion (Mi-MIC)**

1. **Eric Deland** - *Federal Institute for Materials Research and Testing (BAM)*
2. **Annie An** - *Federal Institute for Materials Research and Testing (BAM)*
3. **Ji Zheng Yao** - *Sino-German Joint Research Lab for Space Biomaterials and Translational Technology*
4. **Oded Sobol** - *Federal Institute for Materials Research and Testing (BAM)*
5. **Torben Lund Skovhus** - *VIA University College*
6. **Andrea Koerdt** - *Federal Institute for Materials Research and Testing (BAM)*

Corrosion is a very expensive and serious problem in the different industry sectors, eg. Petroleum, On- and off-shore, infrastructure. It is estimated that 20% of all corrosion damage is caused by microorganisms or microbiologically influenced corrosion (MIC). Several microorganisms are known to cause corrosion, including sulfate-reducing bacteria, nitrate-reducing bacteria, methanogens etc. For several years, methanogens were regarded as a mild corrodor (~0.065 mm/yr), largely due to a lack of detailed investigation on the corrosion mechanism under real-environment simulated conditions. Resulting in the common belief that siderite, a non-conductive compound, is the sole corrosion product (CP) of methanogen-induced MIC (Mi-MIC). To simulate natural environmental conditions, we developed and introduced the multiport flow column system (MFC), a multi-sectional corrosion flow-cell. Using the MFC, we obtained ten times higher corrosion rates than previously reported. With a combination of several analytical techniques, such as ToF-SIMS, SEM-EDS and FIB-SEM, we found strong indication that siderite is not the sole corrosion product of Mi-MIC. The corrosion layers contained phosphorus, oxygen, magnesium, calcium and iron. The differences in the CP between static and dynamic

environments demonstrated the impact of testing procedures on the corrosive potential of methanogens. To further verify and deepen our understanding of Mi-MIC, we are currently studying the influence of additional environmental parameters (e.g. pH, salinity, flow rate) on Mi-MIC. Overall, results of this study will expand the current understanding of MIC from both analytical and mechanistic points of view, thus aiding the development of different mitigation strategies for various industry sectors.

#### **#64 Effects of Extreme Physicochemical Parameters of injected seawater - produced water (ISW-PW) on sulfidogenesis and Microbially-Influenced Corrosion (MIC)**

1. **Mohammed Sindi** - Newcastle University
2. **Xiangyang Zhu** - Saudi Aramco
3. **Angela Sherry** - Newcastle University
4. **Neil Gray** - Newcastle University
5. **Ian Head** - Newcastle University

Microbiologically-influenced corrosion (MIC) is a global issue, influencing the premature failure of metallic infrastructure, accounting for 20% of all internal corrosion. The objective was to understand the influence of physicochemical characteristics of mixtures of seawater and formation water on MIC. Anaerobic microcosms containing mixed ratios and, therefore mixed salinities of injected seawater (ISW) : production water (PW) incubated at (15°C-60°C), were setup. Temporal changes in sulfide, sulfate, volatile fatty acids (VFAs) and microbial communities were determined. No significant sulfide production/ sulfate-reduction were detected (NS, 126 g/L; AG, 212 g/L), (250 days incubations), suggesting the potential inhibition of SRMs, while VFAs were rapidly consumed. Microbial community composition was driven by temperature, with *Halanaerobium* spp. selectively enriched at low temperatures (NS & AG 15- 30°C), distinct from high temperatures (45 and 60 °C) microbial enrichments. *Halanaerobium* spp. enrichment relative abundance (RA) of: ( $\geq 1\%$  start for all to: (NS 15°C: 35%), (NS: 30°C: 50%), (AG 15°C: 20%), and (AG 30°C: 35%), at end of incubations was observed. Negligible corrosion rates (AG (15°C-60°C):  $\leq 0.15$  MPY  $\pm 0.008$ ) (NS 30°C: 0.46 MPY  $\pm 0.072$ ); (NS 60°C MPY  $\pm 0.008$ ),) were detected. SEM detected advanced stage pitting nucleations (NS 60°C: 1400 pits  $\pm 800.8$  per cm<sup>2</sup>; 50  $\mu$ m-150  $\mu$ m) (NS 30°C: 0.17- 0.22 pits (20  $\mu$ m). *Halanaerobium* spp. is implicated in metabolising guar gum in hydraulic fracking & drilling fluids, MIC, sulfidogenesis, and sporulation. Understanding *Halanaerobium* spp. implications will prove beneficial for an array of applications, including: MIC mitigations, and down-hole biocidal applications.

#### **#17 Biofilm Injectivity During Produced Water Re-Injection (PWRI)**

1. **Gunhild Bødtker** - NORCE
2. **Edin Alagic** - NORCE
3. **Janiche Beeder** - Equinor
4. **Bartek Vik** - NORCE
5. **Espen Kowalewski** - Equinor
6. **Dag Standnes** - Equinor

Core floods were performed to assess the combined effect of biofilm growth and particle injection on injectivity during produced water re-injection (PWRI) under elevated temperature. Experiments were performed using outcrop Bentheimer cores and synthetic (SPW) and native produced water (NPW). A core injected with realistic NPW flux showed similar pressure build-up as a core flooded with SPW added quartz particles. Pressure build-up for a core flooded with SPW without particles was 3-4 times higher at similar volumes injected. This result may be explained by particle-

shearing at the sandface preventing build-up of biofilm filtercake. Cell counts showed that bacteria in NPW to a large extent travelled through the core, suggesting that *in situ* biofilm growth was the main cause for bioplugging. This was supported by pressure data from periods with cell free injection. Mitigation of plugging by injection of synthetic sulphate reduced water without nutrients reduced the pressure by 1.5-fold and was probably a result of biofilm starvation. Subsequent transient increases in pressure were assumed to be an effect of biofilm detachment/entrapment. Test of a commercial solvent to mitigate plugging showed limited effect on permeability restoration. The main mechanisms related to biofilm injectivity and brine quality have been identified and the complexity of multiple factors during PWRI has been discussed. Development of cost efficient and realistic injectivity testing of oil field brines is a necessary tool for assessment of injectivity, optimization of water quality and treatment for improved injectivity.

## **#29 An integrated methodology to study reservoir souring at the lab- and field-scale**

1. **Moein Jahanbani Veshareh** - Denmark Technical University
2. **Hamid Nick** - Denmark Technical University

Reservoir souring field scale studies for decades have relied on lab scale batch or flow experiments. Traditionally, Monod kinetic equation is calibrated with a set of experimental data and then is used to predict reservoir souring in the field scale. These studies are done based on the assumptions: a) Monod equation can characterize the kinetic of a metabolism derived by multiple microorganisms; b) Growth yield is constant; c) Biofilm does not have a volume and does not influence porosity; d) Growth temperature dependency is mono modal. Here, these assumptions are discussed. We illustrate the shortcoming of using Monod equation for modelling reservoir souring which is a process derived by a diverse microbial community. We propose a method how to resolve this. We also illustrate that a constant growth yield assumption can cause a significant underestimation of H<sub>2</sub>S concentration. Next, we address whether or not microbial biofilm should be taken into account in reservoir souring simulations. Lastly, using the suggested integrated methodology we compare different reservoir souring mitigation strategies for a Danish North Sea hydrocarbon reservoir.

## **#65 Unmasking the hidden responses of a souring community to repeated glutaraldehyde treatments in sand-packed flow-through bioreactors**

1. **Xiang Shi** - Heriot-Watt University
2. **Kenneth S Sorbie** - Heriot-Watt University
3. **Julia R de Rezende** - Heriot-Watt University

Biocides are applied to control reservoir souring, yet the efficacy of specific biocides is difficult to predict due to a limited understanding of the microbial responses. Here we investigated the development of a souring microbial community in sand-packed flow-through bioreactors undergoing a 57-day continuous flow programme consisting of repeated glutaraldehyde treatment-recovery cycles. We aim to understand how microbial abundance and community structure has responded to the biocide cycles. Besides the routine molecular methods (DNA sequencing and qPCR), we employed the propidium monoazide (PMA) technique to remove DNA from damaged and dead cells before extraction, thus allowing comparison between the *total* microbial community and the *live-only* fraction. Compared to untreated controls, repeated biocide treatment-recovery cycles caused: (i) higher live-only microbial abundance in effluent samples; (ii) a “shelter zone” deep in the bioreactor, within which higher live-only abundance was observed; (iii) a community shift in sand samples towards different SRM populations. All three findings could only be observed after distinguishing the live cells from the

total. Furthermore, we characterised the qualitative and quantitative error resulting from using effluent samples as a proxy for the bulk microbial community (planktonic + sessile), which is important for souring models. Overall, this study highlights several hidden responses of a souring community to repeated glutaraldehyde treatments, some of which could be counter-productive in the field. A conceptual model is proposed to delineate the development of biocide-induced microbial spatial patterns, which is currently being incorporated into our modelling tool to predict the biocide efficacy for souring control.

#### **#11 Severely corrosive sulfate-reducing biofilms contain a diverse multi-heme cytochrome gene cluster**

1. **Sven Lahme** - Exxon Mobil Upstream Research Company
2. **Jaspreet Mand** - Exxon Mobil Upstream Research Company
3. **John Longwell** - Exxon Mobil Upstream Research Company
4. **Ramsey Smith** - Exxon Mobil Upstream Research Company
5. **Dennis Enning** - Exxon Mobil Upstream Research Company

Despite improvements in the monitoring of microbially influenced corrosion (MIC), available technology still offers limited understanding of the actual threat and mechanisms of MIC in oil fields. Sulfate-reducing bacteria (SRB) have been frequently linked to MIC, and a few SRB isolates can severely accelerate corrosion by utilizing cathodic electrons. One of those isolates, *Desulfovibrio ferrophilus* strain IS5, possesses a special multi-heme cytochrome gene cluster with a proposed role in the uptake of steel-derived electrons. Using shotgun metagenomics, we screened microbial communities from oil field samples and corrosive laboratory tests (1.1-2.5 mm/yr), and recovered 17 gene clusters that are homologous to the one in strain IS5. We then designed qPCR assays to target homologs of the gene DFE\_0465 (termed here *micC*) in strain IS5, a putative extracellular c-type cytochrome. In order to develop and refine water-based MIC monitoring strategies, we recently developed custom-built once-flow-through corrosion autoclaves that simulate pipeline-like conditions such as the presence of acid gases, pipe wall shear stresses and pressure. Using this test skid, we applied the *micC* assay on liquid samples in experiments conducted under lithotrophic sulfate-reducing conditions. The tests showed severe microbial corrosion rates of up to 1.6 mm/yr along with  $2.6 \cdot 10^1 - 1.6 \cdot 10^5$  copies of *micC* per mL in the autoclave fluids, suggesting that IS5-like SRB may have contributed to severe MIC under the simulated pipeline conditions. This work indicates that water-based pipeline monitoring using mechanistic biomarkers can provide actionable data in the context of oil field integrity management.

#### **#6 Review of Current Gaps in Microbiologically Influenced Corrosion (MIC) Failure Investigations in Alberta's Oil and Gas Sector**

1. **Andre Abilio** - University of Alberta
2. **Richard Eckert** - DNV GL Ohio
3. **Torben Lund Skovhus** - VIA University College
4. **John Wolodko** - University of Alberta

Microbiologically Influenced Corrosion (MIC) is an interdisciplinary threat to the oil and gas industry. Currently 10-40% of all corrosion issues in the sector are related to MIC. However, due to the unpredictability that microorganisms add to MIC management and diagnosis, MIC is yet not fully understood. The present study was performed to assess the current methods used to diagnose MIC in oil and gas production pipelines. A comprehensive review of 50 failure assessments ran between January 1, 2017, and December 31, 2019 in the Province of Alberta, Canada, was carried out. Lines of evidence related to microbiology, chemistry, metallurgy and

operating factors were reviewed and the frequency in which they were considered was quantified. Biotic independent factors (chemical, metallurgical, and operating) were assessed in more than 90% of the assessments while *only 70%* took microbiological analyses into consideration. Molecular microbiological methods (MMM) were ran for only 6 to 10% of the assessments. Additionally, this study offers a traceable number that can be linked to MIC: 11.7% of corrosion failures in oil and gas production pipelines in Alberta between the 3-year period reviewed was caused by MIC either as the main failure mechanism or as a contributing factor. Therefore, this presentation aims to discuss best tactics for MIC failure investigations and how to integrate the interdisciplinary lines of evidence required to conclusively diagnose MIC. Also emphasizing the need for optimized tools, such as MMM, to bridge the current gap where only 70% of microbiological driven failures were evaluated by microbiological tools.

## **Session 03: Hydrocarbon Biodegradation.**

### **Invited Talk:**

**Deep subsurface microbes involved in the degradation of complex organic materials**

**Yoichi Kamagata, *National Institute of Advanced and Industrial Science (AIST), Japan***

Deep subsurface harbors a vast variety of microbes that contribute to the degradation of hydrocarbons and other complex organic materials. Recent studies are showing unforeseen capacities of microbes. Some methanogens dwelling subsurface are capable of degrading methoxy moieties of a variety of aromatic compounds within coals that would not otherwise be the substrates for methanogens. Over the last decade, extensive studies revealed that the degradation of petroleum hydrocarbons involves a variety of bacteria and archaea including Atribacteria (such as OP9 and JS1) but the organisms remain uncultivated and their degradation mechanisms are still controversial. Together with those microbial studies, we investigated the potential of biological augmentation and stimulation for microbial enhanced energy recovery from crude oil. We obtained a microbial community capable of methanogenic crude oil degradation from oil reservoir A in Japan. We inoculated the microbial culture into production water from the other oil reservoir B in which the indigenous microbial community are unable to degrade crude oil. We found that methane production associated with toluene degradation, indicating that biological augmentation (transplantation of microbes) could be effective for energy recovery.

### **Offered Talks:**

**#63 Syntrophic Hydrocarbon Degradation in a Decommissioned Off-Shore Subsea Oil Storage Structure**

- 1. Adrien Vigneron** - *Newcastle University*
- 2. Nicolas Tsesmetzis** - *Shell International Exploration and Production Inc.*
- 3. Perrine Cruaud** - *Université Laval*
- 4. Ian Head** - *Newcastle University*

Over the last decade, metagenomic studies have revealed the impact of oil production on the microbial ecology of petroleum reservoirs. However, despite their fundamental roles in bioremediation of hydrocarbons, biocorrosion, biofouling and hydrogen sulfide production, oil field and oil production infrastructure microbiomes are poorly explored. Understanding of microbial activities within oil production facilities is therefore crucial for environmental risk mitigation, most

notably during decommissioning. The analysis of the planktonic microbial community from the aqueous phase of a subsea oil-storage structure was conducted. This concrete structure was part of the production platform of the Brent oil field (North Sea), which is currently undergoing decommissioning. Quantification and sequencing of microbial 16S rRNA genes, metagenomic analysis and reconstruction of metagenome assembled genomes (MAGs) revealed a unique microbiome, strongly dominated by organisms related to *Dethiosulfatibacter* and *Cloacimonadetes*. Consistent with the hydrocarbon content in the aqueous phase of the structure, a strong potential for degradation of low molecular weight aromatic hydrocarbons was apparent in the microbial community. These degradation pathways were associated with taxonomically diverse microorganisms, including the predominant *Dethiosulfatibacter* and *Cloacimonadetes* lineages, expanding the list of potential hydrocarbon degraders. Genes associated with direct and indirect interspecies exchanges (multiheme type-C cytochromes, hydrogenases and formate/acetate metabolism) were widespread in the community, suggesting potential syntrophic hydrocarbon degradation processes in the system. Our results illustrate the importance of genomic data for informing decommissioning strategies in marine environments and reveal that hydrocarbon-degrading community composition and metabolisms in man-made marine structures might differ markedly from natural hydrocarbon-rich marine environments.

### **#38 Field Application of Anaerobic BTEX Bioremediation Technologies in Groundwater**

1. **Courtney Toth** - *University of Toronto*
2. **Andrea Marrocco** - *University of Waterloo*
3. **Adam Schneider** - *University of Waterloo*
4. **Bill McLaren** - *University of Waterloo*
5. **Griselda Diaz de Leon** - *University of Waterloo*
6. **Nancy Bawa** - *University of Toronto*
7. **Shen Guo** - *University of Toronto*
8. **Jennifer Webb** - *SiREM Labs*
9. **Rachel Peters** - *Federated Co-operatives Limited*
10. **Kris Bradshaw** - *Federated Co-operatives Limited*
11. **Neil Thomson** - *University of Waterloo*
12. **Sandra Dworatzek** - *SiREM Labs*
13. **Elizabeth Edwards** - *University of Toronto*

Thousands of groundwater sites are contaminated with Benzene, Toluene, Ethylbenzene, and Xylenes (BTEX). BTEX presents significant risks to human and environmental health, especially benzene, a known carcinogen. Many conventional remediation technologies, including aerobic bioremediation, excavation, or pump and treat are not always applicable or effective when applied at anoxic sites. To this end, a collaborative team has spent the past 5 years developing and testing biotechnology tools specifically designed for anaerobic *in situ* treatment of BTEX. Previously, we showcased how anaerobic benzene biodegradation was largely controlled by the abundance of highly specialized hydrocarbon-degrading microorganisms, and that many groundwater sites with persistent benzene contamination harbored low concentrations of these organisms. This year, our presentation will highlight early field trial results of two applications intended to increase the abundance of active benzene and BTEX-degrading microorganisms in contaminated groundwater. Case Study #1 evaluates the use of hydrocarbon-adsorbing materials (carbon-based injectates, CBIs) to sequester petroleum plumes and enrich for intrinsic BTEX degraders. Case Study #2 explores bioaugmentation with microbial cultures into the subsurface to immediately increase the abundance of active hydrocarbon degraders. We are currently evaluating pilot scale field applications of three bioaugmentation cultures (targeting benzene, toluene and o-xylene) at five contaminated groundwater sites across North America. Quantitative

PCR biomarker assays and 16S rRNA gene amplicon sequencing, among many groundwater monitoring tools, are being employed at both case studies to obtain a holistic understanding of the effectiveness of each biotechnology.

#### **#5 Niche partitioning and high replication rates of aerobic microbes promote biogenic methanogenesis in petroleum reservoirs**

1. **Ibrahim Farag** - *University of Delaware*
2. **Glenn Christman** - *University of Delaware*
3. **Zarath Summers** - *Exxon Mobil Research and Engineering*
4. **Jennifer Biddle** - *University of Delaware*

Biogenic methane is generated in petroleum reservoirs, yet its levels vary widely in response to the surrounding ecological conditions. Typically, microbes produce methane under anaerobic conditions through microbial methanogenesis. However, biogenic methane has been detected in reservoirs containing oxygen. The ecological settings and metabolic networks that allow microbial methanogenesis under aerobic conditions remain not fully understood. In this study, we integrate geochemical, metagenomic, and genomic analyses to identify the methane sources and major microbial culprits in biogenic methane production in five oil production wells from an onshore conventional oil reservoir, where three of these reservoirs have detectable oxygen levels. One potential source of oxygen introduced to these wells is the oxygen mixed with CO<sub>2</sub> and water injected into the wells to enhance the oil recovery process. Our analyses indicate that, under complete anoxic conditions, methanogenic archaea are significantly outcompeted by fermentative bacteria for shared substrates leading to reduced biogenic methane levels. While in the presence of oxygen, aerobic bacteria (e.g. Alpha- and Gamma-proteobacteria) replicate at high rates, presumably consuming high levels of oxygen which creates anaerobic niches favorable for methanogens. Unlike anoxic reservoirs, methanogenic archaea encounter less competitive situations with fermentative bacteria, which increase the overall biogenic methane production capacities. Our analysis set a model for the potential microbial activities and metabolic tradeoffs that are important for enhanced biogenic methane production in crude oil reservoirs.

#### **#42 Fibre Highways: translocation of the microbiome for hydrocarbon bioremediation**

1. **Angela Sherry** - *Northumbria University*
2. **Jane Scott** - *Northumbria University*

There is a global legacy of hydrocarbon contaminated ecosystems where options for bioremediation should continue to be a research focus for the foreseeable future. Research into the movement of bacterial communities along fungal mycelium (fungal highways) has previously been shown to facilitate hydrocarbon bioremediation.<sup>1,2</sup> A liquid film surrounds the fungal mycelium in which microorganisms can translocate (move) towards a chemical (pollutant), consequently fungal highways have the ability to improve the bioavailability of pollutants in environments, such as soils.<sup>1</sup> The study expands upon research on fungal highways to investigate 'fibre highways' - the directional movement and dispersal of microbes on a range of natural and synthetic fibres as a tool for targeted bioremediation of hydrocarbons. With cross-disciplinary expertise in environmental molecular microbiology and materials and textile science, the methodologies include a combination of growth experiments, visualisation technologies, next-generation sequencing and bioinformatics. A deeper understanding of the dynamics of hydrocarbon-degrading microbiomes along fibre highways will facilitate the advancement of biotechnological solutions that can be used to remediate polluted sites. Ultimately, outcomes will lead to the development of environmentally responsive textile systems composed of natural and

sustainable materials that can be used to increase the contact time of microbes with pollutants for more efficient bioremediation or to 'seed' polluted sites which are difficult to reach. <sup>1</sup>Furuno *et al.* 2010. Fungal mycelia allow chemotactic dispersal of PAH-degrading bacteria in water-unsaturated systems. *Environ Microbiol*, 12(6), 1391-1398. <sup>2</sup>Kohlmeier *et al.* 2005. Taking the fungal highway: mobilization of pollutant-degrading bacteria by fungi. *Environ Sci Technol*, 39, 4640-4646.

#### **#40 Increasing the rate of anaerobic benzene degradation in enrichment cultures**

1. **Shen Guo** - *University of Toronto*
2. **Courtney Toth** - *University of Toronto*
3. **Xu Chen** - *University of Toronto*
4. **Fei Luo** - *University of Toronto*
5. **Elizabeth Edwards** - *University of Toronto*

Benzene is a widespread and toxic environmental pollutant necessitating clean up. The fate of benzene in anoxic environments such as soils, sediments and groundwater was once thought to be controlled by the abundance of oxygen: benzene is aerobically degraded at high rates by ubiquitous microorganisms. We now know that benzene can also be biodegraded under various anaerobic electron-accepting conditions ( $\text{Fe}^{3+}$ ,  $\text{NO}_3^-$ , and  $\text{SO}_4^{2-}$ ) and fermentatively (i.e., methanogenic conditions). Benzene degradation rates below 1 mg/L have been typical of the enrichment cultures maintained in our lab for decades, while toluene degradation rates in similar anaerobic enrichments were much faster. We suspected a missing essential nutrient or accumulating inhibitor. Here, we present data showing that relatively high rates of benzene degradation can be indeed achieved in these enrichment cultures that derived from sediments from contaminated sites. For over two years, we repeatedly fed a methanogenic consortium (DGG-B) progressively increasing concentrations of benzene (from 5 mg/L to over 150 mg/L) and found that biodegradation rates in experimental replicates increased proportionally, from less than 0.2 mg/L/day to over 10 mg/L/day. We also applied this strategy to a nitrate-reducing, benzene-degrading consortium, and although partially successful, degradation rates never exceeded 5 mg/L/day, perhaps as a result of the difficulties in supplying nitrate consistently. In contrast, the methanogenic consortium was never limited by electron acceptor availability. These data indicate that slow rates of degradation are not related to limiting essential nutrients or inhibitors, but rather to benzene and acceptor availability.

#### **#48 Genome Sequencing and Hydrocarbon Degradation Profiling Reveal Metabolic Role of Fungi in Fuel Degradation and Bioremediation**

1. **Osman Radwan** - *University of Dayton Research Institute*
2. **Oscar Ruiz** - *Air Force Research Laboratory*

Contamination of fuel by filamentous fungi and yeast causes substantial problems to the military and civilian sectors by reducing the quality and stability of fuel, disrupting fuel filtration systems, and degrading polymeric coating and metal alloys. Therefore, understanding the fungal biology and mechanisms underlying their ability to proliferate and degrade fuel will help in designing effective prevention and mitigation approaches. In this study, next-generation sequencing using Illumina platform and a novel bioinformatic pipeline were employed for full-genome sequencing, *de novo* assembly, gene prediction, and annotation of several genomes of filamentous fungi and yeast. These genomes included *Aspergillus versicolor*, *Superstratomyces atroviridis*, *Scedosporium apiospermum*, *Eutypella* sp., *Lecanillium* sp., *Fusarium fujikuroi*, *Byssosclamyces* sp., and *Yarrowia lipolytica* ATCC 20496. The size of genomes ranged

from 20 Mb (*Y. lipolytica*) to 68 Mb (*A. versicolor*), harboring 6,000 to 18,578 genes encoding important proteins involved in biofilm formation, hydrocarbon degradation, and efflux of toxic substances. The metabolic pathways identified in filamentous fungi and yeast genomes include degradation of *n*-alkanes, branched alkanes and aromatic hydrocarbons. The genomic results are strongly supported by growth curves using quantitative real-time PCR (qPCR) and hydrocarbon degradation profiles using gas chromatography-mass spectrometry (GC-MS) that confirmed the ability of fuel-degrading fungi to proliferate and degrade hydrocarbons. Genomic data and GC-MS results reveal a multiplicity of fungal mechanisms to adapt and degrade hydrocarbons. This knowledge will help in developing new approaches to mitigate fuel biocontamination in fuel system and novel ways to apply fuel-degrading fungi for bioremediation of hydrocarbon contaminants in the environment.

### **#59 Hydrocarbon-degrading microbial communities in Arctic sea ice, seawater, and sediment along shipping routes in Canada's Kivalliq region**

1. **Meng Ji** - *University of Calgary*
2. **Casey Hubert** - *University of Calgary*

The extreme environment of the Canadian Arctic has been scarcely studied for its biodegradation potential of oil spills. Reduced ice cover due to the effects of climate change has led to a rise in human activities, which inevitably increases the risk of oil and fuel spills from vessels, posing great risks to the marine ecosystem and Canadian northerners that rely on it. Hydrocarbonoclastic bacteria catalyze bioremediation of oil compounds in marine biomes. Previous studies have indicated that beta microbial communities vary within different marine biomes, and few studies have explored the vertical distributions in the diversity and composition of Arctic marine ice, water, and sediment in a given location. This research investigates bacterial community composition within vertically oriented biomes in the Kivalliq region in Nunavut, from sea ice, through the water column, to the seafloor. *In situ* baseline diversity analyses of surface seawater and sediment using 16S rRNA amplicon sequencing revealed various ZOTUs in water to be more abundant than sediment communities (27.86% vs 5.15%). Baseline samples are complemented by mock oil spill microcosm incubations to assess biodegradation capabilities in surface water and sediment using 16S rRNA amplicon sequencing and epifluorescence microscopic cell counting. Appearance of known hydrocarbonoclastic bacteria such as *Thalassolituus*, *Cycloclasticus*, and *Oleispira* after 42 days correlated with an increase in total cell counts. This research has the potential to incorporate biological diversity into monitoring environmental change and improving the efficacy of oil spill bioremediation strategies in Arctic conditions through cutting-edge genomic technology.

### **#26 Characterization of a predicted necromass-recycling bacterium in a methanogenic benzene-degrading enrichment culture**

1. **Xu Chen** - *University of Toronto*
2. **Courtney Toth** - *University of Toronto*
3. **Shen Guo** - *University of Toronto*
4. **Fei Luo** - *University of Toronto*
5. **Olivia Molenda** - *University of Toronto*
6. **Elizabeth Edwards** - *University of Toronto*

Methanogenic benzene biodegradation is a globally relevant but poorly understood process. To study this metabolism, our laboratory has maintained several anaerobic benzene-degrading enrichment cultures for over 20 years. In one methanogenic consortium, referred to herein as

DGG-B, two bacterial phylotypes have been detected in every subculture for which molecular data is available; the chief benzene-degrading fermenter (*Deltaproteobacteria* ORM2) and a candidate phylum member of unknown function (OD1, now reclassified as *Nelsonbacteria*). Given its persistence, we hypothesized that *Nelsonbacteria*, a member of the candidate phylum radiation (CPR), must therefore serve an important role in DGG-B. Here, we used a combination of growth experiments, molecular analyses and microscopy tools to assign a functional role to *Nelsonbacteria*. We began by cultivating DGG-B on various growth substrates, including acetate and hydrogen and other complex substrates, and found that *Nelsonbacteria* became most significantly enriched on dead biomass. Although the specific dead cell compound(s) used to enrich *Nelsonbacteria* have not yet been identified, our data suggests that the uncultured organism may be involved in necromass recycling. In other words, *Nelsonbacteria* may be utilizing biomass released from dead cells to produce secondary metabolites consumed by other microbes in the culture. Microscopy revealed that *Nelsonbacteria* grows in close association with *Methanosaeta*, a methanogenic archaea essential for completing the biotransformation of benzene to methane. A genomic analysis is now in process to find the evidence of necromass recycling pathways and how *Nelsonbacteria* may potentially interact with *Methanosaeta*.

#### **#61 Importance of investigating the effect of hydrocarbon bioremediation on corrosion**

1. **Susmitha Kotu** - DNV GL
2. **Christopher Kagarise** - DNV GL
3. **Kenneth Evans** - DNV GL
4. **Richard Eckert** - DNV GL

Bioremediation is a process in which hydrocarbon pollutants are removed from contaminated soil and water. Bioremediation is achieved by either adding chemicals to stimulate native microorganisms or by adding microorganisms to degrade the hydrocarbons. Whenever bioremediation is utilized for hydrocarbon degradation, there is a possibility that the threat of microbiologically influenced corrosion (MIC) on surrounding metal structures and pipelines could be increased. Growth of corrosive microbial species can occur if the added biostimulatory solutions provide appropriate nutrients to the native microorganisms or if the added microbial species influence the growth of corrosive species. To prevent unintended microbial growth and MIC, laboratory testing may be performed to assess the effects of hydrocarbon bioremediation on MIC. This presentation provides an example framework for the design of laboratory tests to investigate the effect of hydrocarbon bioremediation on corrosion of buried steel structures. A case study will be presented in which the impact of a biostimulatory solution was tested in the laboratory prior to application for its impact on the corrosion of buried steel structures. Laboratory tests were designed to assess the effects of bioremediation treatment on corrosion initiation and severity of uncoated steel in contaminated soil in the presence and absence of cathodic protection (CP). Data from corrosion rate measurements, soil chemistry, biofilm and soil microbial community abundance, activity and diversity between treated and untreated soil were used to characterize the effects of bioremediation on corrosion.

## **Session 04: The oil and gas microbiome: Problems and Solutions.**

### **Invited Talk:**

**MIC monitoring in Equinor; a historic journey**

**Turid Liengen, Solfrid Molid, Vincent Gregoire and Line Lilleby Bjering, Equinor, Norway**

Statoil, a Norwegian petroleum company, was established in 1972. After merger with Norsk Hydro and several name changes the company changed name to Equinor to reflect the evolution and identity as an energy company for the generations to come. Equinor is today an energy company with more than 21000 employees developing oil, gas, wind and solar energy in more than 30 countries. The first oil field, Statfjord, was put in operation in 1979 and the company grew substantially in the 1980. Focus on microbial influenced corrosion (MIC) was first set late 1980 and our story starts around year 2000. This journey will take us through how sampling and analyses have been done and how interpretation of the results has changed with respect of MIC. In the beginning the samples analysed were mainly water samples but also deposits on biocoupons retrieved from the system were investigated. The analysis method was bacterial culturing. Nutrient content in the form of orthophosphate and dissolved organic carbon (DOC), and sulphate as an electron acceptor were analysed. Trends for the bacterial number and nutrient content were compared with corrosion rates measured with corrosion coupons. Today the bacterial analysis is carried out by qPCR analysis. New generation sequencing (NGS) is also tested out. Systematic analysis and experience provide understanding of the MIC potential. Based on this, the risk of failure due to MIC is calculated. Microbial monitoring with respect of MIC is, however, still not very precise. It is believed that in the future new markers are necessary to enhance accuracy in estimating risk of failure due to MIC. Further collaboration between industry and academia is needed to ensure technology development related to new markers for more precise MIC monitoring.

### **Offered Talks:**

**#33 On the necessity of multi-phase, field scale, and long term simulations in reservoir souring studies**

1. **Ali Mahmoodi** - *Danish Hydrocarbon Research and Technology Centre*
2. **Mohammad Reza Alizadeh Kiapi** - *Danish Hydrocarbon Research and Technology Centre*
3. **Moein Jahanbani Veshareh** - *Danish Hydrocarbon Research and Technology Center*
4. **Hamid Nick** - *Denmark Technical University*

Reservoir souring is a phenomenon in which Sulfate Reducing Bacteria (SRB) produce hydrogen sulfate, a hazardous and corrosive gas, through their activity in oil reservoirs, usually due to seawater-flooding. Several methods such as biocide injection and nitrate, nitrite, and perchlorate injection, have been proposed to mitigate this problem. Injection of biocides, which suppress microbial population and injection of nitrate, or perchlorate, which impact the activity of SRB through numerous inhibitory or competitive mechanisms, are some of such approaches. In this regard, several experimental and modeling studies have been done to help better understand the underlying mechanisms and efficiency of these methods in various scales. While these studies provide valuable information in terms of pathways and processes happening during reservoir souring and mitigation, the results of most of them cannot be directly attributed to the real-world conditions. Scarcity of injected reactants deep in the reservoir due to fast consumption near well-bore, existence of excess amounts of reactants deep in the reservoir, presence of oil and/or gas phase, which can retard or facilitate movement of some components, and total consumption of some reactants by the passage of time are some of the mechanisms we look into in different

scales. In this study, we represent a series of simulations on synthetic models in various conditions to emphasize how neglecting some essential factors, namely reservoir heterogeneity, flow patterns, presence of oil and gas phase, and temperature can severely alter the results, thus resulting in misleading conclusions, if attributed directly to the real-world situations.

## **#12 Application of novel technologies for the detection and monitoring of corrosive microbiomes in oilfields**

1. **Jaspreet Mand** - *ExxonMobil Upstream Research Company*
2. **Sven Lahme** - *ExxonMobil Upstream Research Company*
3. **John Longwell** - *ExxonMobil Upstream Research Company*
4. **Dennis Enning** - *ExxonMobil Upstream Research Company*

The diverse microbiomes inhabiting petroleum-transporting infrastructure can inflict severe corrosion on steel pipelines. Differentiating corrosive from benign microorganisms using microbiome surveys of pipelines remains challenging. We recently identified a biomarker capable of distinguishing corrosive from noncorrosive methanogenic archaea in laboratory samples. Here, we demonstrate the merit of the developed qPCR assay, which targets a specific archaeal hydrogenase (*micH*), for field application. Samples were collected from several offshore oil production pipelines where MIC was suspected to be an issue, as well as from locations with a negligible corrosion threat. In all cases, microbiomes were surveyed using 16S rRNA gene sequencing and with targeted qPCR assays for *micH*. Between  $2 \cdot 10^4$ – $1 \cdot 10^5$  gene copies of *micH*/g of pig debris were detected in a North American pipeline, where in-line inspection (ILI) data indicated ongoing internal corrosion. In contrast, *micH* was undetectable in an adjacent pipeline without active corrosion. The ability to implement this assay on more easily sampled planktonic microbiomes was then tested on African oilfield produced waters. Up to  $4 \cdot 10^2$  gene copies of *micH*/mL were detected in pipelines with a history of MIC, while *micH* was undetectable in wellhead fluids and other infrastructure where MIC was not occurring. The ability of this biomarker to be used as a robust indicator of MIC was demonstrated by correlation of *micH* with active corrosion in oilfield pipelines. Furthermore, detection of *micH* in produced water microbiomes can potentially reduce the reliance on biofilm samples for microbial monitoring, allowing for easy application of this novel MIC detection technology.

## **#14 Metagenome mining hydrocarbon environments for multidrug (biocide) resistance gene sources**

1. **Damon Brown** - *University of Calgary*
2. **Naomi Aggarwal** - *University of Alberta*
3. **Raymond Turner** - *University of Calgary*

Hydrocarbon pipelines are subject to microbiologically influenced corrosion (MIC) where the microbes degrade the metal directly through metabolism or indirectly through the production of corrosive by-products. To treat MIC, biocides are commonly used in batch treatments to control the microbial population. Over time, repeated use of a biocide will show reduced killing efficacy as the microbial community develops tolerance/resistance, resulting from multidrug resistance efflux pump (MDREP) genes being expressed and shared throughout the community. These genes are categorized into six superfamilies, small multidrug resistance (SMR), major facilitator superfamily (MFS), multidrug and toxic (compound) extrusion (MATE), ATP-binding cassette (ABC), resistance-nodulation-cell division (RND) and proteobacterial antimicrobial compound efflux (PACE). These systems extrude biocides such as QACs out of the cell. These genes are frequently located on mobile genetic elements allowing the genes to be shared between different

species, resulting in an overall increased community tolerance. Genes similarly annotated in different species share little nucleotide sequence identity and are susceptible to mutation, further diversifying their nucleotide sequences making them hard to follow/identify. Primers designed to target certain representative genes from these superfamilies were designed from multiple sequence alignments of annotated genes identified in genomes from six species chosen to represent a simplified MIC associated community (*Acetobacterium woodii*, *Bacillus subtilis*, *Desulfovibrio vulgaris*, *Geoalkalibacter subterraneus*, *Pseudomonas putida*, and *Thauera aromatica*). These primers were used to probe various hydrocarbon environments *in silico* and identify potential sources of these genes. Identifying the sources and abundance of these genes can help direct effective biocidal programs for downstream systems.

### **#3 Field Optimization of Biocide Treatment Based on a Novel Sessile Bacteria Monitoring Program**

1. **Amela Keserovic** - *Schlumberger*
2. **Øystein Birketveit** - *Schlumberger*
3. **Lisbeth Iversen** - *Schlumberger*
4. **Marko Stipanicev** - *Schlumberger*

To maintain the reservoir pressure and enhance the oil and gas recovery, a combination of deoxygenated seawater (SW) and produced water (PW) has been injected at an operator's field. A negative side effect of commingling SW and PW is a possible boosting of microbial growth and activity in the injection system due to an increased access to organic nutrients carried by the PW, resulting in biofouling and reduction in water injection rate. Glutaraldehyde-based biocide treatment employed from the field start-up showed a declining effect observed by the pressure increase inside the water injection system. In May 2017 a custom-built Biofilm Monitoring Unit (BMU) was installed at the operator's installation, downstream the PW and SW mixing point to monitor and optimize the biocide treatment and gain the control over the bacteria and biofilm growth. The BMU served as a source of biocide-treated and biocide-untreated biofilm samples that were periodically collected and analyzed. Sessile bacteria present within the samples were enumerated using Microbial InstaCheck. Since the BMU installation, more than 34 biofilm samples were extracted and analyzed. During this period, the biocide has been replaced with a biocide blend consisting of glutaraldehyde and quaternary amine compound, which proved to be a better alternative as it reduced the pressure inside the water injection system. In this paper the authors show an example of a successfully monitored and optimized biocide treatment campaign, as well as the challenges and problems encountered during the program.

### **#66 Selection in microbial islands creates a large core community with variable relative abundances**

1. **Verena Brauer** - *University of Duisburg-Essen*
2. **Lisa Voskuhl** - *University of Duisburg-Essen*
3. **Sadjad Mohammadian** - *University of Duisburg-Essen*
4. **Mark Pannekens** - *University of Duisburg-Essen*
5. **Shirin Haque** - *The University of the West Indies*
6. **Rainer Meckenstock** - *University of Duisburg-Essen*

Ecologists are facing the riddle that many microbial communities are taxonomically unpredictable yet functionally stable. Because taxonomic patterns do not permit inference of the underlying community assembly mechanisms, the relative importance of selection, dispersal, drift, and speciation is still unclear. The influence of selection is particularly obscure, as it can produce the

entire spectrum from constant to erratic taxonomic compositions, even in the absence of environmental disturbance or dispersal. In this study, however, we investigated exceptional mini-ecosystems that allowed unraveling of the underlying processes. We showed that 193 naturally replicated microbial communities that we isolated from  $\mu\text{l}$ -sized water droplets enclosed in heavy oil of the Pitch Lake, Trinidad, had highly variable relative abundances but shared a core community constituting on average  $68.0 \pm 19.9\%$  of the total community. The unique physical isolation of the droplets, as supported by a fluid-dynamics model, excluded dispersal as possible assembly process, and 16S rRNA gene sequence analysis and computational modelling revealed that speciation and ecological drift were unimportant. Hence, the results suggest that selection produces high taxonomic predictability within complex microbial systems in the form of a large core community, although relative abundances may vary.

### **#73 Microbial Communities in Biodiesel Storage Tanks Correlate with Fuel Composition**

1. **James Floyd** - *University of Oklahoma*
2. **Blake Stamps** - *Air Force Research Laboratory*
3. **Caitlin Bojanowski** - *Air Force Research Laboratory*
4. **Wendy Goodson** - *Air Force Research Laboratory*
5. **Bradley Stevenson** - *University of Oklahoma*

We conducted a survey of storage tanks containing biodiesel or ultra-low sulfur diesel across 17 military bases in the US to detect the extent and composition of microbial contamination. Fuel from the bottom of each tank was collected and the microbial communities present were characterized using high throughput sequencing of small subunit ribosomal RNA (SSU rRNA) gene libraries. FAME and *n*-alkanes from contaminated fuels were characterized and quantified using GC-MS. Microbial taxa known to increase carbon steel corrosion were investigated to determine if they were more prominent in contaminated fuels based on the fuel's composition using redundancy analysis modeling. The fungi Trichocomaceae were found to be prominent in fuels containing palmitoleic and myristoleic acid methyl esters while yeasts in the family Debaryomycetaceae were found to be prominent with fuels containing more pentadecanoic and oleic acid methyl esters. Correlations that were obtained using the redundancy models were then tested by generating growth curves for isolates from these fungal families using their preferential FAME as a sole carbon and energy source and determining if these organisms have better fitness than on substrates not predicted to be favorable. Teasing apart relationships between fuel composition and microbial community structure will generate new hypotheses about microbial community function in storage tanks and aid in the development of remediation and preventative efforts in contaminated biodiesel systems.

### **#55 Sulfidogenic microbial communities of the Uzen oil field and their resistance to biocides**

1. **Alexey Ershov** - *Research Centre of Biotechnology RAS*
2. **Diyana Sokolova** - *Research Centre of Biotechnology RAS*
3. **Tamara Babich** - *Research Centre of Biotechnology RAS*
4. **Ekaterina Semenova** - *Research Centre of Biotechnology RAS*
5. **Salimat Bidzhieva** - *Research Centre of Biotechnology RAS*
6. **Denis Grouzdev** - *Research Centre of Biotechnology RAS*
7. **Nurlan Zhaparov** - *Branch of the Limited Liability Partnership "KMG Engineering"*
8. **Tamara Nazina** - *Research Centre of Biotechnology RAS*

Microbiologically influenced corrosion is caused by prokaryotes reducing sulfate and other oxidized sulfur compounds, which produce hydrogen sulfide in the petroleum reservoir. To control sulfide accumulation, injection of biocides is used; however, formation of biofilms increases microbial resistance to biocides and thus prevents suppression of sulfidogenesis. Another way to control sulfide production is injection of a nitrate solution, which stimulates the growth of denitrifying/nitrate-reducing bacteria releasing nitrite into formation water. Nitrite inhibits the activity of sulfite reductase (*dsrA*), thereby decreasing the rate of sulfidogenesis in the reservoir. The aim of this work was to determine potential agents of microbial corrosion in the injection and formation water of the Uzen oil field (Republic of Kazakhstan) and the possibility of suppressing the growth of sulfidogenic prokaryotes in planktonic and biofilm forms. High-throughput sequencing revealed predominance of sulfate-reducing *Desulfonauticus* bacteria and the presence of thiosulfate-reducing bacteria of the genera *Brockia*, *Defluviitoga*, and *Thermosiphon*. Accumulation of up to 450 mg sulfide/L by enrichments was demonstrated by cultural methods. Addition of nitrate decreased sulfide production in a number of formation water samples. A significant increase in the resistance of microorganisms to biocides in the samples, where biofilms has been formed on mineral carriers (carbonate core and steel coupon), was shown. Thus, the prospects of applying both nitrate and biocides to suppress the growth of sulfidogenic prokaryotes and increased biofilm resistance to biocides have been demonstrated. This study was supported by the Russian Science Foundation.

## **#72 Geofluids facilitate a microbial dispersal cycle in the subsurface biosphere**

1. **Daniel Gittins** - *University of Calgary*
2. **Calvin Campbell** - *Natural Resources Canada*
3. **Martin Fowler** - *Applied Petroleum Technology*
4. **Anirban Chakraborty** - *University of Calgary*
5. **Srijak Bhatnagar** - *University of Calgary*
6. **Jayne Rattray** - *University of Calgary*
7. **Carmen Li** - *University of Calgary*
8. **Adam MacDonald** - *Government of Nova Scotia*
9. **Natasha Morrison** - *Government of Nova Scotia*
10. **Pierre-Arnaud Desiège** - *Natural Resources Canada*
11. **Casey Hubert** - *University of Calgary*

The seafloor biosphere is composed entirely of microbial biomass, but little is known about the effects environmental selection and dispersal have on this vast microbial ecosystem. We investigated endospore dispersal facilitated by geofluids originating from oil reservoirs and their persistence during burial in marine sediments from the NW Atlantic Ocean. Structural geology indicating deep subsurface to surface geofluid flow conduits and seabed morphological features that indicate near surface hydrocarbon migration pathways were identified by seismic reflection surveys. The presence of hydrocarbons migrating from oil reservoirs at these sites was confirmed through geochemical assessments of surficial marine sediment (top ~10 m) obtained by piston coring in up to 3,400 m water depth during three offshore expeditions. Heating of these sediments enriched thermophilic bacterial endospores that were assessed by 16S rRNA gene profiling to compare populations in sediments with and without thermogenic hydrocarbons. This revealed 42 unique amplicon sequence variants (ASVs) correlated with seepage. Metabolic features of these thermophiles, revealed by metagenomic sequencing, combined with their biogeography including both oil reservoirs and cold seep sediments, demonstrates a dispersal history that includes deep biosphere habitability followed by subsequent sporulation to survive colder surface environments. Coupled with this, the evidence of constant spore deposition into the seabed and persistence during sedimentation, shown by high levels of dipicolinic acid down core, provides evidence of a

geological cell cycle sustained by dispersal and burial. Our results provide evidence of a dynamic system of microbial transport in the deep biosphere, mediated by geofluids originating from oil reservoirs.

## **#62 Exploring the use of DNA-based monitoring tools in the biological monitoring of a gas pipeline located in the Peruvian Amazon.**

1. **Jose Miguel Seoane** - *Repsol*
2. **Juan de Dios Miranda** - *Repsol*
3. **Kat Bruce** - *Nature Metrics*
4. **Cuong Q. Tang** - *Nature Metrics*
5. **Alexandra Crampton-Platt** - *Nature Metrics*

Increasingly, areas of interest for oil and gas development are also being recognized and valued for their biodiversity resources. Hence, the ability to accurately monitor and preserve biodiversity has become an integral component of the goals of sustainable development acquired by the O&G Industry. Current methods for biodiversity monitoring are still based on direct observation, capturing, and counting specimens which implies high variability, long times and complex logistics. In this work we explore the use of environmental DNA as an alternative in the biological monitoring of a gas pipeline located in the Peruvian Amazon. A range of DNA-based biomonitoring techniques were applied in parallel with ongoing conventional monitoring during the wet and dry seasons, which allowed us to compare both methodologies and its associated field efforts. eDNA provided more unique taxa than conventional methods on fish, and similar levels of detection on major mammals, minor mammals (minus bats) and amphibia while cutting by 50% the sampling efforts when compared to conventional methods. These included more than 12 Red list vertebrate species which couldn't be identified by conventional methods. eDNA could therefore be considered as an alternative/complementary survey method for these taxa in future. For bats, birds, reptiles and insects, eDNA methods detected fewer species than conventional methods but still added new records in each case. In conclusion, eDNA could become the primary method for monitoring fish, major mammals, and minor mammals (minus bats) but a combined approach is likely to be needed for monitoring amphibians, birds, and bats.

## **Session 05: Alternative fuels to oil and gas**

### **Invited Talk:**

**From CO<sub>2</sub> to novel fuels – and beyond**

**Korneel Rabaey, Ghent University, Belgium**

Carbon capture and utilization (CCU) is gradually maturing. In 2022, a 85 kton ethanol plant using CO from the steel industry will be up and running in Belgium. Plans are to construct a second plant, producing methanol at 46 kton scale from H<sub>2</sub> and CO<sub>2</sub> around 2024. In my presentation I firstly want to discuss the nature of these production processes and the rationale for installing them. An unfortunate aspect of producing fuels from CO<sub>2</sub> is evidently the low market value of the initial building blocks. This is where microbial processes can come to the fore. Microorganisms can via diverse pathways incorporate methanol and ethanol and produce biopolymers, protein or more energy rich products such as medium chain fatty acids. The advantage of coupling bioprocesses directly to a CCU process is that the CO<sub>2</sub>, coming from the bioreactor, can be reintroduced into the initial CCU process. In my presentation, I will discuss using microbial protein

as an example how CCU processes can be coupled to microbial upgrading and what key considerations are.

## **Offered Talks:**

### **#15 Microbial communities in alternative aviation fuels**

1. **Ruth Barnes** - *University of Sheffield, Conidia Bioscience Ltd*
2. **Myrsini Chronopoulou** - *Conidia Bioscience Ltd*
3. **Alexander McFarlane** - *Conidia Bioscience Ltd*
4. **Joan Kelley** - *Conidia Bioscience Ltd*
5. **Steven Thornton** - *University of Sheffield*
6. **Stephen Rolfe** - *University of Sheffield*

Alternative fuels (biofuels) will become integral to the aviation industry, reducing the carbon footprint of air travel whilst meeting increased demand. However, there has been little consideration of the changes in the microbial communities that might occur in these new fuels. Shifts in the composition of microbial communities in fuel systems could have significant impacts upon testing and treatment of microbial contamination, needed to prevent system fouling and microbial induced corrosion. Laboratory microcosms were established, using contaminated real-world samples as inoculum, in different biofuels and in current standard Jet A-1, for comparison. Microcosms were sampled after two- and four -weeks. Samples were assessed for visual contamination and total dry biomass weighed. DNA was extracted and subjected to real-time quantitative Polymerase Chain Reaction(qPCR) and Next Generation Sequencing, targeting the 16S rRNA and ITS genes. In addition, organisms were isolated on minimal media with fuel hydrocarbons as the sole carbon source. Bacterial communities were dominated by *Pseudomonas* sp., while *Hormoconis resinae* was dominant in the fungal communities, with only small variations in their relative abundance between differing fuel types. The abundance of bacterial 16S rRNA genes was higher than that of fungal ITS genes. While the types of organisms that grew were similar across the fuel types, a variation in relative abundance of these organisms driven by fuel type was observed. This work demonstrates the application of laboratory microcosms to simulate fuel systems and gain insights into the communities that develop in aviation biofuels, helping to inform testing and maintenance regimes.

### **#58 Geological and microbiological characterization of rocks collected from deep and superficial sites for the study of potential underground hydrogen storage sites**

1. **Pierangela Cristiani** - *Ricerca sul Sistema Energetico -RSE*
2. **Andrea Franzetti** - *Università degli Studi di Milano-Bicocca*
3. **Francesca Pittino** - *Università degli Studi di Milano-Bicocca*
4. **Riccardo Castellanza** - *Università degli Studi di Milano-Bicocca*
5. **Andrea Bistacchi** - *Università degli Studi di Milano-Bicocca*
6. **Fabrizio Balsamo** - *Università di Parma*
7. **Fabrizio Storti** - *Università di Parma*

The storage of excess energy from non-programmable renewable sources using hydrogen as an energy vector is an option of great interest and goes into the direction of a progressive replacement of fossil natural gas with so-called renewable gases. A review of the possible critical issues, arising from this option for gas infrastructures and for hydrogen/methane gas storage highlights issues deserving further studies. To avoid risks and losses, possible sites for underground gas storage must be carefully selected and characterized. Due to the difficulty of

conducting on-site experiments, laboratory analogs of underground reservoirs are sometimes obtained from rock samples collected from underground deposits. However, it is often difficult to realistically reproduce the condition of deep reservoirs, from a microbiological and chemical point of view. Rocks collected from outcrops, hence at the surface, may exhibit different behavior than in deep reservoirs. On the other hand, surface sites can provide preliminary information from simpler and more approachable tests than deep ones. This work aims at identifying types of rocks representative of the condition of some geological sites that could potentially be of interest for hydrogen/methane storage. Differences in microbial colonization of rocks collected from sites at different depths are presented and discussed here. The microbial content of calcarenite rock samples (a detrital porous limestone, common in carbonatic reservoirs) collected from deep and superficial sites (with and without a bituminous component) was analyzed by gene sequencing (NGS Illumina, 16S rRNA marker).

# Flash presentations

## #22 Microbial corrosion under thiosulfate-reducing conditions by microbial communities in hydraulically fractured shale flowback waters

1. **Danika Nicoletti** - *University of Calgary*
2. **Subasthika Thangadurai** - *University of Calgary*
3. **Lisa Gieg** - *University of Calgary*

Infrastructure related to oil recovery by hydraulic fracturing of shale can fall victim to microbiologically influenced corrosion (MIC) by microorganisms that can survive the highly saline conditions of flowback waters. Thiosulfate, a thio-salt released from fractured shale, can be used by some microorganisms as an electron acceptor to produce sulfide, a highly corrosive compound. This study aimed to investigate biogenic sulfidogenesis by thiosulfate reduction and the associated corrosion in a microcosm experiment prepared using microbial cultures from flowback waters of two hydraulically fractured wells. The microbial cultures were incubated with carbon steel beads in a high-salt minimal medium containing thiosulfate, under conditions of either no added electron donor or added yeast extract. Incubations with no added electron donor had corrosion rates that remained below 0.016 mm/year and produced less than 2 mM sulfide. When yeast extract was added, corrosion rates reached 0.225 mm/year with the production of 9.30 mM sulfide and 4.12 mM acetate (a product also implicated in MIC). When the same samples were incubated with biocide (benzalkonium chloride (BAC)), sulfide and acetate production were inhibited. These results indicate thiosulfate-reducing microbial activity that is paired with high corrosion rates in the presence of a readily-utilized electron donor. Microbial community analysis revealed a high relative abundance of *Halanaerobium*, a fermentative, thiosulfate-reducing taxon in the initial cultures. The microbial community analysis of the samples after the experimental incubation period is underway, and will reveal the persistence of *Halanaerobium* or other potentially corrosive organisms that are active under thiosulfate-reducing conditions.

## #16 MIC assessment in Argentina's Unconventional O&G Facilities: critical control points and mitigation strategies challenges

1. **Maria Pagliaricci** - *YTEC*
2. **Juliana Soler Arango** - *YTEC*
3. **Walter Morris** - *YTEC*
4. **Walter Vargas** - *YTEC*

The reserves of shale gas and shale oil in Argentina's unconventional reservoirs represent the second and fourth-largest in the world, respectively. To develop these fields, massive amounts of water are injected into the reservoir by hydraulic fracturing operations. Water flowback associated with the initial production stages has detrimental consequences on wells and surface facilities run life due to Microbiologically Influenced Corrosion (MIC). This work applies molecular biology methods in MIC analysis: ATP determination, Microorganisms genes count through qPCR and Bacterial Diversity with a metagenomics approach. The information obtained is used to review the monitoring procedures and mitigation strategies. The assessment results in three main locations with more than 200 samples lead to the following conclusions: i) the microbiome present in fracturing fluids has a MIC potential and can degrade gel formulations; ii) microbiomes in flowback water are different as they are native to the reservoir, iii) flowback water microbiome contains mainly bacteria and their metabolisms are rather diverse not just limited to sulfate-

reducing bacteria (SRB). The traditional methods of monitoring SRB (culture) and typical kill test to assess microbial activity in unconventional facilities are not enough to understand MIC and its consequences. The challenge is to design specific monitoring strategies for critical microorganisms and develop effective mitigation strategies to control microbial activity and mitigate MIC.

#### **#24 Dispersal of sulfate-reducing biofilms with biocides using flow cell systems**

1. **Gloria Okpala** - *University of Calgary*
2. **Connor Horemans** - *University of Calgary*
3. **Lisa Gieg** - *University of Calgary*

Surface-attached sulfate-reducing microorganisms (SRM) contribute to souring in oil reservoirs, oil pipelines, and storage facilities. Such biofilms are generally resistant to inhibitory agents due to the limited penetrability of the chemical agents into the biofilm strata. The nitrosating compound sodium nitroprusside (SNP) has been shown to be an effective biocide preventing planktonic SRM activity at low concentrations (0.05 mM) but its efficacy to treat already established biofilms is unknown. As it is highly diffusible, SNP may have the ability to penetrate biofilm strata. In this study, the effect of SNP on SRM biofilms grown in flow cell systems vs. planktonically-grown cells was investigated. SRM enriched from pipeline sludge were used for the experiment. Planktonic SRM activity was not affected by the addition of 0.025 mM SNP, only partially inhibited by 0.05 mM SNP, and was completely inhibited at 0.1 mM SNP. In contrast, biofilms were only transiently inhibited by 0.1 mM SNP (the highest dose tested) during biocide injection and sulfide production resumed once biocide injection ceased. Thus, higher concentrations of SNP were required to inhibit sulfide production in SRM biofilms. During treatment of biofilms with SNP, detached clumps of cells were observed, while in the control treatment, this was not observed. The amount of DNA extracted from the bulk fluids of both control (6.0 ng/μl) and treated biofilms (33 ng/μl), suggests that SNP caused dispersion of biofilm cells. Therefore, SNP appears to be at least transiently effective against problematic biofilms that plague the oil and gas industry.

#### **#56 Comprehensive microbial and metallurgic study of water injection cement-lined flanks**

1. **Zakariya Yacoub** - *Saudi Aramco*
2. **Husam Khanfar** - *Saudi Aramco*
3. **Anaam Shaikh** - *Saudi Aramco*
4. **Ammar Alsaqer** - *Saudi Aramco*
5. **Abdulkareem Alqahtani** - *Saudi Aramco*

Comprehensive microbial analysis of Water Injection cement-lined flanks conducted in Research and Development Centre (R&DC) to assess the flanks durability and toleration toward MIC and investigate the root cause of failure in cement-lined water injection flank. The study involved characterization of colonizing microbes together with other supporting analyses to investigate the contribution of microbes to the failure. Different groups of detrimental microbes were characterized using two different and well-established techniques; the gene based quantitative polymerase chain reaction (qPCR), and the culture-based method, phenol red dextrose (PRD) which was used for the detection and enumeration of acid producing bacteria (APB). Along with that, geochemical analysis and metallurgic examination performed to better identify the root cause of corrosion in the flank. Result revealed the presence of planktonic methanogens in the water samples, various types of sessile microbes were also detected including SRB, APB, SRA. The results revealed the colonization of some of the well-known corrosion causing microbes in low to moderate numbers associated with corrosion products detection. The TGA scraps analysis

suggest that the inorganic matters of the material were found to be predominant ( $\geq 86$  wt%) though the scraped powder from the inner surface wall of the flank contained microbial corrosion products (mackinawite) with appreciable amounts of formation rock materials. Morphology of the detached cement fragment shows that the microbes flourish in water stagnation zones located in-between pipe metal wall and the fragile semidetached cement lining, which consequently leads to the formation of local focal corrosion.

#### **#41 Corrosivity of heterologously expressed MIC hydrogenases from methanogenic archaea**

1. **Sherin Kleinbub** - *Federal Institute for Materials Research and Testing (BAM)*
2. **Andrea Koerdt** - *Federal Institute for Materials Research and Testing (BAM)*

Over the last 20 years, it has become clear that, in addition to SRB, several classes of microorganisms are involved in microbiologically influenced corrosion (MIC), e.g. Methanogenic Archaea (MA). However, little is known about the corrosion mechanisms of MA. A recently identified [NiFe]-hydrogenase from *Methanococcus maripaludis* OS7, located in the MIC-Island was identified as the suspected causative agent of methanogen-induced-MIC (Mi-MIC). Another more corrosive MA, *Methanobacterium*-affiliated IM1, harbors genes for these hydrogenases too, with small changes in the amino-acid sequence. To verify whether these changes of the protein structure could be the reason for the different corrosion behavior, we expressed the small and large subunit of the proteins of both strains' codon-optimized in *Escherichia coli*. In the following step, the activity of the expressed proteins will be investigated and compared *in vivo* and *in vitro* for a better understanding of the corrosion mechanisms and corrosivity of different corrosive methanogenic strains.

#### **#19 The potential role of the in-place soured formation water on reservoir souring**

1. **Mahsan Basafa** - *Memorial University of Newfoundland*
2. **Kelly Hawboldt** - *Memorial University of Newfoundland*

Reservoir souring is a widespread phenomenon in seawater-flooded reservoirs where seawater containing sulfate is injected for maintaining the pressure. In this case,  $H_2S$  is generated as a result of the biogenic activity of sulfate reducing microorganisms, and is carried with the produced fluids to the topsides representing a corrosion and safety hazard. Furthermore, a fraction of  $H_2S$  could dissolve into the connate formation water in the reservoir as the reservoir fluid flows to top surface facilities. In theory, the  $H_2S$  dissolved into the static phases of reservoir fluid during the active souring process, could enrich the flowing reservoir fluids with  $H_2S$  even after souring control measures had been put in place upstream. The liberation of this dissolved  $H_2S$  could explain the behavior of  $H_2S$  in reservoirs being treated for souring, where the  $H_2S$  in the topsides production fluids initially drops, indicating the souring treatment is working, and then spikes up. The effect of hydrogen sulfide liberation from a soured aqueous phase present in the reservoir to the reservoir fluid during production on reservoir souring was investigated in this study through evaluating the partitioning behavior of  $H_2S$  in a multiphase system using non-ideal gas and solution thermodynamic models.

## **#25 Efficacy of biocide treatments to control souring under different temperatures and high salinity conditions in oil fields**

1. **Rita Eresia-Eke** - *University of Calgary*
2. **Gloria Okpala** - *University of Calgary*
3. **Lisa Gieg** - *University of Calgary*

Microbial sulfide production in crude oil reservoirs can lead to souring. This unwanted process can be harmful to workers, may lead to infrastructure corrosion, and devalues the quality of crude oil, necessitating its control. In this study, we examined the role of sulfate-reducing microorganisms (SRM) as key contributors to the souring process in a high temperature (~70°C) and high salinity (~1.4 M Eq. NaCl) light oil field (°API of 37.8), along with the efficacy of biocide treatments under these conditions. Samples were enriched for sulfate-reducing microbial communities at temperatures ranging from 30 to 70°C, with souring achieved to date at 30 and 50°C. Soured cultures were subjected to 5 biocide treatments, namely, sodium nitroprusside (SNP), glutaraldehyde (GLUT), benzalkonium chloride (BAC), THPS, and bronopol added at varying concentrations. At 30°C, enrichment cultures were sensitive to biocides with the following minimum inhibitory concentrations (MIC): 30 ppm SNP, 50 ppm BAC, 40 ppm THPS, and 500 ppm GLUT. The enrichments were more tolerant to bronopol, which was effective only at an increased MIC of 1500 ppm. At 50°C, all biocides were effective at same MICs, except bronopol which showed effectiveness at a lower MIC of 300 ppm. At elevated temperatures, bronopol degrades to formaldehyde and nitrite, having a double inhibitory effect on tested enrichment cultures. Ongoing testing at other temperatures will reveal whether similar trends in biocide efficacy are observed under high salinity conditions, and will help to determine biocide MICs that effectively treat souring in different global crude oil reservoirs.

## **#53 Microbial Quality of Produced Water for Reservoir Reinjection**

1. **Sarah Al-Aqeel** - *Saudi Aramco*
2. **Nada Alghamdi** - *Saudi Aramco*
3. **Xiangyang Zhu** - *Saudi Aramco*

Reinjection of produced water (PW) into oil reservoir is a common practice in oil and gas industry for pressure maintenance. However, improper use of PW, especially when it is mixed with other source waters, can have serious consequence to oil production, reservoir souring being one of the examples. In an oilfield system, five different source waters were proposed to be mixed at various ratios for reservoir injection. The source waters include PW from an oil processing facility, Aquifer water (AW) from aquifer wells, and waters from settling tanks (TK), evaporation pond, and API oil-water separator in a dry crude tank farm. The study evaluated the chemical compositions of the source waters and microbial quality in the original source waters and five mixed waters at various ratios. The objective is to determine the optimal mixing ratio for PW reinjection with minimal adverse impact from microbial populations, focusing on sulfate-reducing prokaryotes (SRP). The mixed water at 95% PW and 5% TK water ratio showed the lowest counts of general bacteria and SRP after 3 and 6 weeks of incubation at 45°C. The mixed water at the ratio of 45% PW, 45% WW, and 10% TK water showed high number of general bacteria ( $>10^6$ /ml) and SRP ( $>10^3$ /ml). The results indicated that the microbial quality of source water has to be closely monitored before reservoir injection. As a best practice, a biocide treatment program on reinjection PW should be implemented to protect the reservoir from biogenic H<sub>2</sub>S production and microbial corrosion in long-term.

## **#70 The Effect of a Biocide on Bacteria and Archaea to Mitigate Microbiologically Influenced Corrosion**

1. **Ciara Goldsmith** - *University of Calgary*

2. **Lisa Gieg** - *University of Calgary*

Crude oil transmission pipelines can be at risk for microbiologically influenced corrosion (MIC). Over time, sludge comprised of crude oil, sand, water, and microorganisms may build up within the pipeline which can expedite MIC via under-deposit corrosion if not treated or removed using physical and/or chemical treatment methods. In this study, we investigated the effect of a glutaraldehyde-based biocide (GLUT) on the corrosive capabilities of microbial communities present in two different pipeline sludge samples collected from the nose of a pigging unit used to clean the same section of a transmission pipeline in February and August 2019. Incubations containing sludge and carbon steel beads in the presence or absence of GLUT were monitored over 3 months for microbial activity, community composition using amplicon sequencing, and corrosion. *Acetobacterium*, a H<sub>2</sub>-consuming, acetate-producing taxon, dominated the sludge samples (~50% relative abundance). In the absence of GLUT, the relative abundance of *Acetobacterium* dropped to 10-20% relative abundance. However, in the presence of GLUT, the relative abundance of *Acetobacterium* did not change from that of the original sample, although corrosion rates and microbial activity decreased. A similar study using sludge from the same pipeline also previously showed that methanogens (also H<sub>2</sub>-consumers) remained similarly abundant in the presence of a GLUT-based biocide. Ongoing qPCR assays targeting the 16S rRNA gene, the *mcrA* gene (for methanogens), and *Acetobacterium* in the sludge incubation will help to determine whether H<sub>2</sub>-utilizing microorganisms may be differentially affected by biocide (GLUT) treatment, which may have implications for pipeline treatments.

## **#45 Assessment of anaerobic aromatic hydrocarbon degradation pathways of two metagenome assembled genomes (MAGs) obtained from ad jet-fuel contaminated aquifer**

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2. **Adriana U. Soriano** - *PETROBRAS/ CENPES*

3. **Erika Valoni** - *CENPES*

4. **Marcus P. Baessa** - *CENPES*

5. **Hans H. Richnow** *Helmholtz Centre for Environmental Research (UFZ)*

6. **Carsten Vogt** - *Helmholtz Centre for Environmental Research (UFZ)*

7. **Valéria M. Oliveira** - *University of Campinas – UNICAMP*

Natural attenuation represents all processes that govern contaminant removal in the environment, including microbial-mediated degradation. Its rate and efficiency depend on multiple factors, including the abundance of microorganisms and their degradation genetic potential. This study aimed to reconstruct genomes from metagenome datasets of *in situ* microcosms (BACTRAP<sup>3</sup>s) amended with hydrocarbons (benzene, toluene and naphthalene) exposed for 120 days in a jet fuel contaminated aquifer. An automatic bioinformatic pipeline called *SqueezeMeta* was used, which employs *Metabat2* and *MaxBin* tools for binning approach. Two high-quality Metagenome Assembled Genomes - MAGs (ID1 ~3.9 Mbp, 90% completeness, 0.78% contamination / ID2 ~4.7 Mbp, 98% completeness, 1.82% contamination) obtained through the binning approach were affiliated to *Geobacter* and *Pelotomaculum* genera. Based on the metabolic reconstruction, a model for aromatic hydrocarbon anaerobic degradation in the jet fuel contaminated aquifer was proposed, comprising the complete degradation of benzene activated by carboxylation and of toluene activated by fumarate addition by a *Pelotomaculum* member coupled to sulfate reduction

and/or primary and secondary fermentation by a *Pelotomaculum* member coupled to nitrate or iron reduction by a member of *Geobacter* genus via syntrophic interaction. In summary, by combining *in situ*, high-throughput sequencing and binning, we were able to identify the main microorganisms and infer the metabolisms potentially involved in the activation and biodegradation of benzene and toluene in a jet fuel contaminated aquifer, contributing to the understanding of anaerobic biodegradation processes that take place in subsurface environments.

#### **#46 Structural Diversity and Bioactivity of Novel Antimicrobial Compounds for Mitigation of Microbial Contamination in Fuel**

1. **Osman Radwan** - *University of Dayton Research Institute*
2. **Amanda Barry** *University of Dayton Research Institute*
3. **Oscar Ruiz** *Air Force Research Laboratory*

Microbial contamination in fuel is a challenge faced by the military and commercial sectors alike and demands the development of effective control strategies for mitigation. While broad-spectrum chemical biocides are effective at reducing microbial contamination in fuel, the toxicity and hazardous effects restrict their use. Therefore, alternative eco-friendly biocides are in dire need. This study aimed to identify new antimicrobials produced by environmental microorganisms isolated from fuel systems. Further, we attempt to elucidate the molecular and cellular mechanisms by which these microorganisms employ antimicrobials to gain an adaptive and growth advantage over other members of the fuel-degrading microbial community. A total of five hundred environmental microbial isolates were screened for production of antimicrobial compounds. Our screening identified 15 promising isolates that provided high growth inhibition of fuel-degrading microorganisms including *Pseudomonas putida* (Gram-negative bacteria), *Gordonia* sp. (Gram-positive bacteria), *Yarrowia lipolytica* (yeast) and *Hormoconis resinae* (filamentous fungi). Cell-free culture filtrates, were tested on multiple fuel-degrading bacterial and fungal species showing inhibition of cell growth and spore germination. Currently, we are applying Liquid Chromatography-Quadrupole Time of Flight Mass Spectrometry (LC-QTOF MS) to characterize the antimicrobials. This research will significantly expand our understanding of how microorganisms produce and use antimicrobials as an adaptive mechanism to gain an evolutionary advantage in the fuel-degrading microbial community. This knowledge will be translated to the development of eco-friendly biocides to reduce biocontamination in fuel systems.

#### **#30 Single-step bacterial detection in aviation jet fuel using aptamer-based fluorescence switching**

##### **Authors**

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Bacterial contamination in aviation fuel is a major cause of concern for the aircraft industry. Traditionally, bacterial contamination in aviation fuel is measured using immunoassays, molecular methods and cell culture that need laboratory infrastructure and skilled operator and can take from a few hours up to a few days, leading to longer aircraft downtimes. Faster and simpler tests like lateral flow devices largely lack the sensitivity required for an effective onsite test. Here, we employed an “aptamer switch” that performed bacterial identification and transduction in a single

step by going from a 'Fluorescence OFF' to a 'Fluorescence ON' state on binding with bacteria. 100 µL jet fuel (JET A-1) contaminated with *Pseudomonas aeruginosa* was spiked in a 100 µL buffer solution of an aptamer having affinity against this Gram-negative bacterial species. 600 cfu (colony forming unit) and 60 cfu of the bacteria were detected respectively within 5 min and 60 min of bacterial spiking directly by measuring the increase in fluorescence intensity using a microplate reader. The detection is quantitative as the fluorescence intensity increase is proportional to the bacterial load. These initial results suggest the potential for aptamer-based fluorescence switching to be employed as a rapid, easy, and relatively low-cost onsite test in routine aircraft maintenance for early identification of critical levels of bacterial contamination in aviation fuel. This can allow for timely interventions necessary to pre-empt any severe impact on aircraft performance, safety, operational efficiency, and costs.

## **#78 Genomics and in silico prospecting of biosurfactants from bacteria in the oil industry**

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Biosurfactants are organic compounds capable of decreasing the surface or interfacial tension of liquids, solids and gases, and produced by a wide variety of microorganisms. Faced with the need to search for new compounds that can sustainably meet the high demand for synthetic surfactants, biosurfactants offer a promising alternative, presenting important advantageous characteristics such as biodegradability, adaptation to varied conditions, ecological acceptability and the possibility of being customized through genetic modification. This study aimed to perform genomic prospecting of bacteria from the Petroleum and Energy Research Collection (APPE), previously isolated from environments of the oil industry, for the production of secondary metabolites with biotechnological application, with an emphasis on biosurfactants. Forty-nine strains of bacteria were cultivated and screened for surface tension reduction, emulsification and demulsification, in order to select the best performing strains. Four strains were selected (*Pseudomonas aeruginosa* 2054, *Bacillus safensis* 970, *Bacillus* sp. 1963 and *B. oceanisediminis* 1316) and sequenced through the Illumina platform (Illumina®Sequencing) for further analysis of genome assembly and annotation of predicted genes. A search for metabolic pathways that code for the production of secondary metabolites was carried out through antiSMASH platform and ImproveAssembly program. Consistently with the previous culture-based screening, clusters for biosurfactant production (rhamnolipids, surfactin) were found, as well as antibiotic (plantazolicin, bacilysin, among others) and siderophore (pyochelin, petrobactin, pyoverdine, among others) production clusters. These results highlight the biotechnological potential a microbial culture collection may offer in the petroleum and energy industry, contributing to the bioeconomy and sustainable development of the productive chain.

### #39 Engineering *Bacillus* spp. for in situ Enhanced Oil Recovery

1. **Miguel Cueva** - *Cemvita Factory*
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Microbial enhanced oil recovery (MEOR) is a well-documented technique within the oil and gas industry. One of the limiting factors in MEOR applications is the limited quantities of biosurfactant produced by indigenous in situ bacteria under anaerobic conditions. To increase biosurfactant production beyond what is possible naturally with the indigenous bacteria and under ideal environmental and nutritional conditions, three genes *yerP*, *phrC*, and *comX* were chosen as targets for genetic engineering as means of exploring its potential to overexpress biosurfactant (surfactin) production in the model *Bacillus subtilis*. The genes *comX* and *phrC* encode peptides that positively activate the *srfA* gene which is responsible for producing surfactin while *yerP* is involved in the efflux of surfactin. In abundant concentrations, these peptides significantly increase the surfactin production. The ability to clone and express genes in *B. subtilis* was first confirmed by successful cloning and expression of the green fluorescent protein (GFP). Subsequently, the new *phrC* and *comX* sequences were successfully introduced in the receptor bacteria. Currently we are evaluating the concentration of surfactin produced by the new engineered strain and comparing the production against its counterpart wild type. Experiments are being performed under aerobic and anaerobic conditions. Production of surfactin by the wild type *B. subtilis* ranged from 83.4 and 1.98  $\mu\text{g/L}$  under aerobic and anaerobic conditions respectively as measured by HPLC-MS. Preliminary results point out the natural capabilities of *B. subtilis* to produce biosurfactant and to serve as chassis for potential overexpression of genes of great applicability potential in MEOR.

### #34 A Comprehensive Multi-Phase Pore-Scale Model for Dynamic Biofilm Evolution in Porous Media

1. **Mohammad Reza Alizadeh** - *Danish Hydrocarbon Research and Technology Centre*
2. **Ali Mahmoodi** - *Danish Hydrocarbon Research and Technology Centre*
3. **Moein Jahanbani Veshareh** - *Danish Hydrocarbon Research and Technology Centre*
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Reservoir souring is the generation of  $\text{H}_2\text{S}$  due to microbially induced activities by sulfate reducing bacteria (SRB) in oil reservoirs.  $\text{H}_2\text{S}$  is corrosive, toxic and can cause considerable health and environmental issues. Hence, reservoir souring mitigation is of great importance and has been a popular and desirable research area particularly during the past decade. Numerical models are commonly used to study reservoir souring generation and mitigation processes. These models can be categorized into three different classes from the scale point of view: pore-, core- and field-scale. There exist several models presented in the literature so far for core- and field-scale simulations, however, the numerical studies of reservoir souring at the pore-scale are limited. One of the challenges for the modelling is relating permeability to ever-changing porosity of the domain. Biofilm production due to microbial growth, can change the pore structure and consequently the flow path. The intensity of this change is a function of several parameters such as pore structure, bio-availability, fluid velocity, and temperature among others. In this work, we present a comprehensive multi-phase pore-scale model for studying the dynamics of biofilm growth and its effect on porosity and permeability. In this model, we consider SRB exist originally in the pore space (which is saturated with water and oil) and the required sulfate and volatile fatty acid for SRB's metabolism come from the injecting water phase and the existing oil phase,

respectively. We conduct several simulations to derive an upscaled porosity-permeability relation for various reservoir conditions.

### **#77 Microbiological investigation helps to prevent corrosion risks at Saudi Aramco's Sulfur Recovery Plant**

1. **Husam Khanfar**- *Saudi Aramco*
2. **Alexander Grigoryan**- *Saudi Aramco*
3. **Xiangyang Zhu**- *Saudi Aramco*

A comprehensive compositional and microbiological investigation was conducted to control microbial implications in corrosion processes at a Saudi Aramco Sulfur Recovery Plant (SRP) that supports refineries in sulfur, hydrocarbon and ammonia removal. Medium to high loads of total bacteria and such corrosive microbes as sulfate-reducing bacteria, iron-oxidizing bacteria and methanogens were revealed in the sludge samples from SRP pipeline system. High-throughput sequencing indicated presence of bacterial orders Bacteroidales, Cytophagales, Bacillales, Clostridiales, Ignavibacteriales, Burkholderiales, Rhodocyclales, Desulfuromonadales, Pseudomonadales and Xanthomonadales in examined samples. No bacteria were detected on the surface of SPR's metal vessels with signs of corrosion, however the compositional analysis of inorganic compounds in the sludge samples suggested that these were majorly corrosion products and bacteria might contribute in their formation. While the organic fraction of the sludge samples was of lube oil/grease nature. Further studies linking the microbial activity and corrosion reactions in the SPR system are required to understand the root cause of the potential failures and maintain the pipeline integrity.

### **#67 Environmental Genomics Applications for Environmental Management Activities in the Oil and Gas Industry - State of the Art Review and Future Research Needs**

1. **Marc Skinner** - *Stantec*
2. **Jeffrey B Pollock** - *Chevron Energy Technology Company*
3. **Nicolas Tsesmetzis** - *Shell International Exploration and Production Inc.*
4. **Thomas Merzi** - *Total*
5. **Cyril Mickiewicz** - *Eni Natural Resources*
6. **Anita Skarstad** - *Equinor*
7. **Paola M Pedroni** - *Eni Natural Resources*
8. **Michael Marnane** - *Chevron Energy Technology Company*
9. **Jordan C Angle** - *ExxonMobil U*

The International Association of Oil and Gas Producers (IOGP) Environmental Genomics Joint Industry Program (JIP) was formed in June 2019. The aim of the JIP is to facilitate the development of guidelines for the application of environmental genomics to support environmental management activities in the oil and gas industry. Towards this goal, a white paper summarizing the state-of-the-art in environmental genomics research and how it may be used to advance technology development opportunities for the oil and gas industry was drafted. In addition to the literature review, consultation of professionals from academic, regulatory, and industrial backgrounds with expertise on these topics was conducted. While there was a consensus that the application of environmental genomics has advanced greatly in a short period of time with demonstrable benefit potential, there was acknowledgement that key aspects of best management practices are still lacking. Through the academic, regulatory, and industrial consultation, specific environmental genomics study areas and applications requiring further development and refinement were identified. These include: methodological standardization,

persistence and dispersal of eDNA; eDNA data integration with various other data types; improvement of reference databases; and refinement of molecular indices. Based on the above and considering the most efficient path to greater regulatory uptake for environmental genomic approaches for the oil and gas industry, the JIP's recommendation is to pursue a Common-Garden Experiment. Such experiment should seek the involvement and ultimately endorsement from the Regulators marking the path towards wider regulatory acceptance and uptake of eDNA-based approaches.

# Posters

## #80 Fluid bio-decontamination with biocidal ceramic filters

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Biofouling is a global socio-economic concern allied to premature materials bio-corrosion and high human health risks, particularly in fluid circuit systems (e.g. water circuits). Most effective anti-biofouling strategies comprise the controlled release of toxic bioactive agents into the contaminated surfaces, which imply limited protection, significant ecotoxicity and promotion of microorganism's resistance. This study aimed to investigate the antimicrobial potential of a newly developed non-biocide-release coating strategy<sup>1</sup>, to generate bioactive monolithic filters for bio-decontamination of submerged surfaces. This new antifouling system comprises the grafting of Econea biocide in polymeric coatings, such as polydimethylsiloxane (PDMS) and polyurethane (PU) based marine paints, used to coat ceramic monolithic filters. The antimicrobial potential of the developed bioactive filters was evaluated against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecalis* (*E. faecalis*)<sup>2</sup>. The best results, in terms of antimicrobial activity and biocide release, showed a significative delay and a decrease of up to 66% in the population of MRSA bacteria on ceramic filters coated with PU-based coatings containing grafted Econea biocide, and no evidence of biocide release after being submerged for 45 days in water. Biocidal PU-based surfaces were also less prone to *E. faecalis* biofilm formation under flow conditions showing an average reduction of 60% after 48 h. Biocidal coated filters showed to be a potential eco-friendly alternative for minimizing the environmental risks associated with biofouling formation in fluid industrial systems.<sup>1</sup>Silva E. R. et al., *Science of the Total Environment*, **2019**, 650, 2499-2511. <sup>2</sup>Ferreira O. et al., *Coatings*, **2021**, 11 (3), 323.

## #81 Ecological selection for halotolerant, hydrocarbon-degrading bacteria enhanced biodegrading under saline conditions

1. **Ali Akbari**- *Cornell University*

2. **Subhasis Ghoshal**- *McGill University*

Surface and groundwater resources in oil production sites are often contaminated with salt and hydrocarbons from produced waters. The adaptability of indigenous microbial communities to salinity is a key factor for natural attenuation or bioremediation of co-contaminated sites. Here, we demonstrate enhanced hydrocarbon biodegradation under saline conditions of up to 5% in

soil slurries. Significantly higher mineralization rate (up to 3 times) in saline systems than non-saline systems was associated with significant enrichment of *Actinobacteria* phylum and increased up to 4.8 times in the absolute abundance of the indigenous hydrocarbon-degrading *Dietzia*. Meanwhile, the general population size decreased up to 5.2 times with salinity. The *in-situ* hydrocarbon degradation activity (gene expression assay) of *Dietzia* was also significantly enhanced (up to 16-fold increase) in saline systems. The results provide novel insights into the feasibility of microbiome-informed treatment processes for saline hydrocarbon contaminated sites.

## **#82 Characterization and degradation potential of hydrocarbon-degrading consortia from surface and deep waters of the Eastern Mediterranean Sea**

- 1. Georgia Charalampous** - *Technical University of Crete*
- 2. Efsevia Fragkou** - *Technical University of Crete*
- 3. Konstantinos A. Kormas** - *University of Thessaly,*
- 4. Alexandre B. De Menezes** - *National University of Ireland Galway;*
- 5. Paraskevi N. Polymenakou** - *Hellenic Centre for Marine Research,*
- 6. Nikos Pasadakis** - *Technical University of Crete, Institute of Petroleum Research, Foundation for Research and Technology Hellas*
- 7. Nicolas Kalogerakis** - *Technical University of Crete, Institute of Petroleum Research, Foundation for Research and Technology Hellas*
- 8. Eleftheria Antoniou** - *Technical University of Crete*
- 9. Evangelia Gontikaki** - *Technical University of Crete*

The diversity and degradation capacity of hydrocarbon-degrading consortia from surface and deep waters of the Eastern Mediterranean Sea were studied in time-series experiments. Microcosms were set up in ONR7a medium at *in situ* temperatures, 25 °C and 14 °C for Surface and Deep consortia respectively, and crude oil as the sole source of carbon. The Deep consortium was additionally investigated at 25° C to allow the direct comparison of degradation rates to the Surface consortium. In total, ~50% of alkanes and ~15% of polycyclic aromatic hydrocarbons were degraded in all treatments by day 24. Approximately ~95% of the total biodegradation by the Deep consortium took place within 6 days regardless of temperature, whereas comparable levels of degradation were reached on day 12 by the Surface consortium. Both consortia were dominated by well-known hydrocarbon-degrading taxa. Temperature played a significant role in shaping the Deep consortia communities with *Pseudomonas* and *Pseudoalteromonas* dominating at 25°C and *Alcanivorax* at 14°C. Overall, the Deep consortium showed a higher efficiency for hydrocarbon degradation within the first week following contamination, which is critical in the case of oil spills, and thus merits further investigation for its exploitation in bioremediation technologies tailored to the Eastern Mediterranean Sea.

## **#83 High pressure sampling and experimentation for the study of deep-sea oil spills**

- 1. Fragkou E** - *Technical University of Crete*
- 2. Charalampous G** - *Technical University of Crete*
- 3. Gontikaki E** - *Technical University of Crete*
- 4. Marinakis D** - *Technical University of Crete*
- 5. Kalogerakis N** - *Technical University of Crete, <sup>2</sup>Foundation for Research and Technology Hellas*
- 6. Antoniou E** - *Technical University of Crete*

The degradation of hydrocarbons by indigenous microbial communities from deep-sea environments is studied largely at atmospheric pressure entailing the risk of over- or under-estimating biodegradation rates. We present a system for high-pressure seawater sampling and experimentation, which is based on a 3-step procedure. A high-pressure sampling device (HP-Sampler), equipped with a unidirectional check valve, is set to retrieve seawater at a specific depth range and maintain pressure during retrieval. A known volume of sample from the HP-sampler is then passed into a high-pressure reactor (HP-Reactor) for experimentation via a high-pressure piston pump (HP-Pump) without disruption of the pressure continuum. We used this set-up to study the degradation of crude oil hydrocarbons at plume concentrations by deep-sea microbial communities from the Eastern Mediterranean Sea with and without dispersant application. Un-decompressed seawater from 600 – 1000 m depth was retrieved and successfully incubated for 77 days in the HP-Reactor at 10MPa and *in situ* temperature (14°C). In the first part of the experiment, light Iranian crude oil was added at day 0 and was replenished regularly until day 35. The incubation was maintained for the second part of the experiment (42-77 days), which involved the addition of dispersant (1:25 v/v COREXIT 9500) with weekly replenishment. Subsampling took place to determine alkane and aromatic hydrocarbons concentration. Our results show high capacity of the indigenous microbial community for alkane degradation regardless of dispersant application. Particularly high PAH removal (95%) was achieved only when oil was dispersed.