

ISMOS⁹

Edinburgh, UK

June 27-30, 2023



Abstract Book June 2023

Editors:

Annie Biwen An-Stepec, Julia de Rezende, Torben Lund Skovhus and Corinne Whitby.

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Welcome Note

Dear Delegate,

Welcome to ISMOS-9, Edinburgh, Scotland.

This is the 9th 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 energy sector. 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 encourage beneficial activities (e.g. hydrocarbon biodegradation for bioremediation and resolve potential challenges (e.g. microbiologically influenced corrosion and souring). ISMOS is continually pushing the frontiers in research for the energy sector and as such we introduce for the first time to the ISMOS symposia series two new sessions. The first of our new sessions entitled "*Energy Storage and Transitioning*" addresses the challenges and technological breakthroughs in relation to energy transitioning, for example, the repurposing of existing infrastructures for CO₂/ H₂ storage. Our second new session entitled "*Monitoring, Control, Diagnostics and New Methods for the Energy Sector*" showcases new method developments including the recent major advances in the field of Artificial Intelligence (AI), Genomics and Computer Modelling and how this can be applied in the energy sector.

ISMOS is multidisciplinary, linking biogeochemists, engineers, managers, molecular biologists and microbiologists, with a mixture of high-profile international speakers from industry and academia. The programme includes a workshop on Hydrogen Storage as well as a Career Workshop aimed at Early Career Researchers, relevant to the diverse energy sector.

For the social events we have a Welcome Drinks Reception (Tues 27th June) held at the James Watt Centre at Heriot-Watt where you can enjoy hot fork dinner, drinks and music. On Wednesday (28th) you can take part in an underground tour of the Royal Mile and experience some of the rich history of Edinburgh – make sure to sign up at the reception desk; places are limited! We have a Conference Dinner (Thurs 29th June) hosted at the Ghillie Dhu which includes whisky tasting, 3-course dinner and of course we wouldn't be in Scotland without live music and a ceilidh (traditional Scottish dancing).

We are very grateful to the Technical & Scientific Committee (TSC) and the Local Organising Committee (LOC) for their organisation and support for this conference. We also thank all the sponsors for their support to ISMOS-9.

ISMOS is a time to catch up with old friends and meet new ones! We hope you have an interesting and enjoyable meeting.

Yours,

Julia R. de Rezende and Xiang Shi, Heriot-Watt University, Edinburgh (LOC)

Torben Lund Skovhus, VIA University College, Denmark (TSC Chair)

Corinne Whitby, Essex University, UK (TSC Vice Chair)

Annie Biwen An-Stepiec, NORCE, Norway (ISMOS Webmaster & SoMe Manager)

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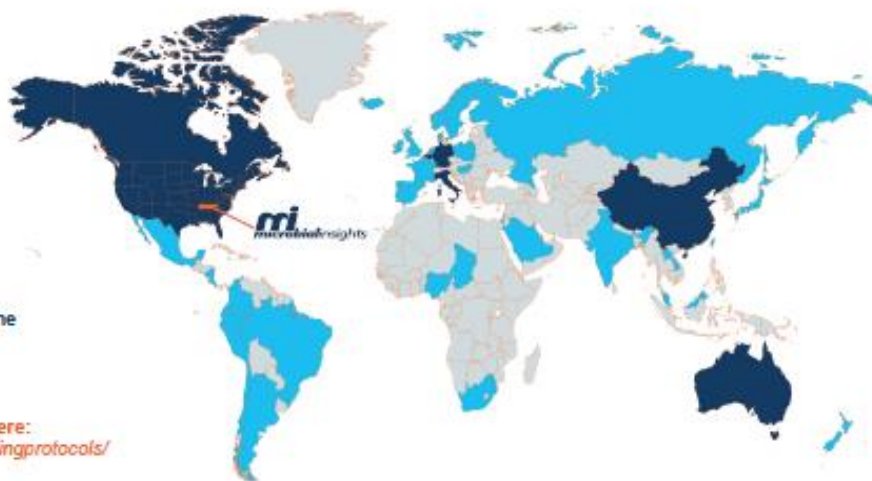
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Progress beyond



“Souring control by implementation of biocide application in response to cessation of nitrate treatment”

Thursday, June 29
11:45 am

*Dr. Renato De Paula
Biocide Technical Lead Advisor
for Solvay Oil & Gas Solutions*

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S05*21:
**Use of adenylate energy charge to
optimize SRP inhibitor and biocide
application for enhanced kill**

Friday, June 30

Danika Nicoletti, Applications Scientist



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Invited speakers:

Addressing the challenges for the energy transition, assessing synergies and minimising conflicts of use of the UK offshore subsurface and seabed.

Akhurst MC, Hough E. British Geological Survey. mcak@bgs.ac.uk

The transition to low-carbon technologies to provide the UK population with heat, power and everyday materials, and reduce emissions has raised challenges for use of the UK offshore subsurface and seabed. The UK subsurface storage resource for the energy transition is reviewed: temporary storage of hydrogen in salt caverns and porous strata; the permanent storage of carbon dioxide. The concept of compressed air energy storage is considered. The UK strategy for decarbonisation of industry, power generation, and supply of low-carbon hydrogen is centred around clusters of industrial CO₂ sources. Case studies illustrate the marked increase in requirement of CO₂ storage for planned large-scale production of hydrogen for low-carbon heating at UK industry clusters. Comparison of the distribution of subsurface strata suitable for storage of gases, the footprint of offshore windfarms and existing uses of the subsurface and seabed has demonstrated potential for conflicts of use. Mapping of offshore storage resources for two industrial decarbonisation clusters illustrates co-location of sites for the temporary storage of hydrogen, the permanent storage of carbon dioxide, and existing uses of the subsurface. The potential for physical interaction with seabed installations is shown by the distribution of existing and planned windfarms. Research to model the effects of subsurface operations, their interaction and impact on seabed infrastructure is outlined. The research objective is to assess the synergies and minimise conflict to manage and optimise use of the busy UK subsurface and seabed.

Adventures in Microbiologically Influenced Corrosion (MIC)

Gieg LM (University of Calgary, Calgary, AB, Canada) imgieg@ucalgary.ca

Microbiologically influenced corrosion (MIC) of metal infrastructure is a degradation phenomenon that must be understood and identified using a combination of microbial, chemical, physical, and metallurgical approaches. MIC has been most frequently studied using defined laboratory systems and pure microorganisms, yielding many fundamental principles governing MIC. However, understanding and diagnosing MIC in complex field systems remains a challenge. A recently completed applied research project aimed at understanding the potential for MIC in oil and gas systems has started to reveal some of the dominant microorganisms and their potential contributions to corrosion across these systems. Combining tools of genomics, chemical analysis, and corrosion experiments, more than 300 samples collected from crude oil reservoirs, offshore platforms, and crude oil transporting pipelines were evaluated for the types and activities of microorganisms that may lead to MIC under different physiochemical conditions. Outcomes of the project also included effective sampling and preservation methods for genomics-based assessments, examining potential MIC diagnostic approaches, and developing predictive models. This talk will highlight some of the key findings of the project that we can now further explore in ongoing scientific 'adventures' to discover and diagnose MIC in oil and gas systems.

Making a living on hydrocarbons

Gutierrez T (Herriot-Watt University) Tony.Gutierrez@hw.ac.uk

Microorganisms are the engines that drive the planet and underpin many of the ecosystem services required to maintain a healthy and sustainable planet. Those that use hydrocarbons, and almost nothing else, as a food source are possibly the most important, let alone intriguing, purging systems in our oceans. These hydrocarbon-degrading bacteria play a fundamental role in the biodegradation of crude oil and its petrochemical derivatives in coastal and open ocean environments, and are at the heart of natural bioremediation processes. This talk will focus on these “gas guzzlers” of the ocean – bacteria with a voracious appetite for hydrocarbons as their principle carbon and energy source. With increasing interests to explore for oil in ever more challenging environments, such as in the arctic and deep water provinces (>1000 m depths), there is a need to improve our understanding on the resilience of these environments to cope with future large spills. For this, we need to better understand the diversity, catabolic potential and response of oil-degrading communities in these types of regions, as well as establish microbial baselines as a reference of a "healthy" system prior to oil spillage or other anthropogenic disaster occurs. This talk will present past and ongoing research to address these goals, as well as touch on the intriguing association of these organisms with eukaryotic phytoplankton, and the natural sources of hydrocarbons entering our oceans that potentially sustain hydrocarbon-degrading bacteria when there is no obvious source of oil pollution.

The microbiology of the engineered subsurface in an evolving energy landscape

Nixon S (Manchester University) Sophie.nixon@manchester.ac.uk

It has long been known that subsurface microbiology impacts on oil extraction. A notable example is the production of sulfide by sulfate-reducing microbes. Over the last decade, an increasing body of literature on fractured shale microbiology has demonstrated similar problematic processes in natural gas extraction, albeit by different groups of microbes and via different metabolic pathways. As we look to the subsurface for permanent storage of CO₂ and transient storage of H₂, now is the time to investigate what impacts the microbiology of target formations may have under a new regime. Perturbation of these communities will inevitably stimulate new metabolic regimes. Are these problematic for storage, or might there be opportunities to enhance CO₂ sequestration? More broadly, how are subsurface microbiological impacts likely to change as we transition away from fossil fuels to a net zero future? In this talk, I will present new research on the microbiology of UK fractured shale which indicates that microbial problems can be significantly limited by choice of input fluid. By placing these UK results in the context of what's known of fractured shale microbial ecology, predictable patterns start to emerge. I will argue that we can be guided by these findings to minimise environmental and economic impacts of extraction of natural gas as we transition to a net zero future. Next, I will present ongoing research that seeks to unearth the potential impacts of CO₂ and H₂ storage on the communities native to oil reservoirs and saline aquifers, and highlight the urgent need for industry collaboration to best understand potential risks and opportunities. Finally, I will show how lessons learnt from the deep biosphere may help us achieve net zero sooner using cutting-edge biotechnology approaches that our group are pioneering in carbon capture and utilisation technologies.

From Town Gas to Geo-Methanation – Microbiology in the context of Energy storage

Pichler M, Hasibar B (RAG Austria AG) Markus.pichler@rag-austria.at

For hundred years energy, in the form of natural gas, has been stored in the subsurface to meet the demand of customers during times of low energy supply. In the beginning these storages were built within depleted gas reservoirs. Afterwards, aquifer storages have been exploited as storage demand increased and the number of gas reservoirs could no longer meet the demand. Everywhere in the subsurface, life in the form of microbes could be found. Either these microbes had been there for millions of years already, or they were introduced artificially via drilling operations, or injection of fluids. Microbes are seen as a problem and measures have been taken to prevent microbial activity wherever possible. More so in recent times as hydrogen storage is seen as a major contributor to a renewable energy system, microbes are perceived as a showstopper. In this talk the activity of microbes in the subsurface should get a more beneficial view. Ten years of experience in storing hydrogen bearing gas mixtures in the research reservoirs of RAG Austria AG have proven, that microbial activity in the subsurface can be controlled and exploited for renewable energy storage. By building on learnings from town gas storage and developing a deeper understanding for behaviour of microbial consortia in porous subsurface reservoirs a method has been developed to convert a one-way industry into a sustainable cycle industry. Furthermore, the learnings from microbial behaviour on field scale give an essential insight on how to handle microbes in hydrogen storage applications.

Is eDNA good enough? Implications for science and society.

Mehrdad Hajibabaei mhajibab@uoguelph.ca

The impacts of biodiversity permeate every aspect of our lives and well-being. However, our ability to measure and study biodiversity has been limited due to the lack of scalable and feasible conventional methods for various applications. The demand for tools and approaches for biodiversity analysis is now more critical than ever, as we confront a "biodiversity crisis" triggered by climate change and other human activities. Over the past decade, methods utilizing environmental DNA (eDNA) have emerged as significant contributors to biodiversity analysis, supported by a vast body of scientific literature. Nonetheless, the application of eDNA tools has predominantly been confined to academic, artisanal, and proof-of-concept scenarios, with limited adoption in real-world, systematic, and ongoing scientific initiatives, as well as environmental assessment and monitoring programs. The absence of an absolute and precise measure of biodiversity in most settings poses challenges for verifying and validating data generated through eDNA studies, especially when compared to traditional approaches. Furthermore, the lack of standardized and interoperable protocols, coupled with the rapid evolution of technologies, has impeded the widespread use of eDNA. While it is crucial to address these concerns through resource allocation and strategic planning, it is equally important to assess whether eDNA fulfills its core promise and meets the demands of the tasks at hand. In my view, when appropriately applied, eDNA tools currently represent one of the most effective options for rapid and accurate biodiversity analysis at the necessary scale to comprehend and mitigate the biodiversity crisis. The recent lessons from the COVID-19 pandemic have shown that solutions that are "good enough" can be justified when the urgency of the situation demands the rapid deployment of the best available tools.

Offered Orals

Session 01: Microbial induced corrosion (MIC) in the Energy Sector:

***53 European MIC Network: New paths for science, sustainability and standards executed via the new COST Action CA20130**

1. Dr. Andrea Koerdt - Federal Institute for Materials Research and Testing, Berlin, Germany
2. Dr. Torben Skovhus - VIA University College

Microbiologically Influenced Corrosion (MIC) is the negative effects to materials caused by the presence of microorganisms and is increasingly becoming a problem for the society. In contrast to USA, Canada, and Australia, the cooperation on MIC in Europe is less developed. Although, several research groups/industrial stakeholders are already dealing with MIC, the discussions are fragmented, and the exchange of information is limited. A true transdisciplinary approach is hardly ever experienced. Consequently, Europe is forced to use methods, prevention measures and standards from other networks, since nothing similar exists according to European standards. This makes Europe a) extremely dependent, and b) in some cases, potential measures/ standards cannot be used due to prohibition by European laws (e.g., use of biocides). In 2021, researchers establish the “EuroMIC”-network, financially supported by the EU-project “COST-Action” to address these issues. Through COST-Action Euro-MIC necessary interaction, communication, knowledge sharing, training of personnel and researchers of different disciplines can be achieved. COST-Action provides support in network activities, Workshops, Training schools, conferences, etc. Euro-MIC wants to bring Europe to a leading position, bringing ideas on an equal level with other nations, considering the values which are important for Europe and representing greater protection for people, property and environment. In the first grant period the project used several networking tools: Meetings/Workshops/Conferences/different mobilization grants and supported young scientist in reaching out to other countries/ experts in the field. The presentation will give the latest news on the current and future activities in the program and how to get involved.

***69 Standardizing Biocide Testing for Biofilm Prevention and Mitigation**

1. Dr. Susmitha Kotu - DNV Energy Systems
2. Dr. Nicolas Tsesmetzis - Shell International Exploration and Production Inc.
3. Dr. Bart Lomans - Shell Global Solutions International B.V.
4. Dr. Renato DePaula - Solvay Oil& Gas Solutions
5. Dr. Jason Lee - Naval Research Laboratory

The integrity of various industrial assets is affected by microbiologically influenced corrosion (MIC) and biofouling. Current industry standards lack a method for selecting biocides that effectively prevent and mitigate biofilm formation. Biocide testing using biofilms is becoming a more prominent topic, as the industry is moving away from its over-reliance on planktonic testing. An industry-wide effort is underway to develop a standard test method for evaluating the effectiveness of biocides in industrial systems, as part of the AMPP TM21495 committee. This standard will provide guidance on experimental design, appropriate experimental setups, analytical methods, and the collection and interpretation of biofilm and planktonic microbiology data along with other application specific data. Once published, the TM21495 standard will assist industry operators, service providers, third-party labs, and chemical manufacturers in selecting biocides to control biofilms in assets experiencing MIC or biofouling, with the ultimate goal of improving safety and sustainability.

***22 Identification of active MIC processes – RNA based sequencing for industry**

1. Dr. Elsemiek Croese - Microbial Analysis b.v.
2. Mr. Rob Elzinga - Microbial Analysis b.v.
3. Ms. Eline Keuning - Microbial Analysis b.v.

Molecular Microbiological Methods are recognized to be the preferred method for detection of microorganisms in relation to MIC. Standards are being developed for implementation of combining QPCR and NGS as standard tools. Although the results provide much more insight in MIC than the more traditional techniques (such as culture based techniques), there is still a gap between the presence of potential capabilities of a species (based on name) and the actual activity of a species in a system. A next step for better understanding actual activity of MIC is to identify the active processes of microorganisms. RNA based analyses offer this information. Using RNA based sequencing (transcriptome analysis), activity of species can be determined. Specifically in systems where MIC species such as SRB can be expected, like in seawater, the knowledge of activity of specific species is crucial. Here we present RNA sequencing data obtained in the field and compare it to lab data where MIC was confirmed. In addition we highlight the steps to be taken to use RNA sequencing data in industrial settings.

***93 Microbial Corrosion analysis of scraped samples from crude oil pipelines**

1. Dr. Aisha Alwuhaib - Saudi Aramco

Microbial Influence Corrosion is one of the major internal corrosion problems in oil and gas industry. As stated in literature, the presence of MIC microbes does not affirm that MIC is occurring, nor does showing that the presence of a given type of microbes establishes a cause-and-effect relationship between the bacteria and metal dissolution. The objective of this paper is to investigate and assess possible contribution of microbial activities to the internal corrosion of the pipeline systems. 30 samples were collected from different crude oil pipelines for microbiological investigation using the qPCR technique and compositional analysis using ESEM and XRD techniques, as well as water chemistry analysis. The qPCR analysis is used for detection and quantification of general bacteria, and microbes with MIC potential such as sulfate-reducing bacteria (SRB), methanogens, and iron oxidizing bacteria (IOB). The microbial analyses detected moderate to high number of general bacteria (103-109 per L of oil or per g of sludge) and high number of corrosive microbes (SRB + IOB + methanogens) (104-108 per L of oil or per g of sludge) in crude oil or sludge samples. Corrosion product pyrrhotite (Fe₇S₈) was also detected with possible microbial origin in the sludge sample collected from one of the crude oil pipelines. One of the crude oil pipelines during one-year investigation showed reduction of number of MIC microbes (SRB) and that could be the result of deployment of MIC management control or enhanced flow rate. The risk pipelines are currently under monitoring program for microbial activities.

***36 Prevention and mitigation of microbiologically influenced corrosion (MIC) using a halophyte-derived natural biocide**

Jakob L. Stein¹, Tanmay Chaturvedi¹, Torben L. Skovhus², Mette H. Thomsen¹

¹ Aalborg University, Denmark.

² VIA University College, Denmark

Sulfate-reducing prokaryotes (SRP) are known to cause microbiologically influenced corrosion (MIC) in the upstream oil and gas industry. SRP notoriously produce and embed themselves within a corrosive biofilm. Conventional biocides in the oil and gas industry have been reported to have a minor-to-no effect on well-established biofilms. However, salt-tolerant plants, called halophytes, produce several extractable bioactive compounds, including natural biocides. The project aims to produce a less ecotoxic alternative to the current biocides used in the oil and gas industry; the bioactive compounds in these

halophyte biocides naturally decompose in the ocean as seasonal coastal plants wither. Previous experiments using static tests in an unrenewed medium established that these natural biocides effectively inhibit SRP [1]. Biofilm-reactor experiments with the natural biocide show the ability to break down an established biofilm. Performance was compared to a common biocide in the oil and gas industry; Tetrakis Hydroxymethyl Phosphonium Sulfate (THPS). 3D surface scanning and weight loss of steel coupons were measured to assess the effectiveness of the biocides in preventing corrosion, and measurements of H₂S concentration and Next Generation Sequencing were used to assess the activity and diversity of microorganisms active in the corrosion process, respectively.

[1] T. Chaturvedi, M. H. Thomsen, and T. L. Skovhus, "Investigation of natural antimicrobial compounds for prevention of microbiologically influenced corrosion (MIC)," in ISMOS-7 Abstract book, 2019, pp. 53–54, Accessed: Nov. 30, 2021. [Online]. Available: http://www.ismos-7.org/wpcontent/uploads/2019/06/Abstractbook_ISMOS7.pdf. Keywords: microbiologically influenced corrosion, MIC, halophyte, natural biocide, corrosion, mitigation, sulfate-reducing bacteria, carbon steel.

***77 Biodiesel storage tanks can be impacted by MIC: what we know, where can we go next?**

1. Dr. Maria Salta - Endures
2. Dr. Nanni Noel-Hermes - Endures

A historical increase in the use of biodiesel, like the one composed of fatty acid methyl esters (FAME) has been observed globally. FAME, is considered renewable, biodegradable, and nontoxic. The chemical composition of FAME and its hygroscopic capacity makes it prone to biological contamination. Water in fuel can be a habitat for microorganisms and it is essential for microbial metabolism and growth. Fatty acids in biodiesel can also be produced as by-products of FAME metabolized by microorganisms which may lead in MIC of steel tanks, while CO₂ production during microbial metabolism may increase the corrosion rate within the storage tank environment. Biofilm accumulation leads to several possible consequences including the formation of galvanic couples, the localized production and concentration of corrosive compounds, and the ennoblement of steel surfaces. Subsequent effects from any of these outcomes would be localized corrosion typically presented by deep, penetrating pits on metallic surfaces that are of great risk to storage tanks and the associated infrastructure. In this work we will review the main parameters used for directly or indirectly monitoring microbial activity within biodiesel storage tanks seeking to address our level of understanding of MIC as an important factor in tank corrosion and whether our knowledge of this environment requires more research. While FAME blends, such as B20, presents new storage challenges to operators, risk assessments informed by further studies and understanding of the microbiome will aid each operator in formulating the appropriate response if contamination is detected.

***17 Expert System for Screening Microbiologically Influenced Corrosion (MIC) as Internal Failure Cause in Oil and Gas Upstream Pipelines**

Andre de A. Abilio¹, John D. Wolodko¹, Richard B. Eckert², Torben L. Skovhus³

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³VIA University College, Research Center for Built Environment, Energy, Water and Climate, Banegaardsgade 2 DK-8700, Horsens, Denmark

The analysis of pipeline failures due to Microbiologically Influenced Corrosion (MIC) is challenging due to the complex interaction of many influencing parameters including pipeline operation conditions, fluid chemistry and microbiology, as well as the analysis of corrosion features and products. To help address this challenge, an expert system was developed to assist non-specialists in screening internal pipeline corrosion failures due to MIC related threats. To accomplish this, 15 MIC subject matter experts (with a total of 355 man-years of accumulated MIC based experience) were recruited to evaluate a total of 65

MIC failure cases based on real-life scenarios. These case study parameters and the expert elicited results were input into an Artificial Neural Network (ANN) model to create a model system which can screen whether a given failure scenario is one of three outcomes: a) failure is likely due to MIC, b) failure is likely not due to MIC, or c) the conclusion is inconclusive (analysis needs more data/information). The model system had an overall accuracy of 74.8% and it showcases that knowledge from subject matter experts can be captured in a reasonably effective way to screen for possible MIC failures. Based on that, this presentation will provide details of the model development process and key results to date. Important considerations regarding the level of confidence of the diagnoses and variation between expert opinion will also be discussed alongside with ideas on how to improve the model for field applicability.

Session 02: Hydrocarbon Biodegradation:

***67 Persistence and Function of Bioaugmented Hydrocarbonoclastic Bacteria in Soils Mediated by Biochar**

1. Dr. Andrew Free - University of Edinburgh
2. Ms. Lyssa Morgan - University of Edinburgh
3. Dr. Clayton Magill - Heriot-Watt University
4. Ms. Zhansaya Assil - University of Edinburgh

Bioremediation is often hailed as an efficient, versatile, cost effective, and environmentally conscious solution for remediation of hydrocarbon contamination. One potential bioremediation strategy is the addition of exogenous microorganisms with defined degradation functions via bioaugmentation. However, issues with persistence of bioaugmented organisms and efficiency of biodegradation of pollutants have resulted in underemployment of this technology for bioremediation applications. The physicochemical properties of biochar have been observed to stimulate biodegradation, proliferation of microorganisms, and improve soil conditions. Here, we investigate the effects of pre-inoculating a consortium of enriched hydrocarbonoclastic bacteria onto softwood pellet (SWP) biochar to test whether it acts as a protectant to the augmented organisms and stabilizes their persistence in autochthonous and allochthonous environments comparative to conventional direct bioaugmentation. We determine community composition differences across various treatment groups and soil types, tracking the fate of individual augmented species in different community contexts and under different treatments using high resolution sequence analysis of amplicon sequence variants (ASVs). Our results suggest that augmenting allochthonous organisms results in successful persistence and establishment of particular species, such as the diesel-degrading strain *Acinetobacter oleivorans* DR1, even in a non-native soil type. Furthermore, we elucidate hydrocarbon remediation efficacy of diesel-amended soil across various treatment groups and soils over 28 days using GC-FID analysis, showing effects of bioaugmentation on straight-chain alkane degradation.

***35 Biodegradation effect on nonmetallic material in oil & gas environment**

1. Mr. Ghazi Qahtani - Saudi Aramco
2. Dr. Budoor Nasser - Saudi Aramco
3. Mr. Mazyad Alyami - Saudi Aramco
4. Dr. Christian CantoMaya - Saudi Aramco
5. Dr. Aisha Alwuhaib - Saudi Aramco
6. Mr. Ghassan Humaid - Saudi Aramco
7. Dr. Hamad Sairi - Saudi Aramco

The growing of the industrial use of composite and nonmetallic materials such as Reinforced thermoplastic pipe (RTP), high-density polyethylene pipe (HDPE), is anticipated to grow over the upcoming years. Particularly in the oil and gas industry, as they provide a suitable environment for

reducing the consumption of other chemicals during the transport and gathering of energies. The primary goal of this study is to understand the mechanism of damage caused by microorganisms in the pipes. This damage could occur as a result of the pipe material providing a source of nutrients and then deteriorating, or it could occur as a secondary effect, as a result of the biochemical activity. An experiment has been conducted for 5 months with materials from the field which are HDPE, and field water. 6 type of biodegradation bacteria were isolated from different oil fields and used in the experiment. The bacteria were given its optimum conditions to see their effect on the surface of the HDPE coupons. The material's physical, chemical, and mechanical changes during biodegradation were observed and monitored each two weeks by performing Scanning Electron Microscope (SEM) and Micro Surface Profiler Instrument. Strain #5 *Pseudomonas* sp. showed high deterioration on the surface when analyzed. The effect of microbial growth and biological activity on HDPE were investigated during the experiment. Therefore, further experiments will be conducted to simulate field condition to study the combined effects of bacteria, temperature and hydrocarbon on HDPE performance in oil and gas environment.

***30 Genome-resolved metagenomics uncovers new hydrocarbon-degradation pathways in uncultured bacteria**

1. Dr. Mirna Vázquez Rosas Landa - Universidad Nacional Autonoma de Mexico
2. Dr. Valerie De Anda - University of Texas at Austin
3. Ms. Georgia Waldram - Heriot-Watt University
4. Dr. Robin Rohwer - University of Texas at Austin
5. Dr. Angelina Angelova - Heriot-Watt University
6. Dr. Tony Gutierrez - Heriot-Watt University
7. Dr. Brett Baker - University of Texas at Austin

Microbes use petroleum as a source of carbon and energy and play an active role in oil spill remediation. Despite this, little is known about the baseline hydrocarbon-degrading communities and the metabolic mechanisms responsible for oil degradation after a spill occurs. We characterized the baseline hydrocarbon-degrading communities of the Faroe Shetland Channel (FSC), a prominent oil production area in the North Atlantic Ocean. We obtained 42 metagenome-assembled genomes (MAGs) from bacteria actively utilizing n-hexadecane via stable isotope probing (SIP) and identified potential novel molecular mechanisms for petrochemical degradation. Phylogenomics revealed 19 genera, including two not previously shown to degrade hydrocarbons: *Lentibacter* (Alphaproteobacteria) and *Dokdonia* (Bacteroidetes). Diversity surveys indicated *Lentibacter* were dominant members, constituting up to 17% of these communities. Most of the SIP-enriched MAGs encoded a complete alkane oxidation pathway containing alkane monooxygenase (AlkB), rubredoxin reductase (AlkT), and rubredoxin-2 (AlkG). However, most Alphaproteobacteria lacked AlkG for electron transfer, yet, we found that they instead encode a novel disulfide isomerase with iron-binding cysteine motifs that are conserved across rubredoxins. *Dokdonia* lacked AlkT and AlkG but had the remaining genes in the alkane-degradation pathway. This study shows that these bacteria are continuously purging hydrocarbons from industrial activities in the North Atlantic Ocean and advances our understanding of the diversity and physiologies of alkane degradation in industrial-impact oceans.

***80 Biodegradation of Crude Oil Associated with Marine Oil Snow (MOS)**

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2. Prof. David Paterson - Scottish Oceans Institute
3. Dr. Tony Gutierrez - Heriot-Watt University

Following the Deepwater Horizon (DwH) oil spill in the Gulf of Mexico, Marine Oil Snow (MOS) received considerable media and research attention due to its apparent ability to remove spilled crude oil from the sea surface. Increased respiration rates associated with MOS in microcosm experiments indicates

that elevated oil biodegradation can occur in these aggregates during sedimentation to the seafloor. However, to date no quantitative data is available that provides insight on the proportion of crude oil removed via biodegradation within aggregates. Using abiotic microcosms, the chemical qualities of bacterial EPS required to trigger MOS formation was assessed. The “artificial” MOS formed from these microcosms has allowed the development of a method to measure crude oil biodegradation from the point of microbial colonisation. Oxygen microsensors were used to determine respiration rates within individual MOS particles and in the surrounding water column. By combining this data with reduction in total petroleum hydrocarbons (TPH), as measured using GC-FID, the biodegradation rates of crude oil within individual MOS particles could be calculated. Determining the biodegradation rate of crude oil during a Marine Oil Snow Sedimentation and Flocculent Accumulation (MOSSFA) event will provide valuable information, not only for better understanding the fate of spilled oil during a major marine spill, but for better computer modelling allowing biodegradation parameters to be applied to ascertain the environmental risk of potential offshore well drilling.

***112 Combined high temperature and high salinity effects on anaerobic microbial communities and metabolic activities in crude oil reservoirs**

1. Mrs. Nneka Jk-Onyeka - University of Calgary
2. Mrs. Rita Eresia-Eke - University of Calgary
3. Ms. Gloria Okpala - University of Calgary
4. Mr. Peter Bell-Scholz - University of Calgary
5. Dr. Lisa Gieg - University of Calgary

Diverse microorganisms inhabit crude oil reservoirs, including sulfate-reducers (SRM) and methanogenic consortia, many of which can biodegrade crude oil or utilize compounds such as volatile fatty acids (VFA). These metabolic activities are known to occur at relatively low salinities and temperatures, but less is understood about these processes when reservoirs are characterized by both higher salinities (>0.6 M NaCl) and temperatures (>50°C). We investigated the potential for sulfate reduction, thiosulfate reduction, and methanogenesis in an oilfield wherein the salinities ranged from 0.9 to 2.5 M and in-situ temperatures were up to 70°C. Incubations amended with VFA or crude oil were established from produced water samples at a range of salinities and temperatures to investigate anaerobic microbial activities. At 30°C, enrichment cultures of SRM and thiosulfate-reducers (TRM) were readily established up to 2.5 M NaCl. At 50°C, SRM cultures were also established at all salinities, while TRM could only be enriched from one field sample. Halanaerobium, Desulfovermiculus, and unclassified Clostridiales dominated the 30°C and 50°C enrichments. Temperatures of >60°C and 0.9 – 2.5 M NaCl impeded the successful enrichment of SRM or TRM. Similar trends were observed under methanogenic conditions. While CH₄ was produced at 30°C under all salinities, lesser CH₄ amounts were produced at 50°C, and no CH₄ was produced at 70°C after 1-year incubation. While all enrichments are ongoing, these initial results suggest that a combination of high salinity and high temperature characterizing some reservoirs may naturally control the activity of microorganisms, minimizing potential reservoir issues such as souring.

***115 Anaerobic methanotrophic archaeal lineages differentiate biogenic and thermogenic hydrocarbon seeps in deep sea sediments**

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2. Dr. Oyeboade Adebayo - University of Calgary
3. Mrs. Deidra Ferguson - University of Calgary
4. Mr. Scott Wang - University of Calgary
5. Dr. Martin Fowler - Applied Petroleum Technology Canada
6. Mrs. Jamie Webb - Applied Petroleum Technology Canada
7. Dr. Calvin Campbell - Geological Survey of Canada - Atlantic
8. Mx. Natasha Morrison - Nova Scotia Department of Natural Resources and Renewables

9. Mr. Adam MacDonald - Nova Scotia Department of Natural Resources and Renewables
10. Prof. Casey Hubert - University of Calgary

Anaerobic methanotrophic archaea (ANME) are key microorganisms in deep sea marine sediments where they oxidize methane alone or in syntrophic association with sulfate-reducing bacteria. Stratified distribution of ANME in marine sediments suggests that environmental parameters select for specific ANME lineages. Here we investigated ANME in deep sea sediments from the Scotian Slope to investigate whether DNA sequencing of the seabed microbiome offers a tool for hydrocarbon seep detection. Piston coring was performed in >2500 m water depth at 19 locations resulting in samples from the upper 10 metres below seafloor (mbsf). Sediments were assessed through conventional geochemical analyses for hydrocarbon liquids and gases that typically signify seepage in seabed samples, with carbon isotope analysis distinguishing thermogenic and biogenic gas. Archaeal 16S rRNA gene sequencing of >400 sediment samples from different locations and depths revealed significantly different microbial communities in the presence of hydrocarbons. Select ANME lineages were observed in high relative abundance in hydrocarbon seepage-positive sediments and were notably absent in hydrocarbon seepage-negative sediments. Furthermore, sediments with biogenic hydrocarbons were characterized by ANME-1a, ANME-2a-2b, and ANME-2c lineages whereas sediments with thermogenic hydrocarbons were characterized by ANME-1b lineages, including an ANME-1b variant that was completely absent in sediments with biogenic hydrocarbons. These patterns suggest that biogenic and thermogenic hydrocarbon seeps enrich for different ANME lineages and that anaerobic methane oxidation pathways among ANME clades may vary.

***14 Investigation of varying microbial redox metabolic potentials to inhibit methane production from oil sands tailings**

1. Dr. Navreet Suri - Postdoctoral fellow
2. Ms. Iram Afzal - Phd student
3. Dr. Alsu Kuznetsova - Research associate
4. Dr. Ania Ulrich - Professor
5. Dr. Tariq Siddique - Professor

Alberta's oil sands contribute 80% of Canada's total oil production. Bitumen recovery from oil sands produces enormous fluid fine tailings (FFT) deposited in ponds. Methane (CH₄) emissions from tailings ponds originate from microbial metabolism of fugitive hydrocarbons (constituents of naphtha, diluent used in bitumen extraction process) under methanogenic conditions in impounded FFT and affect tailings management. Our current research investigates microbial metabolism of petroleum hydrocarbons under other alternative anaerobic (nitrate-, iron- and sulfate-reducing) conditions to prevent methanogenesis. Supplementation of alternative electron acceptors (such as nitrate, iron and sulfate), that are limited in OSTs can inhibit methanogenesis through higher redox energy generation for anaerobic microbial communities. Our preliminary findings of long-term studies reveal different extent of hydrocarbon metabolism under different redox conditions. with negligible CH₄ production. A few hydrocarbons such as toluene, n-decane and 2-methylheptane were consumed under nitrate- or iron-reducing conditions by 400 days of incubation. Whereas, an extensive biodegradation of hydrocarbons such toluene, n-hexane, n-heptane, n-octane, n-nonane and 2-methylheptane was observed with the reduction of sulfate during ~200 days of incubation of FFT amended with naphtha (C₆-C₁₀). This difference could be due to microbial adaptation to lower energy yielding processes (methanogenesis and sulfate reduction) under highly reducing conditions in the absence of sufficient amounts of other electron acceptors (nitrate or less bioavailable iron oxides) in FFT. Our experiments and metagenomic investigations

***49 Field Demonstrations of Successful Benzene and Alkylbenzene Bioremediation using Anaerobic Bioaugmentation Cultures**

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2. Ms. Nancy Bawa - University of Toronto
3. Mr. Shen Guo - University of Toronto
4. Ms. Jennifer Webb - SiREM
5. Ms. Kayla Finney - SiREM
6. Mr. Corey Scales - SiREM
7. Ms. Sandra Dworatzek - SiREM
8. Prof. Elizabeth Edwards - University of Toronto

Bioaugmentation is an environmental remediation technique used to accelerate the clean up of pollutants by adding cultured microorganisms with known degradation capacities into zones of contamination. It is uniquely suited to treat anaerobic environments where alternative remediation strategies (e.g., air sparging, excavation, pump and treat) are ineffective or challenging to implement. Recently we developed three bioaugmentation cultures that degrade benzene (DGG-B), toluene (DGG-T) and o-xylene (DGG-X) completely to methane and CO₂. The cultures are grown at large scale (>100 L) and degrade their target hydrocarbon at rates ranging from 1-25 mg/L/day. Their anaerobic (alkyl)benzene bioremediation performance has been demonstrated in dozens of microcosm and column experiments, highlights of which have been presented at previous ISMOS events. This year, we are excited to share convincing evidence of enhanced anaerobic (alkyl)benzene bioremediation in bioaugmentation field trials. Two examples of these field applications include a decommissioned refinery contaminated with dissolved benzene (79 – 1,800 µg/L), and a chemical manufacturing plant with mixed contamination (alkylbenzenes and chlorinated organic compounds). Sites were bioaugmented with one or more bioaugmentation culture across multiple direct push locations. Significant reductions (up to 99.9%) of dissolved targeted contaminants were observed at both sites after one year, in monitoring wells with coinciding increases (2-4 orders of magnitude) in anaerobic hydrocarbon-degrading bacteria present in DGG-BTM and DGG-TTM, respectively. These results support that enhanced bioremediation was attributed to the growth and anaerobic degradation activity of the bioaugmented microorganisms.

Session 03: Oil and Gas Microbiome, Problems and Solutions

***94 Microbial community compositions and activities differ between sampled reservoir sediments and formation water along a 20 million-year-old pristine oil reservoir**

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5. Dr. Ann-Christin Severmann - University of Duisburg-Essen, Center for Medical Biotechnology, Developmental Biology
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Studies on oil reservoir microbiology typically take samples from producing reservoirs and sample fluids that have been pumped to the surface. This comes with problems since producing oil reservoirs are affected by production processes leading to changes in environmental conditions and the natural microbiome. Hence, pumped samples do not display an unaltered picture of the spatial distribution and composition of the microorganisms in the reservoir. We took 13 samples from a freshly drilled sediment core of a pristine, heavily biodegraded oil reservoir in the North Sea. Core samples originated from above, within, and below the reservoir. 16S rRNA gene amplicon sequencing of the microbiome revealed distinct differences between sediments and formation water, indicating that studies on microbiomes from formation water alone are not necessarily representative for the microbial processes in an oil reservoir. Fluorescence microscopy showed that microorganisms live in small microcolonies on the sediment surface. CT-scanning with image analysis visualized the water phase distribution inside the reservoir sediments and clearly indicated water-filled voids that might be habitable for microorganisms, enlarging the surface for potential biodegradation. Employing microcosm experiments and reverse isotope labelling, we were able to determine the first degradation rates measured from cores above, within, and below a reservoir ranging from 0.05 – 3.73 mM CO₂/(gsediment x year). Results indicate significant degradation potential from autochthonous microorganisms in the reservoir above the water-contact-zone. Evading the general issues of produced oil samples for studying microbiomes results in a more realistic picture of an oil reservoir unaffected by production artefacts.

***95 Taxonomic and functional diversity of microbiomes across hydrocarbon-affected and -non-affected sites in the Barents Sea**

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Despite the role that consumption of hydrocarbons (HC) have in driving climate change, it is still expected that the prospection of HCs will continue in the coming decades. As HC prospection customarily depends on expensive geophysical and geological techniques with concomitant environmental impacts, the EU H2020 Project PROSPECTOMICS (<https://www.prospectomics.eu/>) aims towards verifying the presence or absence of HC reservoirs by harnessing multi-omics microbial signatures in overlying shallow marine sediments. For this study, 50 2-3 m long sediment cores were sampled at HC-affected (n=40) and -non-affected (n=10) sites in the Barents Sea, Norway in October

2021. Following DNA extraction, short-read metagenomes were generated at 30 Gbps per sample using Illumina NovaSeq. Assembly of quality-controlled reads into scaffolds larger than 1000 bp permitted prediction and annotation of genes followed by differential coverage binning. Dereplicated bins were manually curated using uBin into metagenome-assembled genomes (MAGs) of 70 % or more completeness and 10 % or less contamination. Screening of MAG functional genetic content, taxonomy and mean relative abundance across sites provided discriminating evidence for consortia of HC-associated bacterial and archaeal taxa. Putative virus-host relationships were inferred from metagenomes to further understand effects of viral infection in HC-associated microbiomes and biogeochemical cycling. In summary, substantial differences in microbiome composition were detected between HC and reference sites, rendering PROSPECTOMICS' biomolecular prospecting approach crucial in establishing low environmental impact strategies for highly accurate HC prospecting in surficial marine sediments.

***26 Polymer biodegradation by Halanaerobium promotes reservoir souring by providing substrates for sulfate reduction during hydraulic fracturing**

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2. Prof. Anirban Chakraborty - Idaho State University
3. Mrs. Kaela Amundson - Colorado State University
4. Dr. Paul Evans - Chevron Energy Technology Company Pty Ltd
5. Prof. Mike Wilkins - Colorado State University
6. Prof. Casey Hubert - University of Calgary

Hydraulically fractured shale formations have facilitated studies of unexplored niches in the continental deep biosphere, with members of the genus *Halanaerobium* associated with many reservoirs. Polymers like guar gum are used as gelling agents in hydraulic fracturing fluids, and while it is known that these compounds can be fermentable substrates for organotrophic microorganisms in oil fields, the metabolic pathways involved in polymer biodegradation have not been examined in this context. Produced water samples from a hydraulically fractured Permian basin shale reservoir were incubated at 30°C and 60°C to simulate in above-ground storage ponds and downhole conditions, respectively. Metabolism of guar gum coincided with *Halanaerobium* enrichment at 30°C only, with associated metagenomic sequencing pointing to polymer biodegradation through the mixed-acid fermentation pathway. Thiosulfate reduction to sulfide is often invoked to explain the dominance of *Halanaerobium* and their role in souring. However, metagenome-assembled genomes of *Halanaerobium* with high completeness did not uncover complete sets of genes for such metabolism. Incubation at 60°C mimicking downhole conditions showed potential for reservoir souring via sulfate reduction by *Desulfohalobium* and *Desulfovibrionaceae*. Genomes of these sulfate-reducing microorganisms include complete pathways for utilization of mannose and acetate that can be coupled to sulfate reduction. Interpreted together, these results indicate that production of fermentation intermediates (mannose, acetate) by *Halanaerobium* in topsides settings may result in these metabolites being introduced into the subsurface through produced water re-use where they promote reservoir souring by SRB.

***92 In-depth analysis of a pristine oil microbiome sheds new light on the global distribution of indigenous microorganisms, their plasmids, and viruses**

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Oil reservoirs are society's primary resource of hydrocarbons. However, microbial and metagenomic investigations are mainly conducted years to decades after industrial use, allowing water contamination and community exchanges. Here, we present the metagenomic analysis of the pristine Filicudi oil reservoir and its comparison to ten other hydrocarbon-associated sites. We identified 28 different microorganisms belonging to 22 genera in the Filicudi site. Thirteen metagenomic assembled genomes (MAGs) were reconstructed, allowing a detailed analysis of the metabolic capacity of organisms existent in the oil field. Beyond the diversity of microorganisms, we identified 51 plasmids and 95 viruses; the majority of both were presented as novel when compared to elements in public databases. We clustered the microbial single-copy gene *rps3*, plasmids, and viruses from all oil-associated sites and the Filicudi oil, revealing an overlap of elements across geologically distant locations. While *rpS3* genes from Filicudi oil were not found at any other site, three out of the 95 viruses from the Filicudi samples were also detected in a heavily water-flooded oil reservoir in Shandong province in China. We, therefore, conclude that our samples are most likely uncontaminated and represent a pristine microbiome from an oil reservoir. Importantly, we did find an overlap between plasmids from the Filicudi oil and globally distributed sites, indicating that plasmids have fewer barriers when traveling between and settling in ecosystems compared to viruses and microorganisms. Our results shed new light on the genomic capacity and fluidity of oil microbiomes in their natural state.

***98 Metaproteomic investigation of marine sediment to evaluate the metabolic potential for hydrocarbon degradation**

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The EU-funded project PROSPECTOMICS aims to develop a fundamentally new approach for oil and gas exploration in marine environments based on molecular biological techniques. Besides using geological and geochemical parameters, the project partners study the microbial community in marine near-surface sediments by applying several meta-omics approaches to differentiate between sediment sites above hydrocarbon reservoirs and control sites. For the proteomics analysis, several protein extraction protocols for marine sediment were tested and compared for extraction efficiency. We found continuous-elution electrophoresis to be most efficient to extract proteins from sediment samples. Eluted proteins were digested using trypsin and resulting peptides were separated by high-performance liquid chromatography and analyzed via mass spectrometry. Subsequently, proteins were identified using a sample-specific metagenome-based protein database. In first experiments, only a small number of less than 20 proteins could be identified in these challenging environmental samples. Using electrophoresis for protein extraction, significantly more proteins could be recovered from the sediment samples compared to other previously published extraction methods. In the first analyzed sediment core from a hydrocarbon-positive site more than 800 proteins were identified. Most of the proteins could be assigned to Methanomicrobia and were involved in methanogenesis or one-carbon metabolic processes. The investigation of the proteome is of special interest because in contrast to genomic data, which provide information on microorganisms present in environmental biological samples, proteomic data reflect the actual level of metabolic activities in a microbial community. The combination of the meta-omics approaches can thereby provide comprehensive knowledge about metabolic processes in environmental samples.

***108 Response of a produced water enrichment in flow-through sandpicks co-treated with nitrate and biocide for souring control**

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2. Dr. Xiang Shi - Heriot-Watt University
3. Dr. Julia R. de Rezende - Heriot-Watt University

Nitrate and biocide are the most common treatments being used for reservoir souring mitigation. Though nitrate is preferred due to its cost advantages, its field performance is still questionable due to inconsistencies, and strategies combining both are considered. However biocide addition might negatively affect nitrate-reducing populations and consequently enhance sulfate reduction. This study aimed to investigate whether biocide addition was beneficial or detrimental to nitrate-based souring control. Sand-packed bioreactors were inoculated with a microbial community enriched from Malaysian produced water. The bioreactors were shut-in for 12 days before media was continuously flowed through for 34 days. Once sulfide production reached 1mM, 150ppm nitrate was continuously supplied to all bioreactors. Glutaraldehyde was only dosed to 2 bioreactors for 8 hours on days 27 and 34. Besides chemical analyses, effluent samples and bioreactor sand sections were further processed for qPCR and 16S rRNA gene sequencing at the end of the experiment. Continuous nitrate injection was able to suppress sulfide production between 2% and 47% for the first 8 days. The bioreactors that received biocide on day 27 showed on average 23% lower sulfide as compared to bioreactors treated with nitrate only. After 7 days recovery, the 2nd injection of biocide was dosed, and the experiment was terminated to capture the effect of biocide treatment on the microbial community. Ongoing microbial community analysis of the sand biofilms will reveal whether nitrate-reducing and sulfate-reducing populations were equally affected by the biocide and whether biocide affected the spatial distribution of these populations within the bioreactor.

***39 Evaluation of Microbiological and Chemical Characteristics of Water for Oilfield Flooding**

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2. Dr. Xiangyang Zhu - Research and Development Center, Saudi Aramco, Saudi Arabia
3. Dr. Alexander Grigoryan - Research and Development Center, Saudi Aramco, Saudi Arabia

It is a common practice to re-inject the produced water (PW) from crude processing facilities to oil reservoir for pressure maintenance. To meet the increasing demand for oil production, Saudi Aramco is exploring sustainable, environmentally sound, and cost-effective applications of various sources of water for reservoir injection. One such attempt is to use the oily water retrieved from a large tank farm and combine it with PW from two oilfields for re-injection. Mixing of different water sources for re-injection can create many potential complications such as scale formation, biofouling of top-side facilities and injection wells, microbiologically influenced corrosion (MIC), reservoir souring, and formation damage. The study investigated the microbiological and chemical properties of the source waters (hypersaline PW and oily tank farm water), and the changes of these properties after mixing at the ratios representing various operational conditions for the re-injection system design. The study aims to ascertain the optimal mixing ratios or threshold of different types of water and minimize the microbiology-related challenges (corrosion, souring and biofouling). The results from the study provided insights for the potential microbial risks of the mixed waters and the development of mitigation strategies for the water re-injection system.

***52 Souring control by implementation of biocide application in response to cessation of nitrate treatment**

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2. Mr. Chris Jones - Solvay Oil& Gas Solutions
3. Mr. Matt Street - Rawwater
4. Mrs. Muna Mohamud - Rawwater
5. Ms. Leanne Walker - Rawwater
6. Dr. Bob Eden - Rawwater

Reservoir souring remains a challenge for the oil & gas industry, especially in assets where seawater is injected to maintain reservoir pressure. Aside from expensive ultrafiltration systems that remove sulfate to minimize the risk of souring, few chemical solutions are effective to control the activity of SRBs in the reservoir. Nitrate is commonly used to stimulate NRBs to outcompete with the SRB population. However, these competitive exclusion treatments are not always effective due to poor subsurface definition and increasing rates of nitrate injection over time. In this study we evaluated the ability of biocides to replace nitrate injection in conditions where nitrate is no longer able to control souring. For this study, soured sand-packed reactors were treated with batch treatments of a THPS-based biocide for several weeks prior or after nitrate cessation. VFAs, nitrate and sulfide measurements were used to evaluate the microbial souring and genomic analysis was performed to determine changes in the microbial population. The results indicated that biocide treatments were successful to remediate souring in the bioreactors when applied before and after the nitrate treatments were stopped. However, the cumulative amount of generated sulfide was considerably higher when the biocide was applied after cessation of nitrate, indicating that a period of co-injection of nitrate and biocide may be needed to eliminate the risk of a H₂S wave period. Taken together, this study highlights a new option to control reservoir souring and presents a strategy to replace nitrate injection that no longer shows positive effects in the field.

Session 04: Energy Storage and Transitioning

***61 When less is more: How CO₂ and H₂ concentrations affect green methane production in underground gas storages**

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Geo-methanation provides the means to utilize biogenic underground gas reservoirs not only for purpose of storing energy, but also for the conversion of green hydrogen and carbon dioxide to renewable methane. Microbial methanogenesis rates positively correlate with the extent of injected gaseous substrate, however alternative antagonistic metabolisms and/or unfavorable physicochemical effects may also manifest with increasing substrate concentrations, i.e. H₂ and CO₂. This study assesses the methanogenic potential of microbially-active formation brine, which was introduced into sandstone-bearing high-pressure reactors and exposed to mixtures of varying substrate gas concentrations. Ongoing microbial activities were characterized via analytical (HPLC, GC) and molecular (16S amplicon sequencing) methods paralleled by online monitoring of critical parameters such as reactor pressure and temperature. A robust mode of operation could be established, which permits the progressive generation of geo-methane at reasonable conversion rates. However, a four-fold increase of injected carbon dioxide and hydrogen led to a noticeable accumulation of VFAs, which in turn may lead to process instability. The presented findings substantiate the necessity for following an adequate feeding strategy to further optimize geo-methanation rates. Acknowledgments: This work was supported by funds of the Bundesamt für Energie (BFE), Switzerland), the Austrian Research Promotion Agency (FFG) and the Austrian Klima- und Energiefonds, Programme WIVA P&G via the research project Underground Sun Conversion - Flexible Storage (879 311).

***60 Exploring the potential of steel mill off-gas as a geo-methanation substrate at simulated reservoir conditions**

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12. Mr. Andreas Loibner - University of Natural Resources and Life Sciences (BOKU) Vienna, Department IFA-Tulln

Geo-methanation - the microbially-mediated conversion of carbon dioxide (CO_2) and molecular hydrogen (H_2) to methane (CH_4) in natural gas reservoirs - presents a promising solution for large-scale storage of renewable energy. Carbon-rich byproduct gases from industries such as steel production, could be used as a substrate for geo-methanation. This would largely reduce the carbon footprint of these industries and facilitate internal provision of CH_4 for energy-demanding operations. We investigated the potential of steel mill off-gas, containing not only the “conventional” carbon source for geo-methanation, CO_2 , but also carbon monoxide (CO), as a substrate for geo-methanation. For this, reservoir conditions were simulated in the laboratory by operating high-pressure bioreactors. CO_2 and CO in steel mill off-gas were fully converted to CH_4 (albeit slower than the control without CO), confirming its potential as a geo-methanation substrate. A quantitative polymerase chain reaction-based assay was developed to verify the microbial nature of this conversion process. A 16S rRNA gene-based phylogenetic survey revealed noticeable changes in microbial community composition in response to steel-mill off-gas provision. Acknowledgments: This work was supported by funds of the Austrian Research Promotion Agency (FFG) and the Austrian Klima- und Energiefonds, Programme WIVA P&G via the research projects LOCON (874 152) and C-CED (887 638).

***16 Pore-scale study of microbial hydrogen consumption and wettability alteration during underground hydrogen storage**

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2. Prof. Anthony R. Kavscek - Stanford University

3. Prof. Martin Fernø - University of Bergen

4. Dr. Nicole Doffel - Norwegian Research Centre NORCE

Subsurface storage of hydrogen in salt caverns, deep saline formations, and depleted oil/gas reservoirs has been proposed as a reliable and safe technology to store large quantities of hydrogen, which is produced from a surplus of renewable electrical energy. The stored hydrogen, however, may stimulate the growth of hydrogenotrophic microorganisms in the subsurface, with adverse implications for gas storage and withdrawal efficiency including microbial H_2 consumption, gas composition changes, clogging and corrosion. Our pore-scale analysis provided a comprehensive characterization of microbial effects of a typical halophilic sulfate-reducing bacterium during geological H_2 storage operations within a porous network. We observed a significant loss of H_2 from microbial consumption after 2 days following injection into a microfluidic device. Microbial growth in the silicon pore network was observed to change the surface wettability from a water-wet to a neutral-wet state. Due to the coupling effect of H_2 consumption by microbes and wettability alteration, the number of disconnected H_2 bubbles in the pore network increased sharply over time. These results may have significant implications for hydrogen recovery and gas injectivity. First, pore-scale experimental results reveal the impacts of subsurface microbial growth on H_2 in storage, which are useful to estimate rapidly the risk of microbial growth during subsurface H_2 storage. Second, microvisual experiments provide critical observations of bubble-liquid interfacial area and reaction rate that are essential to the modeling that is needed to make long-term predictions. Third, results help us to improve the selection criteria for future storage sites.

***87 A novel halophilic microbial community originating from a salt cavern – Characterization and hydrogen-consumption activity**

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2. Dr. Kyle Mayers - Norwegian Research Centre NORCE
3. Dr. Abduljelil Kadir - Norwegian Research Centre NORCE
4. Dr. Edin Alagic - Norwegian Research Centre NORCE
5. Dr. Janiche Beeder - Equinor ASA
6. Dr. Silvan Hoth - Equinor ASA

Salt caverns have been used for natural gas and oil storage and are now under consideration to store hydrogen, which is needed in large quantities for the Green Shift of industry and society. However, salt caverns are not sterile, and hydrogen is a ubiquitous electron donor for microorganisms. This could entail that the injected hydrogen will be microbially consumed, which will lead to an economic loss and potentially production of toxic H₂S. However, the halophilic communities present in these salt caverns are poorly characterized both on a DNA-level and also with respect to the volume of hydrogen consumption. We sampled brine from a salt cavern in Northern Germany for both community analysis and lab-enrichments. Despite very low DNA amounts we were able to obtain the 16S community which shows the presence of both halophilic Archaea and Bacteria. Most abundant ASV belongs to *Acetohalobium*, which is a chemolithoautotrophic bacteria which is known to consume hydrogen and form acetate. We observed many unknown ASVs, only assigned to Bacteria, which shows that salt caverns contain new and yet to be discovered microbial strains. We transferred the sampled brine in the lab into bottles with a hydrogen headspace, incubating for 170 days at different temperatures. We observed clear microbial induced hydrogen consumption at 30°C with concurrent H₂S formation and pH increase. Our data show that salt caverns harbor diverse microbial communities with the ability to grow on hydrogen. These findings are important to carefully assess the risks related to hydrogen storage in salt caverns.

***62 Microbial fouling in solar energy sector or life on the rocks and other hard surfaces**

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Transforming the energy sector is one of the most important challenges we face today. As new technologies appear, man-made systems across the energy sector become more diverse. For instance, expanding renewable infrastructure provides new and abundant surfaces on which living organisms can settle. Here we present inherent colonisers of solar parks – using a particular example of biofilm-material interactions taking place on solar panels in Europe and Americas. We dissect this interaction by examining both players: the colonised material as well as the microbial settlers. Opportunities for future research in the field will also be discussed. For colonised materials, the effects of biofilm growth on solar panels can be measured. Quantification assays reveal that efficiency reduction can reach up to 10%. This efficiency loss is correlated with an increased proportion of organics in the soiling layer (up to 58% after 18 months of exposure). Further quantification approaches to solar panel biofilms include transmission measurements as well as molecular biological methods. For the colonising microbial biofilms, increasing solar park areas represent unique terrestrial habitats. Rapid changes in the intensity of radiation, temperature, water supply and nutrient availability challenge the survival of microbes – but life on atmosphere-exposed solid surfaces has a long evolutionary history in deserts. Desert-adapted subaerial biofilms appear on solar panel surfaces in their full biodiversity. Such a specialized yet diverse

group of free-living, melanized ascomycetous fungi can invade new solar panel surface niches, representing a potential source of emerging anthropogenic biodiversity.

***103 Defining the impact of microorganisms on subsurface hydrogen storage**

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2. Mr. Adrian Hidalgo Ulloa - Laboratory of Microbiology, Wageningen University & Research
3. Dr. Yehor Pererva - Laboratory of Microbiology, Wageningen University & Research
4. Dr. Bart Lomans - Shell Global Solutions International B.V.
5. Prof. Diana Z. Sousa - Laboratory of Microbiology, Wageningen University & Research

Subsurface hydrogen (H₂) storage in salt caverns, porous reservoirs and aquifers is proposed as a potential solution for balancing fluctuations between the production and the usage of sustainable energy. However, H₂ is also an excellent energy source for diverse anaerobic microbial metabolisms thriving in subsurface environments. Methanogens, sulfate reducers and acetogens could exert a significant detrimental impact on H₂ storage. Through their activities, these microbes could cause a loss of the stored H₂, but also the production of unwanted products like H₂S (leading to the corrosion of the equipment) and the loss of injectivity through accumulation of bio-based solids as biofilms and extracellular polymeric substances. Yet, the extreme conditions of subsurface environments can limit the microbial impact on H₂ storage. In our project, we assess the potential impact of microorganisms on H₂ storage, related to H₂ depletion and formation of unwanted products. Formation water samples from two salt caverns and ten porous reservoirs collected across Europe were incubated in batch lab microcosms. Tests were done at four different temperatures (35, 50, 65 and 80°C), at 1.7 bar with 20%CO₂/80%H₂ or 100% H₂, and with or without trace elements and nutrients amendments. Microbial activities were observed in the majority of the tests for at least one of the tested conditions, with the exception of tests with samples from one of the salt caverns and three porous reservoirs. Acetogenesis was observed up to 50°C and sulfate reduction with sulfide production took place up to 65°C, whereas methanogens showed activity even at 80°C.

***44 Utilization of produced water as a substrate for a microbial electrolysis cell**

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2. Prof. Tanmay Chaturvedi - Aalborg University
3. Dr. Torben Skovhus - VIA University College
4. Prof. Mette Thomsen - Aalborg University

Produced water (PW) is the main waste stream from offshore oil and gas operations, significantly contributing to the environmental impact factor (EIF). Emerging technologies such as microbial fuel cells (MFC) and microbial electrolysis cells (MEC) are currently being investigated for their potential to generate electricity and hydrogen while removing oxidizable compounds from domestic and industrial wastewater. The potential of PW as a substrate for a MEC is evaluated in this work through the optimization of different parameters of the setup and two different inocula (marine sediments and domestic wastewater). The Wadden Sea inoculum contains sulfate-reducing bacteria (SRB) that are known exoelectrogens and thrive in oil-rich and PW environments. Both inocula communities were analysed with 16S rRNA amplicon sequencing. The Wadden Sea inoculum showed a high abundance of *Marinobacter* genus, known for their capacity to degrade hydrocarbons; while the wastewater inoculum was rich in *Methanosaeta* genus demonstrating potential to convert acetate into methane. A hydrogen production of 0.105m³H₂/m³ of anolyte per day was recorded in Single Chamber configuration with SRB inoculum. However, the low current measurements indicated insufficient bacterial activity, which was also verified with the ATP analysis performed at the termination of the experiment. In future work, bacterial activity may be increased by switching to a continuous operation supplying fresh substrate and expanding the experiment length, giving enough time for bacteria to

acclimatize to the medium. A more representative sample of PW, containing higher concentrations of acetate can also lead to increased hydrogen production.

***47 Modelling of microbial effect on H₂ reactivity in underground storages at laboratory and reservoir scales**

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2. Mr. Yann Le Gallo - GEOSTOCK
3. Ms. Nere Ruiz - GEOSTOCK
4. Mr. Joachim Tremosa - GEOSTOCK

In the frame of energy transition, hydrogen is seen as a pillar of a future low carbon energy system. Because a very large volume of hydrogen may be stored, underground solutions for storage as salt cavern or porous media are considered. In porous media (aquifer or depleted gas fields), the stored gas is injected in an underground porous layer and is in contact with the formation water and the surrounding rocks, which may enhance some abiotic reactions. Moreover, this environment can be favorable to microorganisms' development. As an electron donor, hydrogen is a very attractive source of energy for numerous microorganisms. The microbiology reactivity towards hydrogen was investigated within Hystories EU project. Based on the experimental results, a microbial reactivity model was developed using the software PHREEQC to simulate the methanogenesis and sulphate reduction reactions, that were observed at the laboratory scale. This model allowed to establish a kinetic law for hydrogen, sulphate or carbonate consumption over time, but also for minerals dissolution or precipitation and lastly for bacteria growth. Using the software CMG-STARs, these kinetic reactions rates were then upscaled for a 3D synthetic hydrogen storage to estimate the expected hydrogen losses and uncertainties related to reactivity over a few injection/withdrawal cycles. According to the model, most of the reactivity occurs at the start of hydrogen injection as in the current approach the bacteria activity. The model shows that the reaction front follows the progress of the hydrogen front.

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***34 Consolidation of weak sand zones in hydrocarbon reservoirs using microbial biofilms**

1. Dr. Tony Mitchell - Equinor ASA

Production of sand from poorly consolidated hydrocarbon reservoirs is a well-documented phenomenon, usually associated with increasing water production and affects many oil and gas producing fields. Sand production has implications for both integrity (due to the highly erosive nature of sand) and production efficiency (having to reduce liquid production to minimize the effects). Sand management can be achieved by mechanical means (such as sand screens in the lower completion of the well), selective perforation or reservoir management. For retrospective sand control, options are limited, either reducing production or chemical treatments. This opens the possibility for microbial methods to consolidate the sand; this being termed Bioconsolidation. The study aimed to investigate the concept of using in-situ generated biofilm to create structure and stabilise loose sand grains. Using simple up-flow bioreactors under both aerobic and anaerobic conditions, with a range of growth media, inocula and temperature, it was demonstrated that non-carbonate precipitation, biofilm consolidation could be achieved in the laboratory. Measuring inlet pressure and flow rate through the columns, an injectivity index was calculated and used as an indirect indicator of biofilm development, in addition, visual assessment of consolidation was made at the end of each experimental phase. Whilst still a long way from field conditions, the study demonstrated the potential for the concept and opens the way for further development, with the ultimate aim of either treating the reservoir with an inoculum and growth medium, or alternatively stimulating growth of microorganisms native to the reservoir to bring about the consolidation.

***72 Microbial Organic Acid Acidizing: Review on Past Knowledge, Technological Advances, and Implemented Field Trials**

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2. Mr. Mohsen alhumam - Saudi Aramco
3. Mr. Mohammed Bataweel - Saudi Aramco

Hydrochloric acid (HCL) is the most highly used acid for carbonate stimulation. However, due to its high reaction rate that decreases its chance of wormhole penetration and its high corrosivity, organic acids became sustainable alternatives due to their low corrosion rate and retarded reaction rate. Microbes are capable of producing organic acid in situ such as lactic and acetic acid. The in-situ production of organic acid allows the acid to be transported deeply into the formation and hence permits successful acidizing. One of the disadvantages of organic acids is their low dissolving capacity which may still limit deep penetration resulting in ineffective formation stimulation. Hence, several studies have investigated combining HCL with organic acid to achieve wormhole penetration with low well tubular corrosivity. In this review, we present the latest updates regarding the application of bio-based organic acids as a method of carbonate stimulation. The results of some laboratory studies and implemented field trials applying in situ microbial organic acid stimulation were compiled and interpreted. Additionally, this review attempts to address almost all the issues concerning the application of such technology and discuss its future outlook. This paper confirms the credibility of using microbial produced organic acid towards carbonate

***84 The PROSPECTOMICS Project - Using molecular biological techniques for hydrocarbon prospecting**

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2. Prof. Dörte Becher - University of Greifswald
3. Dr. Rolando di Primio - Subsurface Management, Specialist Geology, Aker BP, Norway
4. Prof. Alexander J. Probst - Universität Duisburg-Essen
5. Prof. Thomas Rattei - University of Vienna
6. Prof. Paul Wilmes - University of Luxembourg

Hydrocarbons (HC) will remain an important resource for the next several decades and exploration of new HC reservoirs will have to continue. Given the rapid and unforeseen shifts in supply, economies and the public require a secure and reliable supply, especially in Europe, which is largely dependent on foreign suppliers. However, utilizing EU-borne resources also means increased exploration for HC reservoirs in the world's most densely populated continent with tight environmental regulations, often times public resistance and conflicts with other users of the respective areas, both at the surface and subsurface. PROSPECTOMICS provides a possible solution by deploying a radically new approach based on biomolecule (DNA, RNA, protein) analyses related to hydrocarbon-degrading microorganisms in marine sediment, complemented by detailed geochemical studies. The underlying hypothesis is that all hydrocarbon reservoirs have some leakage and the influx of even minor amounts of hydrocarbons into the relatively nutrient-poor sediment can cause a shift in microbial community structure and metabolic processes. PROSPECTOMICS uses state-of-the-art "omics" techniques and detailed biogeochemistry to develop better understanding of the activity and metabolic potential of microbial life in sediments that are exposed to hydrocarbon seepage. The employed omics techniques will generate vast amounts of data, requiring analysis via machine learning and predictive models. The potential product of PROSPECTOMICS will be a set of biological "fingerprint" markers that can easily and routinely be employed to guide hydrocarbon exploration with minimal environmental disturbance and unprecedented sensitivity.

***121 Machine Learning Models Can Predict The Geochemical Status Of Marine Sediments Using Taxonomic Data**

1. Mr. Rohan Khan - University of Calgary
2. Prof. Anirban Chakraborty - Idaho State University
3. Mx. Carmen Li - University of Calgary
4. Prof. Casey Hubert - University of Calgary

Seabed hydrocarbon seeps are hotspots for microbial activity. Microbiome surveys can determine differences between seabed seep microbial communities and surrounding sediments. Machine learning algorithms can train models that rank sediment sites as hydrocarbon positive or negative using taxonomic and hydrocarbon geochemistry data. Recent studies assessing geochemical and microbial biodiversity show that in deep-sea oil prospective basins, like the eastern Gulf of Mexico and the Scotian Slope, phyla such as Caldiatribacteria are more prevalent in hydrocarbon positive regions. These observations were tested further here by training various machine learning models using taxonomy of 16S rRNA sequences from 172 surface layer marine sediment samples from the eastern Gulf of Mexico. Bacterial phyla Caldiatribacteria and Aminicenantes and archaeal order Thermopfundales were determined to have the largest feature importance scores for predicting hydrocarbon positive samples. Due to less variation in archaeal taxonomy, indicator species were assigned higher importance scores resulting in more accurate predictions by archaeal models. Predicting the hydrocarbon status of sediments using DNA sequencing is valuable, yet certain indicator species may be unique to geologic basins in different parts of the world. If models trained using microbiome data from one region can accurately predict hydrocarbon status in another region, it suggests a common relationship between certain microbial taxa and hydrocarbons. If models can only predict hydrocarbon status of sediments within a region, it can be inferred that despite similar hydrocarbon geochemistry, the other ecological factors also contribute importantly to seabed microbiome compositions.

***57 How does the choice of monitoring methods influence the interpretation of microbiological results?**

1. Dr. Heike Hoffmann - Intertek

Over the last decades, a suite of microbiological and molecular methods has become available to the oil and gas industry to gauge the adverse effect of microbes in oil and gas installations. Extensive effort has been made to understand and act accordingly on the output of these different techniques and interpret results gained from these analyses to best effect. For example, data from qPCR are used routinely as a monitoring technique aiding in understanding the status of the offshore system and guiding the action required to be taken, whilst next generation sequencing (NGS) remains a method used for more in depth testing, such as in failure investigations.

When comparing different methods such as the culture-dependent triplicate MPN methods and the culture-independent method qPCR, the question remains, what is the difference between the two outputs? In this study we collected various samples from offshore installations, the samples were inoculated into appropriate growth media for MPN analysis as well as preserved for subsequent qPCR analysis. Upon completion the samples were also analysed by NGS using the Illumina MiSeq platform, as well as Nanopore MinION to determine any distinct similarities or differences. These results suggest some distinct differences in microbial communities identified between the two sets of samples (cultures versus direct extraction), for example the composition identified from the MPN SRB contained a higher portion of *Desulfovibrio* in comparison to direct DNA extracts, however the overall sulphate reducing community in both sets was similar, more details will be discussed further in this study.

***21 Use of adenylate energy charge to optimize SRP inhibitor and biocide application for enhanced kill**

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2. Dr. Jordan Schmidt - LuminUltra Technologies Ltd.
3. Dr. Lisa Gieg - University of Calgary
4. Dr. Ed Burger - EB Technologies, Inc.
5. Dr. Gary Jenneman - GJ Microbial Consulting, LLC

Adenylate energy charge is a cellular activity measurement based on the abundance of three energy-carrying nucleotides in a sample: adenosine triphosphate (ATP) having the highest stored energy for cellular processes, adenosine diphosphate (ADP), and finally adenosine monophosphate (AMP), containing the lowest stored energy. ATP is consumed in cellular processes as its phosphate bonds are broken, leaving behind ADP and finally AMP. Environmental stressors (for example, change in temperature or salinity, scarcity of nutrients, or chemical treatments), can affect cellular energy and thus the levels of AMP, ADP, and ATP that accumulate within the cell. The concentration of these three energy-carrying nucleotides can be used to calculate the energy charge (EC), a value between 0 and 1 that identifies a microbial community that is active and growing, active but not growing, or dead or dormant. This study investigated the use of the EC parameter to optimize the dosage of the SRP inhibitors, nitrite and anthraquinone, along with the biocide glutaraldehyde for the enhanced kill of sulfate-reducing prokaryotes (SRP). A sulfate-reducing enrichment culture dominant in *Desulfomicrobium* was grown to 10⁸ cells/mL and challenged with either SRP respiratory inhibitors having different modes of action. The EC was measured over time in response to the application of the inhibitor to select optimized exposure timepoints for biocide application. Subsequent enhanced kill experiments were performed, and enhanced kill was measured by MPN and AxP concentration. This work will investigate whether EC is an effective parameter to optimize chemical treatments for enhanced kill.

***54 A temporal assessment of sulphate reducing microorganisms and reservoir souring in North Sea oil wells**

1. Dr. Lloyd Potts - Intertek
2. Dr. Heike Hoffmann - Intertek

Seawater injection to enhance oil recovery presents the risk of reservoir souring due to increased H₂S production, which is often associated with the activity of a specialised group, such as sulphate reducing microorganisms. Wells from an ageing oil field in the North Sea where seawater injection has been extensively adopted have exhibited rising H₂S concentrations over the course of 7 years. It is proposed that the rise in H₂S is indeed linked to the presence and increased abundance of sulphate-reducing microorganisms. To better understand this theory nine producing wells were sampled at different time points over several years. Total Bacteria, SRB and SRA were quantified (MPN and/or qPCR) and microbial communities were sequenced (Illumina and Nanopore). Firstly, it was determined that the highest SRB and SRA qPCR readings did not always correlate with high H₂S concentrations. Further, SRA were often enumerated higher than SRB, highlighting the importance of archaea in oilfield microbiology. Sequencing of samples taken several years apart indicated the consistent presence of *Marinobacter*, *Acinetobacter*, *Geoalkalibacter*, *Pseudomonas* and *Pelobacter*, plus the archaea *Archaeoglobus*. However, recent routine MPN analyses suggested a more pronounced rise in SRB abundance than previously recorded. This was further investigated with an intense sampling campaign over two weeks to assess deviation in sulphate-reducer abundance and the shift in microbial composition. Overall, this study provides insight to microbial composition dynamics and sulphate-reducer abundance in ageing wells over several years and explores the reliability of data from portable sequencing technology (MinION).

***89 Advancing Environmental Genomics in the Oil and Gas Industry: Current Progress and Future Directions of IOGP JIP34**

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2. Dr. Jordan Angle - ExxonMobil Upstream Integrated Solutions
3. Dr. Gustavo Estrada - Eni Natural Resources
4. Dr. Harvey Johnstone - International Association of Oil & Gas Producers
5. Dr. Ane Kjølhamar - Equinor ASA
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Environmental genomics is a rapidly advancing field that promises to revolutionize the way in which industry conducts biodiversity monitoring. The International Association of Oil and Gas Producers Environmental Genomics Joint Industry Program (JIP) was formed in June 2019 with the aim of facilitating the development and uptake of environmental genomics within the oil and gas industry. Towards this goal, a white paper was produced that summarizes the state-of-the-art in environmental genomics research, and the opportunities and limitations of applying environmental genomics within industry. The white paper included a comprehensive literature review, and importantly, involved consultation with professionals from academic, regulatory and industry backgrounds from across the globe that had expertise in environmental genomics applications. While this consultation revealed a consensus that the application of environmental genomics has advanced greatly in a brief period, with demonstrable benefits, there was an acknowledgement that key aspects are still lacking that would allow confident application of genomics approaches within industry. Through the review and consultation process, a range of knowledge gaps and areas requiring further development were identified. To elucidate which of these areas were most critical to the successful application of environmental genomics within industry, the JIP is drafting guidance that describes sampling design considerations, minimum standards for laboratory analyses and approaches to genomics data interpretation. Through the drafting of guidance, the JIP hopes to determine which gaps are most critical, enabling these to be prioritized for targeted research. The guidance will then be updated regularly to capture the latest research outcomes.

Poster Presentations

***9 Methanogenic Biodegradation of Anionic Polyacrylamide in Oil Sands Tailings**

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Anionic polyacrylamide (A-PAM) is a flocculant used to improve the settling and dewatering rate of oil sands tailings. Although A-PAM is an effective treatment, little is known about its environmental fate specifically its susceptibility to microbial degradation in oil sands tailings. Microcosms were established to investigate the ability of A-PAM (400 and 1000 mg/L tailings) to be used as a nitrogen source, carbon source, carbon and nitrogen source, and co-metabolite. Due to the dominance of methanogenesis in oil sands tailings, microcosms were monitored under anoxic conditions for methane production. The degradation of A-PAM was determined using size-exclusion chromatography (SEC). Nitrogen source and co-metabolite treatments were supplemented with acetate as a carbon source. Microcosms were analyzed for microbial community structure. After 231 days, cumulative methane production for nitrogen source treatments was 2.02 mmol for negative controls (no A-PAM), 14.57 mmol at 400 mg/L, and 17.87 mmol at 1000 mg/L. This suggests that methanogenic activity was higher when A-PAM was provided as a nitrogen source. Cumulative methane production for co-metabolite treatments was 29.28 mmol for negative controls, 23.67 mmol at 400 mg/L, and 25.33 mmol at 1000 mg/L. Methane production was not observed in other treatments. For 1000 mg/L doses, A-PAM removal efficiency was 19.25% and 41.83% for nitrogen source and co-metabolite treatments, respectively, while 400 mg/L doses had pore water concentrations below the SEC detection limit. These results suggest that A-PAM can potentially be used as a nitrogen source and may only serve as a carbon source under co-metabolic processes.

***11 Microbial Influence Corrosion Investigation for Refinery Oil Plant Processing**

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A comprehensive MIC study was conducted in Crude Stabilization operations where H₂S and light hydrocarbons are removed from crude oil. The aim of this work was to determine the root cause of repetitive failures in the stabilizer unit. Gas plant -2 feed to NGL Plants 20A and 41 was observed to be extremely high in both salts and water. Eleven days later, a tube leak developed in a Stabilizer Reboiler; the first of thirteen failures in Refinery would experience over the next 29 months for these two plants which had previously been very reliable. Plants 20-A and B41 each consist of two operations, degassing and crude stabilization. All of the observed corrosion has been in the Crude Stabilization operations where H₂S and light hydrocarbons are removed from crude oil supplied by other gas plant. The feed enters Stabilizer Columns 41-B-C-1 and 120A-C201 at the top tray of the 17-tray columns and the separation occurs using heat provided by two thermosiphon reboilers, 41B-E-1A/B and 20A-E-201A/B using 60 psig steam. Failures also exhibited severe saucer-shaped pitting. As results, Refinery Operations collected samples from 7 locations in Plant 41-B and submitted them for MIC investigation. The results confirmed the suitability of the operating conditions for microbial growth. The total bacteria (TB) indicated low to high number range from 2.02×10^2 to 7.64×10^8 cell/mL provided water sample and oil samples. The Sulfate-reducing bacteria (SRB) indicated below detection limit to high number up to 1.34×10^7 . The IOB showed below detection limit to moderate number up to 4.63×10^5 . The Acid producing bacteria (APB) and Sulfate-reducing Archaea (SRA) and Methanogens Archaea showed below detection limit. This indicates severe Microbial influence corrosion (MIC) in the system these groups of microorganisms can separately or collectively fail a system with the generation of highly aggressive species such as sulfide. This paper presents conducted work, analytical techniques, interpretation of data, and discussion of findings and suggests recommendations to help mitigate this type of failures and prevent its re-occurrence.

***12 Develop a computational model to predict biocorrosion progression in oil and gas industry**

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Uncontrolled microbial activity, and the consequent microbial corrosion, is one of the leading causes of pipeline failure in oil and gas industries worldwide particularly in non-scrapable pipelines including flowlines, trunklines, test lines and tie-in lines with limited information on the internal integrity. 20% of corrosion issues is caused by Microbial corrosion, recently, due to oilfield aging and increased water injection as a means of the secondary oil recovery, the water cut of crude oil is increasing and production rate (hence, the flow rate in the pipeline) is decreasing over time. The resulting dropout and accumulation of water and solids at low-lying sections of the pipelines cause under-deposit corrosion and microbial corrosion. Controlling the microbial corrosion by proper pipeline design and apply physical and chemical mitigations is key factor to maintain the pipeline integrity. However, the threat of corrosion and frequent leaks in non-scrapable and untreated pipeline is higher. Therefore, a development of a reliable hybrid mechanistic and risk factor MIC model that is able to predict the microbial effects on pipelines integrity with accurate prediction level is being investigated. While it is common practice for some pipeline to use chemical and physical method to inhibit the growth of the biofilm, a preventive approach to reduce the occurrence events of corrosion and potential leaks will have an impact on asset integrities, operation continuity and maintenance cost reduction.

***17 Expert System for Screening Microbiologically Influenced Corrosion (MIC) as Internal Failure Cause in Oil and Gas Upstream Pipelines**

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The analysis of pipeline failures due to Microbiologically Influenced Corrosion (MIC) is challenging due to the complex interaction of many influencing parameters including pipeline operation conditions, fluid chemistry and microbiology, as well as the analysis of corrosion features and products. To help address this challenge, an expert system was developed to assist non-specialists in screening internal pipeline corrosion failures due to MIC related threats. To accomplish this, 15 MIC subject matter experts (with a total of 355 man-years of accumulated MIC based experience) were recruited to evaluate a total of 65 MIC failure cases based on real-life scenarios. These case study parameters and the expert elicited results were input into an Artificial Neural Network (ANN) model to create a model system which can screen whether a given failure scenario is one of three outcomes: a) failure is likely due to MIC, b) failure is likely not due to MIC, or c) the conclusion is inconclusive (analysis needs more data/information). The model system had an overall accuracy of 74.8% and it showcases that knowledge from subject matter experts can be captured in a reasonably effective way to screen for possible MIC failures. Based on that, this presentation will provide details of the model development process and key results to date. Important considerations regarding the level of confidence of the diagnoses and variation between expert opinion will also be discussed alongside with ideas on how to improve the model for field applicability.

***18 Effect of different bioremediation approaches on the soil microbiome of fuel-affected areas**

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Fossil fuel and/or its derivatives have caused environmental impact and driven society in search of alternatives aiming to reduce pollution and promoting sustainability. Biofuels, such as ethanol and biodiesel, are some of the alternatives in Brazil. However, with the increased use of fuel/biofuel blends, cases of pollution with biofuels in the environment are expected. Four areas were impacted for experimental purpose with different blends named Area 4 (Ethanol-10%– Gasoline-90%); Area 5 (Biodiesel-20%-Diesel-80%); Area 7 (Ethanol-25%-Gasoline-75%); and Area 8 (Biodiesel-20%-Diesel-80%). Bioremediation strategies were further implemented, as follows: injection of electron acceptors in areas 4 and 7; and air injection plus bioaugmentation and biostimulation using autochthonous microbial consortium in areas 5 and 8. Soil samples from the source-zones were taken before the intervention for microbial community assessment using next-generation-sequencing. One year after the intervention, the soil microbiome was assessed again to unravel the effect of the bioremediation approach. Results showed that the community varied according to the blend. However, a core microbiome of hydrocarbon degraders could be identified, encompassing taxa such as *Extensimonas* and *Acidocella*, among others. Anaerobic bioremediation strategies influenced the microbiome composition and structure at a greater extent than aerobic strategies. Overall, blend composition did not influence specific degradation functional profiles in the areas. Nonetheless, genes for aerobic and anaerobic degradation were enriched in all areas after the bioremediation. Results obtained so far contribute to the understanding of the microbial dynamics in areas polluted with different blends and allow us to gain insights about the impacts caused by the bioremediation approaches. Keywords: Ethanol, biodiesel, electron acceptors, biostimulation, biodegradation, microbiome.

***23 Improved degradation of petroleum hydrocarbons by co-culture of fungi and biosurfactant-producing bacteria**

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Microbial remediation has proven to be an effective technique for the cleanup of crude-oil contaminated sites. However, limited information exists on the dynamics involved in defined cocultures of biosurfactant-producing bacteria and fungi in bioremediation processes. In this study, a fungal strain (*Scedosporium* sp. ZYY) capable of degrading petroleum hydrocarbons was isolated and co-cultured with biosurfactant-producing bacteria (*Acinetobacter* sp. Y2) to investigate their combined effect on

crude-oil degradation. Results showed that the surface tension of the co-culture decreased from 63.12 to 47.58 mN m⁻¹, indicating the secretion of biosurfactants in the culture. Meanwhile, the degradation rate of total petroleum hydrocarbon increased from 23.36% to 58.61% at the end of the 7-d incubation period. In addition, gas chromatography – mass spectrometry analysis showed a significant ($P < 0.05$) degradation from 3789.27 mg/L to 940.33 mg/L for n-alkanes and 1667.33 µg/L to 661.5 µg/L for polycyclic aromatic hydrocarbons. Moreover, RT-qPCR results revealed the high expression of *alkB* and *CYP52* genes by *Acinetobacter* sp. Y2 and *Scedosporium* sp. ZYY respectively in the co-culture, which correlated positively ($P < 0.01$) with n-alkane removal. Finally, microbial growth assay which corresponded with Fluorescein diacetate hydrolysis activity, highlighted the synergistic behavior of both strains in tackling the crude oil. Findings in this study suggest that the combination of fungal strain and biosurfactant-producing bacteria.

***24 The effectiveness of cathodic protection (CP) on microbiologically influenced corrosion (MIC) control**

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Cathodic protection (CP) is an electrochemical method, to manage corrosion in different industries, especially in buried and sub-merged environments. In those environments microorganisms are present and can cause microbiologically influenced corrosion (MIC). Most of the industry standards recommend performing CP using -800 mV (Ag/AgCl), however, if MIC-microorganisms are present, for instance sulfate reducing bacteria (SRB), it is recommended to use even more negative potentials. It is assumed that this will provide adequate protection of the metal. There currently is no information or valid data in context of CP, on the level of MIC threat and the extent to which more negative potentials can be used to provide adequate protection and not over-protection (due to hydrogen embrittlement threat). Conflicting statements can be found in the literature regarding the effectiveness of CP on MIC, from reducing biofilm attachment to increasing bacterial activity and biofilm attachment. Recently, the development and lower price of molecular microbiological methods (MMM) have opened the door for more effective studies of the MIC mechanism along with other electrochemical methods and surface analysis. In this work, the genetic functionality of biofilms formed in the laboratory under CP conditions is investigated using transcriptomics. Gene expression of SRB biofilms under different CP potentials (-800, -850 and -900 mV) will be studied; comparison with control will allow us to distinguish the specific genes that are differentially expressed, leading to a better understanding of the mechanism of CP to affect bacterial activity and diversity. Keywords - Cathodic protection, Microbiologically influenced corrosion, Molecular microbial methods.

***25 *dsrAB* gene expression - new approaches for biocorrosion studies**

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Corrosion induced by microorganisms is an already known fact that affects, among other industries, the oil and gas sector. Its understanding and deep knowledge can avoid material and physical damage to those involved in the related processes. Originally a culture quantification method, the Most Probable Number (MPN) is used to indicate the treatment against sulfatereducing bacteria (SRB), one of the most important group of bacteria in biocorrosion process. However, there are no studies demonstrating the activity of these bacteria in the field or in vitro. Understanding molecular mechanisms involved in microbial activity, especially the expression of important enzymes such as sulfite reductase (*dsrAB*),

present on sulfate-reducing bacteria (SRB) may help reduce the damage caused by this bacterial group. For this, a dose response study was conducted using five short-chain carbon sources to stimulate a single strain of SRB (*Desulfovibrio desulfuricans*) aiming to associate gene expression, bacterial quantification and H₂S production. These molecules (carbon sources) can be detected in high concentration carbon fluids, such as fuel. Between acetate, butyrate, glucoses, lactate and propionate, lactate and propionate showed a positive fold-change in gene expression against a non-stimulated group (control). By the time acetate and glucoses has similar data against control and butyrate had a negative fold-change. In conclusion, this study may provide a more sensitive tool to analyze the biocorrosion activity caused by SRBs, relating metabolic source, activity versus bacterial quantity allowing the early detection of corrosion events, which would ultimately reduce treatment costs and structural recovery measures.

***27 Microbial corrosion of copper alloys in a Methanobacterium enriched media**

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In this work, Methanogen-induced microbiologically influenced corrosion was characterized from an electrochemical point of view on two copper alloys (pure Cu and an $\alpha+\beta$ brass) in a test lasting two weeks. The alloys were tested in a Methanogen-rich solution producing methane by a rich pool dominated by *Methanobacterium*. Electrochemical investigations were performed using a threeelectrode cell that consisted of a working electrode (tested alloy), a reference electrode (standard calomel electrode - SCE), and a counter electrode (SS). Investigations included monitoring the open circuit potential (OCP), electrochemical impedance spectroscopy (EIS), and potentio dynamic polarization. Chemical characterization of the corrosion products and post-experiment observations with SEM and micro-Raman spectroscopy (μ RS) were performed. Molecular analyses by next generation sequencing were performed sampling the material's surface and identifying the constituent microorganisms of the microbial communities. Results indicate a higher corrosion rate for both the alloys and selective corrosion of the areas under the biofilm attachment. Copper oxides, chlorides, and phosphates were detected.

***29 Natural attenuation is an effective response to diesel spills under a wide range of environmental conditions in temperate coastal waters**

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Diesel fuel is one of the more common types of oil spilled into the marine environment, although in most cases, there is little response, leaving natural weathering, including natural attenuation, to remove the contamination. The rate that hydrocarbons enter the water column and are degraded by microbes depends on several factors. Season, which affects temperature, nutrients and community composition, can have an impact, as can mixing energy. The impact of these factors on how much diesel hydrocarbons enter the water and the microbial community response was explored in a series of mesocosm experiments. While mixing of any amount contributed to hydrocarbons entering the water

column, the composition of the initial diesel fuel was very important. Higher initial contributions of monocyclic and polycyclic aromatic hydrocarbons in the spilled fuel resulted in higher water column concentrations. There were minimal differences among seasons due to high variability within experiments. The microbial response was robust, regardless of the concentrations of hydrocarbons, with indications of biodegradation occurring within 4 d of exposure. Increases in prokaryotes occurred in the presence of diesel, with a shift in the community to higher relative abundances of common hydrocarbon degrading bacteria. As concentrations decreased over time, the eukaryote community shifted from the initial community to one which may be composed of organisms with some resilience to hydrocarbons. This series of experiments demonstrates the wide range of conditions under which leaving diesel fuel to natural attenuation is an effective response.

***36 Prevention and mitigation of microbiologically influenced corrosion (MIC) using a halophyte-derived natural biocide**

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Sulfate-reducing prokaryotes (SRP) are known to cause microbiologically influenced corrosion (MIC) in the upstream oil and gas industry. SRP notoriously produce and embed themselves within a corrosive biofilm. Conventional biocides in the oil and gas industry have been reported to have a minor-to-no effect on well-established biofilms. However, salt-tolerant plants, called halophytes, produce several extractable bioactive compounds, including natural biocides. The project aims to produce a less ecotoxic alternative to the current biocides used in the oil and gas industry; the bioactive compounds in these halophyte biocides naturally decompose in the ocean as seasonal coastal plants wither. Previous experiments using static tests in an unrenewed medium established that these natural biocides effectively inhibit SRP [1]. Biofilm-reactor experiments with the natural biocide show the ability to break down an established biofilm. Performance was compared to a common biocide in the oil and gas industry; Tetrakis Hydroxymethyl Phosphonium Sulfate (THPS). 3D surface scanning and weight loss of steel coupons were measured to assess the effectiveness of the biocides in preventing corrosion, and measurements of H₂S concentration and Next Generation Sequencing were used to assess the activity and diversity of microorganisms active in the corrosion process, respectively.

[1] T. Chaturvedi, M. H. Thomsen, and T. L. Skovhus, "Investigation of natural antimicrobial compounds for prevention of microbiologically influenced corrosion (MIC)," in ISMOS-7 Abstract book, 2019, pp. 53–54, Accessed: Nov. 30, 2021. [Online]. Available: http://www.ismos-7.org/wpcontent/uploads/2019/06/Abstractbook_ISMOS7.pdf. Keywords: microbiologically influenced corrosion, MIC, halophyte, natural biocide, corrosion, mitigation, sulfate-reducing bacteria, carbon steel.

***37 Industrial flow rate experiments revealed severe corrosion damage by *Desulfovibrio ferrophilus* IS5 and *Methanobacterium*-affiliated IM1**

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The service life of metallic components used in industry is determined by complex mathematical calculations. In practically every industry, it has been observed that the calculated lifetimes have not

been achieved. Microorganisms can have a direct negative influence on a material (microbiologically influence corrosion; MIC) to shorten the lifetime. Most MIC research has been done on sulfate-reducing bacteria (SRB); but methanogenic archaea (MA) have received increasing attention. In a previous study, we showed that higher corrosion rates (CR) can be obtained for MA in dynamic test systems. Deeper investigations showed that even higher flow rates (0.1 mL/min) further increase the CR by MA. To shed more light on the parameter Flowrate, a semicircular flow cell was used to study the corrosion behavior of *Desulfovibrio ferrophilus* IS5 and *Methanobacterium*-affiliated IM1. In the presented study flowrates of 4.6L/min were tested. We again observed increased CR. The average abiotic corrosion was 0.1 ± 0.01 mm/year and was four to six times lower than the average biotic corrosion rate of IS5 and IM1 (0.43 ± 0.07 mm/year and 0.66 ± 0.04). IS5 showed 1.23- and 4.3-fold higher average corrosion compared to the static condition and in our MFC, respectively. The average CR for IM1 was 4.4 and 3.3 times higher than in the static condition and in our MFC. The results show 1.) the importance of simulating environmental conditions in laboratory experiments to obtain meaningful results, and 2.) for each strain certain changes in parameter can have a high impact on the CR.

***38 Development and Modelling of the Test Methodology for Iron Sulphide (FeS) Scale Prediction**

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Iron Sulphide scale is widely present in oilfield (sour) systems and causes a range of production and HSE problems. Despite substantial progress, continued efforts are needed to precisely understand and predict FeS scaling. The aim of this work is to present a robust model for FeS scale prediction, verify this model with experimental results and compare the modelling results to commercial scale prediction software. Static tests were conducted for two types of Fe²⁺ salts with Na₂S.9H₂O for a range of initial pH values of each solution. Solutions and the morphology of the FeS precipitate were analysed using Inductively Coupled Plasma (ICP), Environmental Scanning Electron Microscopy/Energy Dispersive XRay (ESEM/EDX), respectively. We observed two different types of FeS scale precipitated, although [Fe²⁺] from both Iron(II) salts were the same. ESEM images of the FeS complex from Mohr salt indicated small crystals, whilst that of FeCl₂.4H₂O showed larger crystalline structures. In addition, both pure Sulphur and FeS were observed in the precipitate, as measured by EDX analysis. There was good agreement between the experimental results and our analytical model. Moreover, some differences were found between our model and the commercial code results due to difference in equilibrium constants and calculated activities of the various species. This study (I) contributes to our understanding the morphology of different structures of FeS which can assist production engineers with selection and design of inhibitor treatments. (II) compares both models with experiment, and (III) gives the lab experimentalist a simple tool which models the FeS formation quite well.

***40 The biodegradation of polymers used in EOR and the increase of MIC phenomena in O&G facilities**

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The microorganisms present in the O&G facilities and, mainly, their biofilms, are responsible for the MIC phenomena. In the past, MIC-generating microorganisms subscribed to only a few metabolisms, mainly Sulfate-Reducing (SRB) and Acid-Producing (BPA) Bacteria, whose counts were determined by culture techniques. Currently, thanks to the development of molecular biology techniques, through the study of DNA, it is possible to know the microbiome present in its entirety, increasing our knowledge about microorganism-environment interactions in O&G, breaking several paradigms. The enhanced oil

recovery (EOR) is no stranger to the microbial effect on O&G. In this oil extraction system, polymers are used to increase the viscosity of the injection water and thus improve permeability. Currently the most widely used polymer is HPAM (high molecular weight hydrolyzed polyacrylamide). The problem arises from the biodegradability of this additive. This study demonstrates the change in the microbial composition in the O&G consortia and its MIC condition in the presence of HPAM, as the only carbon source, from in vitro culture tests. The following methodologies were used to understand behavior were: characterization of the microbiome by metagenomic study, microbial counts by ATP and qPCR. In addition, the loss of viscosity in the evolution of the crops due to biodegradation was observed.

***42 Automated Bacterial Bioburden Measurement Device for Laboratory and Field Evaluations**

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This study reports on a laboratory and field evaluation of an automated bacterial bioburden measurement device, which should satisfy the increasing need for multiskilled staff, efficient workflows, and moving existing processes into digital technology. Timely delivery of bacteria population estimates, and the capability to conduct on-site bacteriological analysis, are essential to carry out bacterial risk assessments and to evaluate chemical biocide applications. This study reports on an automated device based on an established enzyme assay method that integrates a concentration step using a fluorophore-labeled enzyme substrate and a highly sensitive fluorescence reading. The device can analyze up to 12 water samples in 24 hours. Data on operational reliability, maintenance requirements, reproducibility, and repeatability are presented. Installation and long-term operation under rugged field conditions are also demonstrated in two different water types, a biologically potent water type, and potable water.

***46 Establishment and evaluation of a composite exogenous microbial oil displacement system**

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Aimed at the poor effect of in-situ microbial flooding in X oilfield of China, a microbial oil displacement system based on the synergistic effect of endogenous and exogenous multifunctional microbiome to enhance oil recovery was studied. Metagenomic analysis revealed that, genes involved in the pathway of anaerobic fermentation, acetotrophic methanogenesis were less abundant, and methanogens were also low in abundance. Therefore, we selected a complex exogenous multifunctional microbiome that was highly efficient in producing surfactant and capable of anaerobic fermentation to continuously activate methanogens. This microbiome was composed of 4 exogenous microbes including *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Ochrobactrum* sp. and *Geobacillus pallidus*, which showed no mutual competitive inhibition in low-nutrient inorganic medium. Compared with single strain, the microbial culture broth of the exogenous microbiome presented the lowest surface tension (25.5 mN/m), interfacial tension (0.69 mN/m), oil particle size in emulsion (average 10 μ m), and the highest emulsification index (EI₂₄ 75%), wax degradation rate (45.3%). The dominant physiological groups investigation in culture broth of the exogenous microbiome and formation brine under different oxygen conditions showed that, in aerobic stage the most dominant bacteria group was the one that can degrade hydrocarbon and produce surfactant, in the oxygen-limited stage it was fermentative microorganisms, while methanogens were activated successfully and became the dominant bacteria in the anaerobic stage. Enhanced oil recovery injected with fermentation liquor of the exogenous microbiome was 8.42%, meanwhile, methane was detected in the produced gas. These results revealed that the exogenous microbiome exhibited a tremendous potential for MEOR application in X oilfield.

***48 The Effect of Organics on Microbiologically Influenced Corrosion**

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Microbiologically influenced corrosion (MIC) is one of the asset integrity threats more difficult to predict in production systems. In part, the difficulty in assessing this corrosion threat lies in the lack of a complete understanding of the complex interactions between microorganisms, environment, and corrosion. There has been a lot of research looking at these factors from an independent perspective, but not enough to understand the interactions among them and how these influence MIC rates. For instance, the effect of nutrients on the risk of MIC has been considered in the prediction models but not from an overall perspective. Although some microorganisms can extract electrons from metals in nutrient-limited environments, models rank assets at a low-risk level when nutrients are below 20 ppm. In this study, we investigated the effect of organic carbon concentration on the corrosion rate triggered by *Shewanella chilikensis*, a bacterium with the potential to use Fe⁰ as the electron donor. Corrosion rates of carbon steel immersed for 7, 14, and 28 days in biotic and abiotic reactors with different concentrations of organics in the range of 0 to 1600 ppm were estimated. Results demonstrated that there is not a linear correlation between nutrient concentration and MIC rate. Interestingly, corrosion rates in the reactors with higher concentrations tended to decrease over time, whereas in the lower concentrations tended to increase. This investigation provides a better understanding of the effect of nutrients on MIC and suggests how nutrients should be incorporated into upcoming models for more accurate predictions.

***51 Antibacterial and antifungal epoxy coatings with improved aesthetic properties**

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Protective coatings with a "barrier effect" such as epoxy paints are often used, accompanied by cathodic protection, in pipelines and tanks to avoid corrosion. The incorporation into these coating types of a synthetic zeolite containing biocidal cations such as Ag⁺ inhibits the biofilm formation and, consequently, the microbiologically influenced corrosion. However, coatings with Ag⁺-exchanged zeolite depict a darkening of the epoxy film. This effect is more pronounced with time, affecting the visual appearance of the film. In this context, the aim of this work was to formulate epoxy coatings modified with increased levels of Zn²⁺ in Ag⁺-exchanged zeolite A, that provide adequate biocidal performance without affecting the aesthetic properties of the film. Factors such as Ag and Zn concentrations, the sequence of cation addition and the influence of ammonium as a complexing agent were considered in the ion exchange procedure. Zn²⁺/Ag⁺-exchanged zeolite A powder showed a white color for zinc concentration above 4% in samples containing 25% of silver cations. The zinc cations position in the framework could avoid the migration of silver species (Ag⁺ cations, Ag_mⁿ⁺ clusters) hosted in the zeolitic cages and its consequent photo-oxidation. Coatings formulated with higher levels of Zn²⁺ in Ag⁺-exchanged zeolite and exposed to environmental conditions for five years did not show darkening and maintain the biocidal capacity. These results are promising for applications that use these zeolites with high rehydration capacity, which simultaneously require long lasting biocidal activity while preserving aesthetic properties.

***64 Looking into the Halo: insights on the osmoregulation of halophiles and their implications in the underground hydrogen storage**

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Underground hydrogen storage (UHS) in high saline geological formations, such as salt caverns, porous media and aquifers, are ideal options for mid- to long-term containment solutions. However, enabling such large operations are challenged by several factors, including the unpredictability of microbial activities. As one of the most accessible electron donors, hydrogen (H₂) is used by many anaerobic microorganisms as an energy substrate, including sulfate-reducing bacteria (SRB), methanogenic archaea (MA) and acetogens (APB). Within high-saline UHS, the reactivity between H₂ and microorganisms are currently largely unknown, and this is further complicated by the fact that only halophilic species are predicted to survive in such osmotically challenging environment. Halophiles are specifically adapted to conditions with high osmotic stress through either the production of compatible solutes or intracellular accumulation of potassium chloride (KCl). Thus, this study focuses on characterizing and understanding the role compatible solutes play within the H₂ consumption process of halophiles. To approach this, different groups of halophilic species will be grown (either pure strains or environmental isolates) with common compatible solutes, such as glycine betaine and glycerol, with H₂ as the electron donor, and determine the rate of H₂ consumption in comparison to cells without additional supplements at two separate temperatures (30 °C and 60 °C). Overall, knowledge generated within this study will provide valuable insights on the physiological and metabolic potential of microorganisms within the high saline UHS or other similar subsurface environments.

***65 Study of Microbiologically Influenced Corrosion in Real-World Samples Using Zero Resistance Ammetry**

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Oil and gas assets are susceptible to microbially influenced corrosion (MIC). Detection of MIC is difficult because it involves a combination of weight loss analysis and specialized molecular techniques to correlate the presence of specific microorganisms to corrosion. This work focuses on detecting internal microbial corrosion in real world samples using split-chamber zero resistance ammetry (SC-ZRA). In SC-ZRA, two electrochemical chambers, containing identical electrodes of the same material, are separated by a salt bridge, and electrically connected through a ZRA. One chamber contains unaltered sample and the second chamber contains a sterilized sample. The transfer of electrons (current) through the ZRA depends solely on the activities of microorganisms in one chamber and their influence on corrosion can be quantified. In this study, eleven produced oil field water samples were collected to determine the efficacy of the SC-ZRA technique at monitoring microbial corrosion. We measured current and validated the results using weight loss analysis. Additionally, SEM and molecular biological analysis were conducted to determine the presence/absence of microorganisms. SEM revealed 50% of the samples showed biological growth on the electrode surface. However, only 20% of the samples showed biotic corrosion rates above 1 mpy. For those samples, the SC-ZRA technique predicted uniform weight loss with 60% accuracy compared to controls. The remaining samples showed no SC-ZRA current or weight loss. These results corroborate laboratory experiments that indicate the SC-ZRA can be used to

differentiate when microbial growth is causing corrosion and the potential of a system to experience MIC.

***74 A Laboratory-Based Case Study of Microbial Assessment and Metallurgic of Crude Oil from Refinery**

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Microbiologically induced corrosion (MIC) and Rietveld quantitative phase analysis are used to identify the root cause of the corrosion deterioration mechanisms at refinery and gas plants (Khanfar and Sitepu, 2021). The failed reboilers and preheaters are caused by the high-water level and excessive amount of sediment and salts in the feed crude. Hence, the microbial activities were investigated with a great care, and the findings revealed that: • Bacteria counts and compositions at gas plants varied widely from batch to batch. • Sour feed crude received at refinery contained high number of corrosive SRB ($10^4 \sim 10^5$ /L crude) and/or IOB ($\sim 10^4$ /L). • High number of SRB ($10^4 \sim 10^5$ /L crude) was detected in the samples collected from drawoff water, crude after degassing, crude entering reboiler, and the final stabilized crude products. • The solids collected from the failed section pipe showed moderate number of corrosive SRB ($>10^5$ /g). • The water used for hydro jetting was detected to contain moderate number of corrosive bacteria, mainly IOB ($\sim 10^4$ /ml). • The solids from the failed section pipe consisted of iron oxide corrosion products. These fluids are heavily contaminated with corrosive bacteria. They are mainly SRB from sour feed crude, draw-off water, crude after degassing, crude entering reboiler, to the final stabilized crude products. References: Khanfar, H.S. and Sitepu, H. (2021). ACS Omega Journal, 6, 11822–11831.

***75 Identification and validation of newly discovered phenanthrene biocatalysts**

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Polycyclic aromatic hydrocarbons (PAHs) threaten human lives since they are highly toxic and carcinogenic. They are widely distributed in the environment and important compounds in oil. However, oxygen is rapidly depleted in water-saturated sediments containing PAHs, making anaerobic microorganisms responsible for biodegradation. So far, only the anaerobic degradation pathway of naphthalene as a model compound has been studied in more detail but the anaerobic degradation of larger PAHs such as phenanthrene is still unknown. We elucidated the function of four newly identified type III aryl-CoA reductases from the TRIP1 enrichment culture involved in anaerobic phenanthrene degradation. The corresponding gene clusters were heterologously expressed in *Escherichia coli*, the enzymes purified, and their catalytic activity toward 2-phenanthroyl-CoA was confirmed by Liquid chromatography– mass spectrometry and UV-vis spectroscopy. The oxygen-sensitive, ATP-independent enzymes reduced 2-phenanthroyl-CoA to dodecahydro-2-phenanthroyl-CoA with dithionite-reduced methyl viologen as electron donor. The reactions occurred in consecutive two-electron reduction steps each catalyzed by one enzyme. Surprisingly, the four enzymes together could also reduce the last benzyl-ring of the former phenanthroyl-CoA, which is thermodynamically very challenging. The molecular weight of the four expressed enzymes was about 72-78 kDa. The four enzymes belong to the old yellow enzyme family, which are characterized by the presence of flavin cofactors, flavin mononucleotide and flavin adenine dinucleotide. Iron content analysis and structural homology modeling confirmed that the four enzymes contain an iron-sulfur cluster which mediates electron transfer. In conclusion, we demonstrated that the ATP-independent NADH-Flavin oxidoreductases are responsible for phenanthrene ring reduction and belong to the recently discovered type III aryl-CoA reductases.

***76 Assessing antibiotics resistance genes in oil-contaminated, bioremediated and pristine soils.**

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Aquatic and terrestrial habitats are increasingly contaminated with hydrocarbons, heavy metals, antibiotics and other contaminants that are released from households, hospitals, industries and as agricultural run-off. The degradation of these contaminants by microorganisms naturally present in the environment is often slow and limited, hence the need to enhance bioremediation, for example through biostimulation, which is the addition of nutrients and electron acceptors. This can be done with the use of organic materials, such as compost and manure. However, depending on the material used for biostimulation, there is an emerging concern that bioremediation enhancement may need to be balanced with the risk of enrichment of antibiotic resistance genes (ARGs). The purpose of this study is to investigate the occurrence of ARGs in soil exposed to different anthropogenic contamination and bioremediation amendments and to identify their attributable factors. Hydrocarbon-contaminated soil samples will be collected from different sites in Nigeria and the United Kingdom. The occurrence of ARGs will be compared in both countries and assessed based on pollutants, bioremediation strategy, microbial community and possible contributory soil parameters. ARGs will be assessed on soil with/without oil contamination and with/without addition of organic and inorganic materials as biostimulation amendments. This will test our hypothesis that ARG abundance will be significantly higher in soils impacted by oil and treated with organic waste such as manure. We will present the overview of this study and preliminary data on the ARG survey across oil contaminated soils.

***81 Method for DNA extraction from crude oil and application on pristine oil reservoir samples**

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Microbes from oil reservoirs shape petroleum composition through processes such as biodegradation or souring. Such processes are considered economically detrimental and might pose health and safety hazards. It is therefore crucial to understand the composition of a reservoir's microbial community and its metabolic capabilities. However, such analyses are hindered by difficulties in extracting DNA from such complex compound as crude oil. Past studies used pre-treatments involving surfactants or solvents. Here we present a novel DNA extraction method from oils with a wide API gravity range. We tested the ability to extract cells from the oils with different solvents and surfactants, the latter both non-ionic and ionic. Furthermore, we tested three DNA extraction methods. Overall, the best DNA yields and the highest number of 16s rRNA reads were achieved with isooctane as solvent, followed by an ionic surfactant treatment with sodium dodecyl sulphate (SDS) and DNA extraction via the PowerSoil Pro kit (Qiagen). The results from the non-pristine testing oils showed signs of human contaminants and showed no correlation between API gravity and DNA yield. The final method was then applied to various oils from pristine oil reservoirs (i.e. not seawater flooded). Despite the expected low cell density of 10^2 - 10^3 cells/ml, the new method yielded satisfactory results with average 16S rRNA sequencing reads in the order of 41000(\pm 8800). Thermophilic, halophilic and anaerobic taxa, which are likely to be indigenous of the oil reservoir, were found in all samples.

***82 Development of a model system to investigate the effects of surface roughness and media on marine biofilm formation and microbiologically influenced corrosion**

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The energy sector continues to face corrosion challenges, with significant pipeline failures due to microbiologically influenced corrosion (MIC). This study aims to develop a representative model system in which inoculae relevant to operating pipelines can be cultured to investigate biofilms and MIC on carbon steels. Two identical anaerobic CDC reactors ran simultaneously for 28 days; one inoculated with a multi-species marine consortium and the other uninoculated. Carbon steel (UNS G10180) discs were used with two surface roughness profiles, Ra of $1.33\pm 0.71\ \mu\text{m}$ and $0.44\pm 0.03\ \mu\text{m}$, as received and polished, respectively. Test media were either artificial seawater supplemented with yeast extract (1 g/L) or ATCC 1249 growth media. Molecular microbiological assessment, plus optical analysis and electrochemical tests were performed (see Figure 1). As expected, biofilms have a marked impact on the corrosion mechanism and reactor environment. Sulfide concentrations initially increased in the inoculated reactors ($523\pm 118\ \mu\text{mol/L}$). Additionally, there was a negative shift in corrosion potential, attributed to microbe attachment and biofilm formation/growth. Localised and shallow pits (see Figure 2) were clearly discernible in the biotic media, whereas only uniform corrosion was evident for the abiotic media. Electrochemical impedance was used to characterize the interfacial properties. This study provides insight into the role of biofilm formation on MIC and the importance of using multiple lines of evidence (MLOE), incorporating a multidisciplinary approach to develop understanding of the mechanistic relationship between the biofilm and metallic degradation. These insights will support a move towards evidence-based biocide dosing and influence recommendations for new industry standards.

***83 Singled out: The case for reference organisms in the study of MIC**

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Bibliographic analyses have counted well over 5,000 publications on microbiologically influenced corrosion (MIC), with this number growing daily (Hashemi et al., 2017). Despite this wealth of information, some experts claim that surprisingly few field-applicable insights into MIC have been gained and that true innovation in MIC management has been limited over the last decades (Little et al., 2020). We argue that much of the research on MIC has occurred in silos, generating breadth rather than depth of information. A larger number of concerted and long-term research initiatives, focused on fewer and particularly relevant microbial species and degradation mechanisms, could be a promising route towards improvements in MIC management in the energy sector. This interactive presentation will briefly review how work, carried out at independent international universities and companies using the same microorganisms such as *Desulfovibrio ferrophilus* or *Methanococcus maripaludis*, has coalesced,

leading to palpable innovation in biocide testing and advanced MIC diagnosis and monitoring. We then introduce the concept of reference organisms, i.e., strains declared to be particularly relevant to material degradation, and propose dedicating a culture collection to such microorganisms. Such an initiative - it is believed - would help facilitate future research efforts towards strains and mechanisms of significance, thereby accelerating progress in the field. It may also attract scientists from adjacent disciplines to MIC and contribute to a truly interdisciplinary approach to biocorrosion research. Organizational formats for selecting, publicizing, and maintaining reference organisms will be outlined. Lastly, the expert audience will be asked to provide their feedback.

***85 Microbiological impacts of geological CO₂ storage in depleted oil reservoirs**

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Carbon Capture and Storage (CCS) is a continuously growing sector at the heart of the global transition to net zero emissions. Depleted oil reservoirs represent lucrative geological storage facilities, complete with existing injection infrastructure which can be repurposed at minimal cost compared to building new facilities (e.g., in saline aquifers). However, while CO₂ injection for enhanced oil recovery has been employed for some time, research into the effects of CO₂ on the residing microbiota is sparse. Prior research suggest CO₂ injection leads to major compositional changes to microbial communities, and indicates the potential for enhanced methanogenic and sulphate-reducing activity; both of which often yield corrosive products that threaten CCS safety. Alternatively, microbial carbon fixation may present a means of immobilising CO₂ in situ, while simultaneously reducing formation porosity, and thus minimising leakage. Here, we present a powerful metagenomic toolkit to investigate oil reservoir microbial communities and put their genomic potential for enhanced CO₂ sequestration (or perhaps negative 'biofouling' processes) into the spotlight. The focus will be on carbon fixation genes that encode for CO₂-driven autotrophy, but also the potential for knock-on heterotrophic activity, including CO₂-emitting metabolism. As such, we will further existing knowledge on potential microbiological impacts of geological CO₂ storage, which may have significant implications for the CCS. In the coming years, our metagenomic investigations will be extended to laboratory-based subsurface simulation experiments that incorporate ¹³C-tracers to unearth the fate of the injected CO₂ into depleted oil reservoirs under in situ conditions.

***91 Microbial Monitoring and Mitigation in Cementing Operations M Alhumam Saudi Aramco**

Microbial contamination and bacteria growth in the cementing makeup water impact cementing operation and integrity. To avoid the premature cement setting caused by microbial growth, the water is usually treated with biocides for microbial control before mixing. This treatment will also prevent biodegradation of the added polymers and stabilize the slurry rheological properties. Make-up water and cementing mix samples were collected from active drilling rigs in the field. In 12 hours biocide addition program, the

tested biocide at 50, 100, 250, and 500 ppm for cementing operations revealed acceptable control of both GAB and SRB numbers in one Field, but not in another Field. In 24 hours biocide addition program, revealed varying levels for control microbial contamination in cementing operations, with higher efficiency with Field B samples opposed to field A, and with better control at higher concentrations of tested biocide at 250, and 500 ppm. As a recommendation therefore, usage of another biocide with a different chemistry at Field A is encouraged once a new biocide is selected.

***96 Understanding biofilm-mediated corrosion of carbon steel coupons with metagenome-resolved pathway analysis**

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Microbially influenced corrosion (MIC) is a well-reported problem in the petrochemical industry, with billions of US\$ losses annually. However, the exact mechanisms of how microorganisms cause MIC have not been defined, only speculated and actively debated. In this study we gain insights into the molecular mechanisms of MIC through the characterization of the metabolic pathways encoded in the microbes' DNA. Artificial Sea Water media was inoculated with anaerobic sediment from Galveston Beach, Texas, and then recirculated in an anoxic continuous flow loop embedded with X52 carbon steel coupons. After a few weeks, biofilm had grown on the steel coupons. The biofilm was harvested, their DNA extracted and then sequenced using Oxford Nanopore long-read sequencing technology. Some samples were treated with biocides to prevent growth; control samples had a mean corrosion rate of 110mpy, while biocide-treated samples had less than 10mpy. From the sequence data, high quality metagenome-assembled genomes (MAGs) were recovered. The corrosive biofilms were dominated by *Desulfocapsa*, a sulfate reducing bacteria often targeted as a culprit of MIC. The differential abundance analysis performed on the MAGs identified 247 genes statistically over-represented in the biocide treated samples. Among these were genes directly involved in electron cycling (e.g. Cytochrome C) in addition to key genes involved in siderophore-mediated iron acquisition. The impacts of these findings will be discussed.

***97 Biocide resistance evolution in sulfate-reducing bacteria mediating microbially influenced corrosion**

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Sulfate-reducing bacteria (SRB) are environmentally and industrially important microorganisms. The disadvantage of their metabolic activity, such as sulfate reduction, results in the formation of toxic sulfide that leads to microbial Influenced corrosion (MIC). SRB have been responsible for biocorrosion of ferrous metal structures in different industries e.g. petroleum and paper industry, and waste water treatment. One of these control measures is the use of biocides. However, it has been shown that various bacteria develop resistance or tolerance to biocides. Thus, a deeper understanding of the molecular mechanisms and evolutionary trajectories towards biocide resistance of SRB are necessary. We apply three commonly used biocides, tetrakis(hydroxymethyl) phosphonium sulphate (THPS), benzalkonium chloride, and glutaraldehyde to investigate the susceptibility of two SRB that are known to cause MIC, namely *Desulfovibrio alaskensis* G20 and *Desulfovibrio vulgaris* Hildenborough. We

determined the minimum inhibitory and bactericidal concentration, and time-kill kinetics for the three biocides. The susceptibility data is used to conduct ongoing evolution experiments to determine the evolution of resistance towards biocides of SRBs. For both SRB strains, genome-wide, barcode-tagged transposon mutant libraries are available. Both transposon mutant libraries have been exposed to sub-inhibitory biocide concentrations, and the abundance of mutants will be detected via Tn-Seq. The combined data from the evolution and the transposon mutant library experiments will provide to a deeper understanding of the genes involved in biocide resistance. The outcome of this work will shed light on the basic stress response of SRB and improve the management of MIC.

***102 Utilizing Adenosine Triphosphate (ATP) to Enhance Microbial Control Treatment in Oil and Gas Industry**

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Microbial contaminations in the petroleum industry have produced and continue to engender a massive loss in capital, either by damage caused by MIC, filter plugging, clogging of valves or by operational delay after a failure occurrence in water systems. Biocide is commonly used in different oilfield applications to eliminate significant financial and operational setbacks. However, Microbes usually develop resistance to biocide treatment over time, therefore its behavior needs to be monitored and controlled in a timely plausible manner in order to mitigate its detrimental effects. Conventional biocide selection for field application is culturebased method (CBM) that can be altered by the high salinity of the system's water (SW). Therefore, it is imperative to use an effective alternative method that is independent to the CBM. In this work, a luminescence-based ATP method was used to quantify the efficacy of the biocide treatment in high salinity water injection system. ATP was investigated in comparison with the CBM to monitor the survival of sessile and planktonic bacteria before and after biocides administration. SW were tested in the lab for SRB and GAB using both techniques before administration of the biocides and regularly after biocide dosing at 1, 2, and 4 hours. Results shows that ATP method can detect the post-treatment surviving microbes that resist the killing effects of the biocide but simultaneously not detected on CBM because they are non-culturable microbes. Biocide assessment using the described ATP method allows proactive steps and rapid response to be taken.

***104 Salinity and Temperature Gradients of Production Water-Reinjection (PWRI) Effects on Sulfidogenesis and Microbial Community Dynamics**

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Reservoir souring induced by Sulfate-Reducing Microorganisms (SRM) is a global issue in oil reservoirs impacting operational safety, metallurgy, capital and operational expenses (Johnson, et al., 2017). The objective of this study was to develop an understanding of the influence of temperature and salinity gradients, that are generated as a result of seawater injection or Production Water Reinjection (PWRI), on reservoir souring and microbial community dynamics. Anaerobic microcosms were incubated at 30°C and 60°C for 250 days. Temporal changes in sulfide, sulfate, volatile fatty acids (VFAs) and microbial community dynamics were determined. Microbial community composition was driven by both temperature and salinity, with *Halanaerobium* sp. observed as a prevalent organism in 30°C incubations and exhibited higher relative abundance in higher salinity incubations (107g/L-150 g/L). This contrasted

with the 30°C incubations at lower salinity (42 g/L-64 g/L); where the predominant organisms were SRM from the genera *Desulfobulbus*, *Desulfobacter*., and *Desulfotignum*. High temperature (60°C), low salinity incubations (42 g/L-64 g/L), revealed a selective enrichment of, *Desulfomaculum* sp., and *Pelotomaculum* sp. Formaldehyde-treated controls curtailed the growth and activity of the PWRI systems' enriched *Halanaerobium* sp., and the SRM communities. Developing the multiple layers of evidence synergies will help towards expanding the fundamental science underpinning reservoir souring, sulfate-reduction, and VFAs production/consumption. This ultimately will aid in devising a streamlined biocide field program; utilising chemistries effective in curtailing the field-specific salinity and temperature gradients microbial communities. Key Words: Reservoir souring, Production Water Re-Injection (PWRI), Sulfate, Volatile Fatty Acids (VFAs), and Next-Generation Sequencing (NGS).

***107 Corrosive bacteria fingerprinting in oilfield wellhead samples**

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In order to understand microbial implications in corrosion processes and design preventive measures for pipeline system at Saudi Aramco, the bacterial abundance and diversity in wellhead crude samples collected from different producing wells in a Central Arabian oilfield has been studied. The qPCR assays revealed 10⁴ -10⁸ /L of general bacteria (GB) in crude. Corrosive sulfate-reducing bacteria (SRB) were detected in six of nine crude samples where their number was at the range of 10² -10⁷ /L of oil. Number of GB and SRB in the only water separated from crude samples were ≤10² /mL and ≤10¹ /ml, respectively, indicating that bacteria were largely associated with oil droplets. Produced waters were found to resemble a normal seawater by TDS and sulfate concentrations, suggesting that environmental conditions unlikely barrier the growth of SRB and other bacteria in the oilfield. Next-generation sequencing data for 16S rRNA gene libraries showed that bacteria of orders Bacillales, Halanaerobiales, Pseudomonadales, Sphingomonadales, Betaproteobacteriales, Oceanospirillales, Syntrophobacteriales, Rhizobiales, Frankiales, Myxococcales, Xanthomonadales, Caulobacteriales and Campylobacteriales were predominant in the wellhead crude. It is proposed that these bacteria shaped a halotolerant microbial community that likely relied on the syntrophic oil oxidation. Microorganisms of particular interest include detected bacteria of families Haloanaerobiaceae (*Halanaerobium* sp.), Syntrophobacteraceae (*Desulfoglaeba* sp. and *Thermodesulforhabdus* sp.), Campylobacteraceae (*Arcobacter* sp.) and some others that can additionally participate in redox transformations of sulfur species with release of corrosive byproducts (i.e., sulfide, polysulfides, etc.) to formation water.

***113 Using microbial ecology methods to evaluate short- and long-term souring control effectiveness in a flow-through sandpack experiment**

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Biocides and nitrate are the two most adopted methods to control reservoir souring. We explored the performance of combining biocide and nitrate to mitigate souring through a 17- day continuous flow programme consisting of a 7-day combined glutaraldehyde (150 ppm) and nitrate (200 ppm) treatment to sand-packed flow-through bioreactors that hosted an active souring microbiota. We evaluated the effectiveness of the treatment by monitoring sulfide concentration. Uniquely, we further employed molecular methods (DNA sequencing and qPCR) together with the propidium monoazide (PMA) technique and applied advanced microbial community analysis tools (e.g. Network analysis, Coda-lasso analysis) to uncover the details of the responses of the live fraction of the microbial community that survived the treatment. While sulfide concentration remained stable in the control (nitrate-only treatment), it decreased from 200 ppm to 50 ppm when glutaraldehyde was added. Alpha diversity

analysis revealed that the combined treatment led to a higher Shannon index and a lower BergerParker index value, indicating a counter-intuitive increase in the community evenness. A conceptual model of the “shade effect” was proposed to explain such a phenomenon. Although a clear shift in the community structure driven by Bacteroidales could be observed during the combined treatment, microbial network analysis and Coda-lasso analysis indicated that the core microbiome and the stability of the community were not affected by the treatment, implying that the community maintained the ability to resume souring. Overall, the study suggested that the combined treatment might not be sufficient over time, albeit souring was successfully controlled in the short-term.

***116 Environmental selection and biogeography shape the microbiome of subsurface petroleum reservoirs**

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Petroleum reservoirs within the deep biosphere are extreme environments inhabited by diverse microbial communities and represent biogeochemical hotspots in the subsurface. Despite the ecological and industrial importance of oil reservoir microbiomes, systematic study of core microbial taxa and their associated genomic attributes spanning different environmental conditions is limited. Here we compile and compare 343 16S rRNA gene amplicon libraries and 25 shotgun metagenomic libraries from oil reservoirs in different parts of the world to test for the presence of core taxa and functions. These oil reservoir libraries do not share any core taxa at the species, genus, family or order levels, and Gammaproteobacteria was the only taxonomic class detected in all samples. Instead, taxonomic composition varies among reservoirs with different physicochemical characteristics, and with geographic distance highlighting environmental selection and biogeography in these deep biosphere habitats. Gene-centric metagenomic analysis reveals a functional core of metabolic pathways including carbon acquisition and energy-yielding strategies consistent with biogeochemical cycling in other subsurface environments. Genes for anaerobic hydrocarbon degradation are observed in a subset of the samples and are therefore not considered to represent core functions in oil reservoirs despite hydrocarbons representing an abundant source of carbon in these deep biosphere settings. Overall, this work reveals common and divergent features of oil reservoir microbiomes that are shaped by and responsive to environmental factors, highlighting controls on subsurface microbial community assembly.

***118 The importance of field samples and material selection when conducting laboratory investigations of MIC in oilfield installations**

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Laboratory experiments using anaerobic batch reactors were carried out to determine whether corrosion of X-60 and X-65 grade carbon steels varied when the alloys were exposed to pigging debris collected from two carbon steel production pipelines located in the Norwegian Sector of North Sea, that differed in corrosion (low corrosion-LC and high corrosion-HC). Coupons manufactured from the X-65 and X-60 grade carbon steel pipe were inoculated with aqueous suspensions of debris representing outermost and innermost pigging material recovered from each pipeline system. Specimens exposed in sterile reactors served as controls. Pigging material was analyzed using metabolic profiling with advanced mass spectrometry techniques. The debris harbored complex bacterial and archaeal microbiomes. Light, scanning electron microscopy and Auger-nanoprobe analyses demonstrated marked differences

in steels microstructure. Corrosion morphology varied between test coupons exposed to LC- and HC-debris and between the innermost- and outermost piggings material from the same system. In both the LC and the HC systems, more pronounced corrosion was noted when using the innermost piggings material as reactors inocula, irrespective of the steel grade. The study revealed that (i) metabolomic profiling discriminated between the severity of corrosion, thus can serve as a tool in corrosion management of oilfield installations, (ii) the selection of field samples is crucial when conducting laboratory investigations aimed to confirm whether and to what extent MIC is a threat and that (iii) the choice of test coupons that match composition and microstructure of pipe material(s) is of paramount importance when conducting laboratory and field MIC testing.

***119 Microbiologically Influenced Corrosion of Copper and Its Alloys in heat exchange power plant cooling tower brass tube**

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Microbially induced corrosion (MIC) causes immense financial and assets loss in oil and gas industry. Copper and its alloys are used widely in several applications like heat exchanger and marine pipelines because of their firmness, thermal conductivity, and corrosion-resistant properties due to patina formation on the outer surface that reduces the corrosion onsets. In this work we present case of repetitive brass tube failure in heat exchanger of power plant cooling tower. quantitative polymerase chain reaction (qPCR) method was used for the microbial analysis. Microbial DNA was extracted either from solid or water samples and subsequently used for qPCR analysis to enumerate total bacteria (BA), sulfate-reducing bacteria (SRB), Iron-oxidizing bacteria (IOB), and acid-producing bacteria (APB). X-ray diffraction (XRD) and Wavelength Dispersive X-ray Fluorescence Spectroscopy (WDXRF) techniques were used to determine the chemical composition of solid samples of foulants. Deposits from the internal surface of the examined brass tube contained a moderately high number of general bacteria (107 /g) and high number of corrosive bacteria (106 /g), including corrosive IOB and SRB. Deposits on the internal surface of the examined brass tube comprised of copper oxides (Cuprite [Cu₂O] and Tenorite [CuO]), and some potentially exogenous products of iron oxidation, for instance, Iron Magnetite [Fe₃O₄] and of Iron Oxyhydroxide [FeO(OH)]. Copper and its alloys are also prone to microbial corrosion like other metals.

***120 Understanding corrosion forms produced by oil field microbiology**

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Oil and gas industry are facing many challenges in terms of increasing productivity, production cost, process interruptions for both upstream and downstream operations. Biofouling, microbial corrosion, reservoir plugging, and souring have been cited to indigenous microbes in oil fields and associated water systems. This work was initiated to understand the mechanism of localized and general forms of corrosion in microbial containing environments at different conditions. The microbial consortium used in this study composed of field sulfate reducing bacteria (SRB) and general aerobic bacteria (GAB). Two different tests were conducted; dynamic and static testing system using biotic and abiotic setups. The general corrosion rate was estimated using weight loss method while the localized corrosion (pitting depth and density) was assessed by metallographic instrument and scanning electronic microscope (SEM). The study concluded that maximum pitting penetration rate (localized corrosion) was recorded in sea water medium inoculated with SRB. Moreover, the pitting depth was low in case dynamic test compared to static test. On the other hand, it was observed the microbial consortium concentration level has no impact on the general corrosion rate, which was mainly driven by medium's salinity level.

***125 A comprehensive review of microbiologically assisted cracking for pipeline steels**

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Microbiologically influenced corrosion (MIC) is believed to be a pervasive problem in many fields such as the oil and gas industry, water utilities, and sometimes biomedical implants. For decades, sulfate reducing bacteria (SRB) have been extensively studied as the significant MIC causative bacteria in MIC research. In addition, microorganisms can participate in the stress corrosion cracking of steels in the field. SRB were suspected to be an important factor in the cracking and may participate in and intensify the pipeline pitting corrosion and crack initiation. The cracking process induced or enhanced by microorganisms is known as “microbiologically assisted cracking” (MAC). This paper focuses on the MIC risen from soil environment in contact with pipe wall. The role of SRB in MAC is deeply discussed from three aspects of materials, microorganism and stress. We attempt to give a theoretical interpretation of SRB-assisted cracking using Ee-pH diagrams. In addition, the multiple factors influencing external corrosion and possible forms of MAC of pipeline steels in the soil are reviewed and analyzed in depth. Modes and mechanisms for both aspects have been comprehensively reviewed and discussed. The conclusions and recommendations for future research in the prevention and prediction of MAC are presented at the end.

***126 Preliminary Execution Of Molecular Microbiological Method (MMM) For Microbiological Induced Corrosion (MIC) Monitoring And Control In Malaysia Offshore Oilfield**

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Microbiological Induced Corrosion (MIC) is one of the major threats to oilfield operators due to its potential to affect the asset's integrity. Statistics show that MIC was responsible for up to 20% of all corrosion-related cost in oil and gas industry, and contributed up to 50% of pipeline service deteriorations. In Malaysia offshore oil field more than 100 nos of pipelines in operations are exposed to MIC threat. In managing this issue, two most important key activities are; monitoring and controlling the MIC. Current approach of MIC monitoring, i.e. using bacterial enumeration kit, which based on most probable number (MPN) was found to be less accurate, time consuming and prone to error, either due to human or environment condition (temperature, preservation, and chemical). Additionally, since 99% of microorganisms are unculturable, MPN method could missed most of the information from the samples. PETRONAS is currently looking into a better method in order effectively monitor MIC by embarking on molecular monitoring methods (MMM), comprises of quantitative polymerase chain reactions (qPCR) and new generation sequencing (NGS). During our pilot execution of these methods, qPCR provides quantity of specific microorganisms, while NGS give deeper insights on the microbial diversity and dominance in the samples. Higher accuracy of both methods assist relevant parties in oil field operations to make decisions to control MIC at specific fields. Besides, these methods also gave indicative insights on the suitable treatment and its effectiveness. Keywords : Microbiological Induced Corrosion, MIC, Biocorrosion, Biocide, MIC control, MIC monitoring, qPCR, Molecular Microbiology Methods, New Generation Sequencing

***127 Study On the Change of Microbial community from Injection water to Produced water in oil fields**

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To enhance oil recovery through microbial technique is to increase the recovery efficiency of water-flooded oil fields. The key of this technique is to study the growth and adaptability of bacteria available and their microcosmic mechanism. This paper discusses the transmutation of microbial community structure from injected water into three block oil reservoirs in X Oilfield of China. Based on the results of microbial diversity analysis in three oil blocks, we compared the abundance and species proportion of common operational taxonomic units (OTU)/bacteria between injected and produced water. We used dominant index to express the dominance of the OTU /bacteria in the same sample, discussed the environmental adaptability of common bacteria (especially available bacteria). Most bacteria that entered into injected water and that could adapt to the oil reservoir environment are the dominant ones. But it was not the case in L block, where indigenous microbial enhanced oil recovery had been implemented by injecting nutrients and air to change reservoir environment artificially in order to ensure microbes to grow easily. The bacteria which can adapt to oil reservoir environment were the non-dominant bacteria. Common bacteria which came with injected water and survived in produced water increased in number, and this led to the decrease of Shannon Index of produced water. There are commonly bacteria with hydrocarbon degradation and biosurfactant production functions in oilfield water samples, such as Rhodobacteriaceae, Pseudomonas, and Bacillus. In addition, we found for the first time that some new genus- Armatimonadetes, Caldiseica, Fibrobacteres and Elusimicrobia- could survive in the reservoir.

***128 Impact of decommissioning on benthic microbial communities in the North Sea**

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There are currently >2100 offshore installations in the North Sea, which require full removal at the end of their service. Information on impacts of decommissioning on benthic microbial communities is limited, however, especially those microorganisms involved in macronutrient cycling, such as ammonia-oxidizing Archaea (AOA) and bacteria (AOB) which are involved in nitrification- a key process of the nitrogen (N) cycle. Two North Sea oil and gas platforms: Northwest Hutton (NWH) and Miller, (decommissioned in 2009 and 2018 respectively), were investigated across an increasing distance gradient away from the platforms, from 50 to 3200m, to understand the impacts on sediment microorganisms. Bacterial 16S rRNA gene abundances increased 10-fold at Miller and significantly decreased 10-fold at NWH with increasing distances from the platform. Archaeal 16S rRNA gene abundances remained stable at Miller but decreased 100-fold with increasing distance at NWH. Both AOA and AOB ammonia monooxygenase (amoA) genes decreased in abundance, whilst ammonium and nitrate concentrations increased (~8- and 16-fold respectively) with increasing distance from NWH. However, at Miller, AOA amoA gene abundances remained similar, but AOB increased ten-fold, whilst ammonium and nitrate concentrations remained unchanged. The legacy of operations and decommissioning of platforms can still be detected in sediment microbial communities nearly 15 years

after decommissioning, including functionally important microorganisms involved in N-cycling, suggesting potential impacts on ecosystem function. Further work will assess heavy metals and hydrocarbon concentrations, and microeukaryotic communities. Information on decommissioning impacts on benthic ecology is crucial to better manage these structures and their legacy in marine environments

***129 Optimising soil RNA extraction for high quality long-read sequencing.**

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The soil environment is arguably the most complex terrestrial environment on earth making the study of microbial-associated processes such as nutrient cycling (Delgado-Baquerizo et al., 2017), carbon storage (Overy et al., 2021) and greenhouse gas production (You et al., 2022) very challenging. Meta-transcriptomics has the potential to go beyond inferred function derived from molecular pathways present in the genomes of microbes that form the soil community and improve our understanding of how those key molecular pathways are regulated in the soil microbiome (Sharuddin et al., 2022). The primary barrier to informative soil metatranscriptomics is the challenge of extracting high quality RNA of sufficient yield to perform representative sequencing. Extracting RNA from soil is notoriously difficult with the presence of PCR inhibitors effecting downstream processing (Carvalhais et al., 2012). Here we present a comparison of the yield and quality of soil RNA extractions using commercially available extraction kits with a focus on maintaining transcript integrity. We found that kit selection has a significant impact on RNA yield and quality and discuss trade-offs in throughput, yield and automatability. Long-read sequencing has the potential to circumvent the need for an assembly-based approach to studying the soil meta-transcriptome and capture full length transcripts directly; towards this goal Oxford Nanopore Technology library preparation methods have also been investigated to optimise sample preparation for read length and depth. We identify RNA quality as a key metric for successful library preparation and outline areas that require further improvement to ensure improvements in extraction yield and quality translate into a more representative meta-transcriptomic dataset. References Carvalhais, L.C., Dennis, P.G., Tyson, G.W., Schenk, P.M., 2012. Application of metatranscriptomics to soil environments. *J. Microbiol. Methods* 91, 246–251. <https://doi.org/10.1016/j.mimet.2012.08.011> Delgado-Baquerizo, M., Trivedi, P., Trivedi, C., Eldridge, D.J., Reich, P.B., Jeffries, T.C., Singh, B.K., 2017. Microbial richness and composition independently drive soil multifunctionality. *Funct. Ecol.* 31, 2330–2343. <https://doi.org/10.1111/1365-2435.12924> Overy, D.P., Bell, M.A., Habtewold, J., Helgason, B.L., Gregorich, E.G., 2021. “Omics” Technologies for the Study of Soil Carbon Stabilization: A Review. *Front. Environ. Sci.* 9. <https://doi.org/10.3389/fenvs.2021.617952> Sharuddin, S.S., Ramli, N., Yusoff, M.Z.M., Muhammad, N.A.N., Ho, L.S., Maeda, T., 2022. Advancement of Metatranscriptomics towards Productive Agriculture and Sustainable Environment: A Review. *Int. J. Mol. Sci.* 23. <https://doi.org/10.3390/ijms23073737> You, X., Wang, S., Du, L., Wu, H., Wei, Y., 2022. Effects of organic fertilization on functional microbial communities associated with greenhouse gas emissions in paddy soils. *Environ. Res.* 213, 113706. <https://doi.org/10.1016/j.envres.2022.113706>

***130 Towards Net Zero: Lowering Steam to Oil Ratios with Thermophilic Bacteria and N₂ Biogas**

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Microbial metabolism in oil reservoirs generates biogas that can play a role in subsurface pressure maintenance. Biogas can play a role in microbial enhanced oil recovery (MEOR) and in some cases can lower the emissions profile of oil production by displacing reliance on steam or methane injection. Among the many different biogas options that microbes produce, nitrogen (N₂) offers both economic and environmental benefits. N₂ has similar properties to other non-condensable gases, such as methane, without being a greenhouse gas. MEOR using N₂ biogas generation to lessen steam or methane requirements can lower the emissions per barrel of oil production. In oil sands produced by steam-assisted gravity drainage or other thermal methods, N₂ generation by thermophilic bacteria can significantly lower an operation's steam-to-oil ratio. In these applications, reservoir zones that receive steam heat up to >200°C whereas in adjacent zones that steam does not penetrate, gradual warming occurs via conductive heating. Deep biosphere zones experiencing conductive heating can spend years at temperatures permissive for thermophiles that would otherwise remain dormant at lower ambient temperature. Since endospores of thermophilic bacteria are ubiquitous in sediments, the combined effect of conductive heating and exogenous nutrient injection can stimulate anaerobic metabolism and biogas generation. This was demonstrated by collecting samples of oil sands (ambient temperature 10°C) and incubating them at 50°C with nitrate under organotrophic conditions. This stimulated endospore germination among thermophilic denitrifying bacteria that can rapidly convert aqueous nutrient cocktails into large volumes of biogas.

***131 Electrochemical behaviour of sulfate-reducing strains in coculture**

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Microbiological Influenced Corrosion (MIC) phenomenon impacts many industrial sectors. In Oil and Gas industry, Sulfate-Reducing Bacteria (SRB) are usually the most incriminated in MIC phenomena. The metabolic activity of some SRB could accelerate the corrosion process by directly using electrons resulting from the electrochemical corrosion process of steel. Electrochemical tests by chronoamperometry were conducted to provide excess electrons to the surface of a carbon steel coupon in the presence and absence of two SRB strains grown alone or mixed at different ratios. A cathodic potential was imposed, and the resulting cathodic currents were monitored. The results obtained show the formation of a cathodic current with one of the SRB, while no cathodic current was observed in abiotic environment or with the other SRB belonging to the same genus. Moreover, the coculture of these two SRB strains leads to a higher current. Therefore, a cathodic reaction occurs on the surface of the steel coupon in the presence of one of the SRB strains, and the presence of two SRB strains can cause a synergy effect on cathodic current increasing further this phenomenon. The microbiological analysis used during these tests did not allow to quantify each strain on the surface of the coupon to evaluate the composition of the biofilm. Therefore, new quantification techniques must be developed for each strain. Further analyses to understand the increase of the cathodic current in pure culture or coculture will improve the understanding of MIC in presence of SRB.

132 30 years old fuel tank case study I: isolation and characterisation of the corrosive and hydrocarbon degrading potential of the diesel fungus *Amorphotheca resinae

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Microorganisms are known to use materials as an environmental niche. Following the history and the underlying physiological mechanism of such colonisation can nourish our understanding of material biodeterioration. Here we present a case study of a 30-year-old diesel tank with metal walls that served in a vehicle since 1988 but that was taken out of service in 2020. Zones of interest for further investigation were identified by endoscopy. Sensor measurements of humidity and gas content of the headspace have been performed. Using minimally invasive sampling methods, inner aged liquid including biological deposits was sampled and studied with cultivation and molecular biological methods. DNA was extracted and amplified with 16S rRNA gene and ITS primers. Several anaerobic taxa were observed, including sulfate-reducing bacteria, thiosulfate-reducing bacteria and methanogens: all known culprits of microbiologically influenced corrosion (MIC). Moreover, 95% of fungal sequences could be identified as a single taxon associated with diesel contamination: the diesel pest *Amorphotheca resiniae*. So far cultivation attempts resulted in three isolates of *Amorphotheca resiniae*, one of them studied in detail. The isolate can use B7 diesel as a carbon source, does not strongly acidify its growth medium, and does not produce a strong iron chelator. Batch corrosion experiments with steel coupons have shown us however that this fungus had a higher carbon steel corrosion rate than the abiotic control. Here we present an application driven corrosion study that shed new insights on the role of fungi on metal corrosion within fuel systems that aid the development of novel mitigation strategies.

***134 Bibliometric Analysis on Microbiologically Influenced Corrosion in Oil and Gas Systems**

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A bibliometric analysis on Microbiologically Influenced Corrosion (MIC) of oil and gas industry was performed utilizing Scopus and Web of Science. The documents published on the topic until May, 2023 were extracted from both databases and merged, which resulted in 603 documents after removing duplicates. Bibliometrix package and Biblioshiny interface in R studio were used for the bibliometric analysis. The first document on the topic was released in 1982 and there was about 9.5% annual growth of publications until 2023 year. The core group consisted of 14 out of 277 sources with NACE International Corrosion Conference Series as the most active in the field (98 documents). The highest number of documents was from the USA (146 documents), China (87 documents) and Canada (48 documents). The most active countries in the field published mainly single country documents and demonstrated very low to no international collaboration on the domain. The Sankey plot projected that besides MIC authors from the USA, China and Canada were also publishing on sulfate reducing bacteria, biofilm and biocide as sub-topics. The most used author keywords mapped after MIC were biofilm, sulfate reducing bacteria, biocide, carbon steel and pitting corrosion which also represent the current direction of the research field. Collaboration network analysis resulted in only ten unique authors and six institution sub-networks with at least two collaborative papers. This study sheds light on the evolutionary nuances of the area while displaying trend topics and research gaps, as well as hidden collaboration networks among the actors. Keywords: co-word analysis, science mapping, thematic evaluation, network analysis, bibliometric study, oil and gas Acknowledgement This article/publication is based upon work from COST Action “CA20130 Euro-MIC” (<https://www.euro-mic.org>), supported by COST (European Cooperation in Science and Technology). COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation.

***135 A deep dive into anaerobic hydrocarbon biodegradation: Metagenome analysis of a methanogenic toluene-degrading enrichment**

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The methanogenic biodegradation of hydrocarbons is a process of great importance in deep subsurface crude oil-bearing reservoirs and in shallow contaminated subsurface environments. With some exceptions, this metabolic process occurs in the absence of external electron acceptors and requires a syntrophic partnership wherein at least one bacterium facilitates the activation and subsequent degradation of a hydrocarbon while partnered with a methanogen(s) that consumes small metabolites and allows the reaction to be energetically favourable. Toluene, a monoaromatic hydrocarbon, has served as a model compound to understand how methanogenic hydrocarbon biodegradation can occur. Previous research in our group showed evidence for toluene activation via benzylsuccinate synthase. Bacteria such as *Desulfosporosinus* and the methanogen *Methanomicrobia* were identified as probable contributors to the toluene biodegradation process. Here, we build upon this initial finding by performing metagenome sequencing using the Illumina NovaSeq 6000 platform. This analysis generated thirty-five high-quality metagenome-assembled genomes (MAGs) (>90% completeness, <10% contamination) and thirty medium-quality MAGs (>70% completeness, <10% contamination). Members of several phyla harbouring genes associated with methanogenic toluene degradation were identified in the MAGs, including those from the Firmicutes (*Syntrophobotulaceae* and *Desulfosporosinus*), *Desulfobacterota* (*Desulfarculaceae* and *Syntrophorhabdaceae*), *Halobacteriota* (*Methanoculleus* and *Methanoregula*) and *Methanobacteriota* (*Methanobacterium*). Further analysis of the MAGs revealed an abundance of electron transfer genes encoding various hydrogenases, formate dehydrogenases, ferredoxin oxidoreductases, NADH-quinone oxidoreductases, and electron transfer flavoproteins (ETF $\alpha\beta$), that are associated with syntrophic metabolism. This study highlights the importance of syntrophy in anaerobic hydrocarbon biodegradation and the use of metagenomics to identify the potential genes and processes involved.

***136 Meta-omics analysis for a deep subsurface microbial community degrading toluene under methanogenic condition**

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Microbial crude oil degradation coupled with methane production has attracted much attention as a potential technology for microbial enhanced energy recovery (MEER). Our previous study demonstrated that methanogenic crude oil degradation could be induced by injecting a crude oil-degrading microbial community into another reservoir lacking its ability. However, the main players and metabolic pathways contributing to the degradation of crude oil constituents in the microbial community remain unclear. In this study, we performed meta-omics analysis for the further enriched microbial community exhibiting methanogenic toluene degrading activity. Amplicon sequencing of 16S rRNA genes showed that the microbial community was dominated by hydrogenotrophic and acetoclastic methanogens, *Peptococcaceae* bacterium, and *Atribacterota* bacterium (class JS1), suggesting that these microorganisms are mainly involved in methanogenic toluene degradation. Furthermore, we recovered

high-quality metagenome-assembled genomes (MAGs) of these microorganisms from the toluenedegrading microbial community. Metatranscriptomic analysis revealed that the Peptococcaceae MAG highly expressed genes encoding the conventional benzyl succinate synthase-mediated toluene degradation pathway. In addition, the JS1 MAG also highly expressed genes encoding benzyl succinate synthase, indicating that these bacteria play a main role in toluene degradation. However, the JS1 MAG lacked the downstream metabolism from benzyl succinate, suggesting that the organism may possess a yet-to-be identified pathway to convert toluene. This study provides insights into the functional role of the oil reservoir microorganisms in the conversion of crude oil to methane for practical MEER.

***137 Baseline study to identify oil and gas seepage from natural and anthropogenically influenced sites from Danish North Sea**

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Oil and gas reservoirs host unique microbial communities that differ from other sedimentary or aquatic assemblages. A second community type can develop where natural gas seeps from the sea floor. Such characteristic patterns can be used to identify the presence of hydrocarbons and recognize their possible sources. This study covers the near surrounding of two production platforms and one natural seepage area from the North-Sea. We aim to use microbial abundance, community composition and concentrations of light aliphatic hydrocarbons as indicators to establish the difference between hydrocarbon seepage from natural and anthropogenically influenced sites. The goal is to develop tools to monitor the integrity of abandoned reservoirs based on the microbial community composition at the sediment-water interface at abandoned production sites. For this we collected samples of surface sediment within 500m from currently active platforms and suitable reference sites. We analysed the number of prokaryotes via using q-PCR of 16S genes, community composition via amplicon sequencing of 16S genes, gas composition via gas chromatography and the isotopic composition of methane via isotope ratio mass spectrometry. This data was correlated with seismo-acoustic data from the underlying seabed, analyses of the bivalve community as well as basic sediment characteristics. The data show distinct correlations between the community composition and active gas seepage.

***138 Development of Molecular Probes for Thiosulfate and Iron Reducing Bacteria**

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Microbiologically Influenced Corrosion (MIC) activities are mostly monitored by using the canonical culture-based methods. However, these methods detect only culturable bacteria or require months of incubation to get the results and hence are not suitable for making immediate decisions. To overcome the limitations, molecular detection methods were developed for many of the MIC-causing microbes but not available for Iron Reducing Bacteria (IRB) and Thiosulfate Reducing Bacteria (TRB). IRB and TRB are among the dominant group of microbes involved in corrosion and souring oil and gas infrastructures. In particular, TRB accounts for about 99% of the total microbial population observed in highly saline environments and is considered to be the most problematic for gas storage in salt caverns. In the present study, molecular probes were developed for IRB and TRB by targeting specific regions of putative genes implicated in iron and thiosulfate reduction, respectively. Candidate sequences were identified by searching putative genes in genome repository databases of pure isolates or in metagenomic database of environmental samples. Retrieved sequences were aligned using bioinformatic software and primer-based probes were developed from conserved regions. Specificity and efficiency of the probes were

validated by amplifying the sequences of target and non-target microbes using Quantitative Polymerase Chain Reaction (qPCR) methods. The probes successfully detected candidate microbes in biocorrosion samples collected from various gas processing facilities. The identified primer-based probes will help oil and gas industries to monitor MIC activities and develop timely management strategies to prevent undesirable microbial activities while improving system integrity and safety concerns.

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