ISMOS-7 ABSTRACT BOOK

18th-21st June 2019

Halifax Convention Centre,

Halifax, Nova Scotia, Canada

Symposium and Poster Sessions

Editors: Sean Caffrey, Andrew Stone, Corinne Whitby & Torben Lund Skovhus.

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DuPont Microbial Control is a leading provider of biocide and antimicrobial solutions that control and can help prevent the growth of nuisance and dangerous micro-organisms. Our products and technologies can be found in a wide range of industries. DuPont Microbial Control helps customers to go beyond biocides and ask more of their microbial control solutions. We offer world-class products, service and information to our customers, including:

- Process preservation chemistries
- Formulation expertise
- In-can preservatives and dry film fungicides/algaecides
- Water treatment chemistries
- Sanitizing and disinfection chemistries
Silver Sponsors

Microbial Insights, Inc. (MI) is a woman owned small business specializing in the development and application of cutting edge molecular biological tools (MBTs) to describe and quantify microbial communities. MI is dedicated to providing superior genetic and chemical diagnostic tools to aid our clients in understanding and managing biological processes for a wide range of areas including environmental remediation, microbial induced corrosion, and microbial source tracking.

LuminUltra is a global leader in the development and delivery of user-friendly microbiological monitoring solutions to laboratories, service providers and asset owners in the oil & gas market, in efforts to help them identify and address the root cause of Microbiologically Influenced Corrosion (MIC), Souring and Biofouling. Our best in class portfolio of testing methods and software tools – including 2nd Generation ATP® and now our GeneCount™ DNA-based products and services – provide customers with an accurate, fast, and comprehensive picture of how many microorganisms and what types of microorganisms are in their processes. Customers benefit by being able to quickly and accurately quantify both total and specific microbes to understand cause-and-effect relationships that leads to more targeted treatment and proactive decision-making, supported by our expert team and digital engagement platforms. More data on the microorganisms in your system means faster, better decision making for greater efficiencies.

Nalco Champion, an Ecolab company, provides safe, sustainable chemistry programs and services to the upstream and midstream oil and gas industry, refineries and petrochemical operations. Through onsite problem solving and the application of innovative technologies, we maximize production, optimize water use and overcome complex challenges in the world’s toughest energy frontiers.
Bronze Sponsors

COVE brings together people, ideas, industry and research to help our community and members to work in new ways. Together, we are a catalyst in creating the world’s next practical, commercial and revolutionary ocean tech advances.

DNV GL is a leading provider of risk management, technical advisory and technical assurance services to the oil and gas industry, specializing in challenging operating environments and mediums in both onshore and offshore applications. Our science and engineering expertise can be applied to a wide range of disciplines related to materials / technology (testing, selection, verification), degradation (including MIC and biofouling), integrity management, flow optimization, forensic investigation and management systems. DNV GL maintains a highly skilled and experienced staff backed by one of the premier materials testing laboratories in North America. We help operators implement and optimize internal corrosion management practices, including biocide and inhibitor selection and advanced monitoring technologies, including molecular microbiological methods.

University of Calgary offers students a high-quality educational experience that prepares them for success in life, as well as research that addresses society’s most persistent challenges. Our creation and transfer of knowledge contributes every day to our country’s global competitive advantage and makes the world a better place. We do this in Calgary – Canada’s most enterprising and caring city, where the Western can-do spirit, sense of family, and youthful energy permeate everything we do. We’re pioneers at heart, innovators by necessity and desire, community-builders because it’s in our DNA. We see potential where others balk at challenge and we know how to cooperate to “git’er done.”
Genome Canada is a not-for-profit organization, funded by the Government of Canada. We act as a catalyst for developing and applying genomic-based technologies to create economic and social benefits for Canadians. We:

- connect ideas and people across public and private sectors to find new uses for genomics
- invest in large-scale science and technology to fuel innovation; and,
- translate discoveries into solutions across key sectors of national importance, including health, agriculture and agri-food, forestry, fisheries and aquaculture, the environment, energy and mining. Learn more about us and discover the role Genome Canada plays in harnessing the transformative power of genomics for the benefit of Canadians.

Welcome Reception Partner

The Nova Scotia Department of Energy and Mines brings all of the province’s experts in subsurface resources together in one department. The merger will also provide opportunities for the energy and mining files to explore collaborative opportunities to better promote the province’s energy and mineral resources.

Nova Scotia Kitchen Party Sponsor

Genome Alberta is a publicly funded not-for-profit corporation which initiates, funds, and manages genomics research and partnerships. We strive to be the leading source of information and administration related to genomics, metabolomics, bioinformatics, bioethics, and other omics-related research in Alberta.
Baker Hughes, a GE company (NYSE:BHGE) is the world’s first and only fullstream provider of integrated oilfield products, services and digital solutions. Drawing on a storied heritage of invention, BHGE harnesses the passion and experience of its people to enhance productivity across the oil and gas value chain. BHGE helps its customers acquire, transport and refine hydrocarbons more efficiently, productively and safely, with a smaller environmental footprint and at lower cost per barrel. Backed by the digital industrial strength of GE, the company deploys minds, machines and the cloud to break down silos and reduce waste and risk, applying breakthroughs from other industries to advance its own. With operations in over 120 countries, the company’s global scale, local know-how and commitment to service infuse over a century of experience with the spirit of a startup – inventing smarter ways to bring energy to the world.

The mission of Saint Mary’s University is to:
- Offer undergraduate, graduate and life-long learning programs.
- Engage in research and disseminate its results.
- Serve the community from the local to the international level.

Dalhousie University is proudly located in Halifax, Nova Scotia which is ranked one of the friendliest cities in the world. Founded in 1818, we’re one of Canada’s oldest universities, attracting more than 18,800 students from around the world. The university blends transformative academic programs with pioneering research on Canada’s East Coast.

As Newfoundland and Labrador’s only university, Memorial has a special obligation to the people of this province. Established as a memorial to the Newfoundlanders who lost their lives on active service during the First World War and subsequent conflicts, Memorial University draws inspiration from these sacrifices of the past as we help to build a better future.
Best Poster Sponsor

**SPRINGER NATURE** Springer Nature is the world’s largest academic book publisher, publisher of the world’s most influential journals and a pioneer in the field of open research. The company numbers almost 13,000 staff in over 50 countries and has a turnover of approximately EUR 1.5 billion. Springer Nature was formed in 2015 through the merger of Nature Publishing Group, Palgrave Macmillan, Macmillan Education and Springer Science+Business Media.

Prof. Gerrit Voordouw Best Presentation Award

CRC Press/Taylor & Francis is the leading publisher of engineering, science, and technology books. See our references, textbooks, and professional works in materials science and chemical engineering, including cutting-edge titles on corrosion, oil, and gas, at www.crcpress.com.

Supporters

Supporters are organizations providing logistical and technical support for ISMOS.
Dear Delegate,

Welcome to ISMOS-7, Halifax Convention Centre, Halifax, Nova Scotia, Canada.

This is the 7th meeting of the International Symposium on Applied Molecular Microbiology in Oil Systems (ISMOS).

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

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

The meeting is multidisciplinary, linking biogeochemists, engineers, molecular biologists and microbiologists, and will include a mixture of high profile international speakers from industry and academia. We will have an end user workshop, which focuses on specific case studies “From lab to field” relevant to the oil and gas industry.

For the social events there is a Welcome Drinks Reception ( Tues 18th June), Kitchen Party at Cove (Wed 19th June) and Gala Dinner (Thurs 20th June).

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 the sponsors (Baker Hughes, COVE, CRC Press/ Taylor & Francis, Dalhousie University, DNV GL, DuPont, Microbial Insights Inc., LuminUltra, Memorial University, Nalco Champion, Nova Scotia Department of Energy and Mines, Genome Alberta, Genome Canada, Saint Mary’s University, Springer Nature, University of Calgary) for their support to ISMOS-7.

We hope you have an interesting and enjoyable meeting!

Yours,

Andy Stone, Genome Atlantic (LOC)
Torben Lund Skovhus, VIA University College (TSC Chair)
Corinne Whitby, Essex University (TSC Vice Chair)
Sean Caffrey, University of Toronto (ISMOS webmaster)
# ISMOS-7 Program Overview

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<td>08:00</td>
<td>Registration</td>
<td>Breakfast</td>
<td>Breakfast</td>
<td>Breakfast</td>
<td>Opening Ceremonies</td>
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<tr>
<td>09:00</td>
<td>Session 1: Current Trends in Physics and Technology</td>
<td>Session 2: Advanced Materials</td>
<td>Session 3: Quantum Computing</td>
<td>Session 4: Space Exploration</td>
<td>Session 5: Renewable Energy</td>
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<tr>
<td>10:30</td>
<td>Coffee Break</td>
<td>Coffee Break</td>
<td>Coffee Break</td>
<td>Coffee Break</td>
<td>Lunch Break</td>
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<tr>
<td>11:00</td>
<td>Session 6: Nanotechnology</td>
<td>Session 7: Artificial Intelligence</td>
<td>Session 8: Robotics</td>
<td>Session 9: Biotechnology</td>
<td>Session 10: Data Science</td>
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<tr>
<td>13:00</td>
<td>Lunch</td>
<td>Session 11: Climate Change</td>
<td>Session 12: Sustainable Energy</td>
<td>Session 13: Space Telescope</td>
<td>Session 14: Advanced Materials</td>
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<td>16:00</td>
<td>Coffee Break</td>
<td>Coffee Break</td>
<td>Coffee Break</td>
<td>Coffee Break</td>
<td>Closing Plenary</td>
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<td>17:00</td>
<td>Session 20: Robotics</td>
<td>Session 21: Artificial Intelligence</td>
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<td>Session 23: Climate Change</td>
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Note: The schedule is subject to change. Please check the official website for updates.
Symposium Program

Tuesday, June 18, 2019

14:00 – 19:00 Registration & Information Desk Open (Convention Level Foyer)

TUESDAY 18th June 2019

15:00 Session 01: Workshop “Science-based oilfield management - From the lab to the field”.

Chairs: Torben Lund Skovhus (VIA University College)/ Richard B Eckert (DNV GL)

Invited Speakers:
15:05 Tom Coolbaugh (ExxonMobil) “Microbial Degradation of Crude Oil and the Value of Dispersants for Offshore Oil Spill Response”
15:45 Kelly Wrighton (Colorado State University) “Biogeography and environmental conditions shape taxonomic membership and souring potential across the fractured shale microbiome”
16:05 TJ Tidwell (Nalco Champion) “Implementation of Microbial Control Strategies that Work: Lab to Frac, Lab to Production”

16:20 BREAK

Offered paper
16:30 Nicolas Tsesmetzis (Shell Technology Center) *165 “MetaHCR: a web-enabled metagenome data management system for hydrocarbon resources”

16:50 Q&A and DISCUSSION

17:30 End of Session

18:00 – 20:00 ISMOS-7 Welcome Reception, Room 605, Summit Level, Halifax Convention Centre
Sponsored by the Nova Scotia Department of Energy and Mines
Wednesday 19th June 2019

NOTE - All ISMOS-7 Oral Presentations will take place in Room C2 – Convention Level (1), Halifax Convention Centre

07:00 – 17:00 Registration & Information Desk Open (Convention Level Foyer)

09:00 Welcome Introduction
Andy Stone, Genome Atlantic
Torben Lund Skovhus, VIA University College
Corinna Whitby, University of Essex

09:20 PLENARY: Adam MacDonald/ Kim Doane (Nova Scotia Department of Energy and Mines)

“Petroleum Resources Offshore Nova Scotia – Microbiology and the Play Fairways Analysis”.

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

Chairs: Torben Lund Skovhus (VIA University College)/ Marcia Lutterbach (INT)

10:05 Invited Speaker: Dennis Enning (Exxon Mobil)

“Insights from microbial community analysis as part of a pipeline corrosion root cause investigation”.

10:35 NUTRITION BREAK IN POSTER & EXHIBIT AREA
Sponsored by DNV GL

Offered papers

Speaker 1
11:05 Kathleen Duncan (University of Oklahoma)

“System-wide analysis of shale gas/liquids processing from an MIC point of view”.

Speaker 2

11:25 Mohammed Taleb-Berrouane (Memorial University of Newfoundland)

“Predicting Sessile Microorganism Populations in Oil and Gas Gathering and Transmission Facilities”. 
Speaker 3
11:45 Andrea Koerdt/ Annie An (Bundesanstalt für Materialforschung und -prüfung (BAM))

*71 “From iron to gas: methanogen-induced microbiologically influenced corrosion (Mi-MIC) cause high corrosion in dynamic environments”.

12:05 LUNCH
Sponsored by Microbial Insights

Speaker 4
13:25 Jaspreet Mand (Exxon Mobil)

*84 “Corrosion inhibitors influence the occurrence of highly localized MIC features”.

Speaker 5
13:45 Mohita Sharma (University of Calgary)

*138 “Characterization of a highly corrosive sample enriched from nitrate injected produced waters after long-term incubation in nutrient limited conditions using metagenomic, electrochemical, and surface analyses”.

Speaker 6
14:05 Nicolas Tsesmetzis (Shell Technology Center)

*166 “Contrasting pathways for anaerobic methane oxidation in Gulf of Mexico cold seep sediments”.

Speaker 7
14:25 Joseph Moore (DuPont Microbial Control)

*151 Highly Resolved Sampling and Analysis of a Hydraulic Fracturing Operation: A Case Study in Effective Microbial Control

14:45 NUTRITION BREAK IN POSTER & EXHIBIT AREA
Sponsored by University of Calgary

15:05 POSTER SESSIONS

17:00 End of Session

17:45 Meet, Argyle Street Lobby, Halifax Convention Centre, to walk to boat departure for evening activity

18:00-21:00 Nova Scotia Kitchen Party, Centre for Ocean Ventures and Entrepreneurship Sponsored by COVE and Genome Alberta
Thursday 20th June 2019

08:30 – 17:00 Registration & Information Desk Open (Convention Level Foyer)

Session 03: Hydrocarbon Biodegradation

Chairs: Max Frenzel (Oil Plus Ltd)/ Nicolas Tsesmetzis (Shell Technology Center)

09:00 Invited Speaker: Ian Head (Newcastle University)
“Controls on anaerobic biodegradation of crude oil hydrocarbons”

Offered papers

Speaker 8
09:30 Courtney Toth (University of Toronto)
*46 “Anaerobic BTEX Bioremediation is a Number’s Game”

Speaker 9
09:50 Gloria Okpala (University of Alberta)
*129 “Microbial degradation of residual recalcitrant hydrocarbon in mined tailings under different redox conditions”

Speaker 10
10:10 Charles Greer (National Research Council)
*174 “Genomics-based analyses to evaluate natural microbial assemblages and their response to petroleum hydrocarbons in Canadian marine environments”

10:30 NUTRITION BREAK IN POSTER & EXHIBIT AREA
Sponsored by COVE

Speaker 11
11:00 Connor Dalzell (Saint Mary’s University)
*127 “Thermochemical Oil Formation in Hydrothermal Vent Sediments at Guaymas Basin, Gulf of California and the Search for a Deep Biosphere Hydrocarbon Fingerprint”.

Speaker 12
11:20 Gareth Thomas (University of Essex)
*60 “Effect of dispersants and biosurfactants on crude oil biodegradation and microbial ecology”.

Speaker 13
11:40 Ali Akbari (Biofilm Center, University of Duisburg-Essen Campus Essen)
*117 “Necromass Fermentation Contributes to the Recycling of Carbon and Nutrients in Environments with Elevated Concentrations of Oil Compounds”.

15
Speaker 14
12:00 Thusitha Gunasekera (Air Force Research Laboratory)
   *150 “Use of transcriptomics and genomics to understand the underlying mechanisms of bacterial adaptation to jet-fuel”.

12:20 LUNCH
   Sponsored by LuminUltra Technologies Ltd.

13:40 POSTER SESSIONS

15:10 NUTRITION BREAK IN POSTER & EXHIBIT AREA
   Sponsored by Genome Canada

Thursday 20th June 2019 Continued

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

Chairs: Nicole Dopffel (BASF)/ Marko Stipanicev (Schlumberger)

15:40 Invited Speaker: Sabrina Beckmann (University of Delaware)
   “Long-term succession in a gas and oil microbiome during in situ stimulation of methane using conductive Neutral Red Crystals”.

Offered papers
Speaker 15
16:10 Lisa Kroll (University of Duisburg)
   *69 “Tiny microbial ecosystems isolated from oil help understanding microbial community assembly”.

Speaker 16
16:30 Geert van der Kraan (Dow Chemical Company)
   *92 “Phylogenomic and Metagenomic analyses of oilfield microbial communities shows they genetically differ and align with the differences in the chemical engineering parameters of top side asset elements”.

Speaker 17
16:50 Sarah Eisenlord (Gas Technology Institute)/ Kara Tinker (NETL)
   *99 “Microbiological assessment of 3 hydraulically fractured wells at the HFTS (Hydraulic Fracture Test Site), Permian”.

Speaker 18
17:10 Casey Hubert (University of Calgary)
*156 “De-risking off-shore reservoir exploration through DNA sequencing and Machine Learning”.

17:30 End of Session

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**Friday 21st June 2019**

08:30 – 16:00  Registration & Information Desk Open (Convention Level Foyer)

Session 04 (continued) Oil and gas microbiome: Problems, control and opportunities

Chairs: Nicole Dopffel (BASF)/ Marko Stipanicev (Schlumberger)

**Offered papers continued**

Speaker 19

09:05 Gunhild Bødtker (Norce Research)

*108 “Influence of water quality on biofilm growth and injectivity”

Speaker 20

09:25 Na Liu/ Gunhild Bødtker (Norce Research)

*110 “Microfluidic studies on mechanisms of biofilm formation and its effect on flow properties in porous media”

Speaker 21

09:45 John Coates (UC Berkeley)

*133 “Dissecting the inhibition of sulfidogenic communities by the specific inhibitor perchlorate”

10:10 NUTRITION BREAK IN POSTER & EXHIBIT AREA, POSTER SESSIONS

*Sponsored by Genome Atlantic*
Friday 21st June 2019 continued
Session 05: Emerging Innovation and Applications in petroleum microbiology

Chairs: Renato De Paula (Nalco Champion)/ Corinne Whitby (University of Essex)

11:15 Invited Speaker: Zarath Summers (ExxonMobil)
“Detecting the Signal through the Noise: Identifying Subsurface Organisms through Machine Learning”.

Offered papers

Speaker 22
11:45 Deidra Ferguson (University of Calgary)
*116 “Marine bacterial diversity in seafloor sediments for environmental baseline monitoring prior to deep sea offshore oil exploration”.

Speaker 23
12:05 Jayne Rattray (University of Calgary)
*104 “Dipicolinic acid as a tracer for thermospores and oil seeps in marine sediments”.

Speaker 24
12:25 Elvira Mitraka (Dalhousie University)
*115 “MICON: an ontology to unify the disparate field of Microbiologically Influenced Corrosion”.

12:45 LUNCH
Sponsored by NALCO Champion

Speaker 25
13:45 Daisuke Mayumi (aist)
*137 “Enhancement of methanogenic crude oil degradation using organic nutrients toward biotechnology applications in oil reservoirs”.

Speaker 26
14:05 Breda Novotnik (University of Calgary)
*160 “Microbial metal dependent conversions of hydrocarbons for zero CO₂ emission energy extraction”.

Speaker 27
14:25 Akhil Agrawal (Central University of Rajasthan)
*158 “Assessing wettability alteration potential and rheological properties of novel polymer for development of biochemical enhanced oil recovery (BcEOR) technology”.

Speaker 28
14:45 Nicole Dopffel (BASF SE)
*111 “Seven years of Microbial Enhanced Oil Recovery (MEOR): Lessons learned on the way to application”.

15:05
KEYNOTE: Kenneth Lee (Fisheries and Oceans Canada)

“Microbes will Influence the Effectiveness of Future Operational Oil Spill Countermeasures”

15:50 CLOSING REMARKS and ANNOUNCEMENT OF ISMOS-8
(Casey Hubert, University of Calgary and Torben Lund Skovhus (VIA University College)

16:00 END OF MEETING
ABSTRACTS

Oral Program:

Session 01: Workshop “Science-based oilfield management – From the lab to the field”.

INVITED SPEAKERS

“Microbial Degradation of Crude Oil and the Value of Dispersants for Offshore Oil Spill Response”

Author
1. Tom Coolbaugh (ExxonMobil)

Oil spill response strategies are designed to minimize environmental impacts to the extent possible. Each response option must be evaluated for operational limitations (e.g., sea state), potential effectiveness, environmental impacts of the response option itself, and applicability under various oil spill scenarios (e.g., size and location of the spill) in addition to health and safety of the responders. Although mechanical recovery is often favored for its ability to directly remove oil from the environment, it has long been recognized that for large offshore spills this technology has significant limitations. In addition to known operational limits in the presence of currents and waves, the dynamic-nature of offshore oil slicks, i.e., rapid spreading and movement, has resulted in mechanical recovery only treating a small fraction of spilled oil in the past. Because of these limitations, the oil and gas industry has worked to develop alternative response tools that can be used in addition to mechanical recovery to more effectively treat large offshore oil spills. This presentation will provide a review of the primary oil spill response options, a discussion that addresses misperceptions and misunderstandings about the use of dispersants, their primary mode of action, and a description of dispersant use during the Deepwater Horizon incident including the important health and safety aspects of subsea dispersant injection.


Author
1. Lisa Gieg (University of Calgary)

Microbiologically influenced corrosion (MIC) in the oil and gas industry is a degradation phenomenon wherein microorganisms play a key role in reducing the integrity of carbon steel infrastructure. However, attributing an infrastructure failure solely to the action of microorganisms can be challenging because physical and chemical conditions also play important roles in driving detrimental microbial activity. Thus, a holistic approach should ideally be used to provide multiple lines of evidence that MIC has occurred. Such evidence should include measurements to identify microbial communities and/or their activities, underlying chemical conditions, and the presence of corrosive products. If possible, confirming MIC potential through controlled laboratory tests can provide an additional line of evidence. As a critical first step for assessing MIC, high quality samples must be obtained. This talk will overview the
importance of obtaining robust field samples for assessing MIC potential, and will present a case study wherein a holistic approach was used to assess whether a pipeline failure could be at least partially attributed to MIC.

“Biogeography and environmental conditions shape taxonomic membership and souring potential across the fractured shale microbiome”

Author
1. Kelly Wrighton (Colorado State University)

The transmission and subsequent proliferation of surface microbes into deep fractured shale has impacts regarding energy recovery, sustainability, and additionally poses significant safety risks. An important knowledge gap that remains poorly studied is identification of surface contaminant inputs and overall functional and taxonomic composition across geographically distinct shales. Leveraging temporal sampling approaches, we analyzed over 350 surface and produced fluid metagenomes, many with paired chemical data, from 63 wells across Permian, Eagle Ford, STACK, Marcellus, Utica, DJ Basin, and Duvernay shale plays. To identify sources of persisting well microorganisms, microbial communities colonizing all major equipment surfaces topside, as well as all downhole fluids, were inventoried during completion on two well pads. These audit results revealed critical control points for microbial management including drill muds and fully formulated frac fluids, which housed organisms with souring potential that later became established, persisting members of produced fluid communities. Data analyses indicate that biogeographical patterns of produced fluids were shaped by shale play, and were largely influenced by salinity and temperature gradients. Functional gene analyses revealed that across all produced fluids, thiosulfate reduction is a primary, and currently underappreciated, driver of souring, especially in wells with higher salinity fluids. The capacity for microbial sulfide production from thiosulfate was evaluated in the laboratory with bacteria isolated from produced fluids, where sulfide yields were influenced by nutritional regimes and growth stage. Ongoing work is leveraging this extensive shale-derived meta-omic database to develop a model to predict biotic (taxon, functional, geochemical) patterns that accurately classify well performance and risk factors. Elucidating the genetic and taxonomic landscapes of fractured shale environments pose important practical implications for management, with insights from this approach widely translatable to other hydrocarbon systems.

“Implementation of Microbial Control Strategies that Work: Lab to Frac, Lab to Production”

Author
1. TJ Tidwell (Nalco Champion)

The implementation of microbial molecular methods (MMM) has revolutionized the ways that risk is assessed and how the value of a proper microbial control strategy is assigned. These technologies coupled with advancements in microbial activity monitoring, such as ATP/AMP testing, have made the experimental work completed in the lab more easily translatable to field processes. In fracturing and completion applications, the prevention of microbial contamination has previously been undervalued, but observations that microbes can create long-term problems,
particularly in water reuse applications, has driven the implementation of advanced programs to treat and prevent the proliferation of microbes. Additionally, MMM have allowed for more robust diagnosis of existing microbial control issues, presumably from poor completions practices, and helped justify more aggressive microbial control strategies to remediate troubled wells and assets. In this talk, we will look at multiple examples of microbial control strategies solved in the lab and scaled up to field wide applications where MMM helped prove both the necessity and value of such programs.

**Offered paper**

*165 “MetaHCR: a web-enabled metagenome data management system for hydrocarbon resources”*

Author

1. **Nicolas Tsesmetzis (Shell Technology Center)**

The ever-increasing metagenomic data necessitate appropriate cataloguing in a way that facilitates the comparison and better contextualization of the underlying investigations. To this extent, information associated with the sequencing data as well as the original sample and the environment where it was obtained from is crucial. To date, there are not any publicly available repositories able to capture environmental metadata pertaining to hydrocarbon-rich environments. As such, contextualization and comparative analysis among sequencing datasets derived from these environments is to a certain degree hindered or cannot be fully evaluated. The metagenomics data management system for hydrocarbon resources (MetaHCRs) enables the capturing of marker gene and whole metagenome sequencing data as well as over 300 contextual attributes associated with samples, organisms, environments and geological properties, among others. Moreover, MetaHCR implements the Minimum Information about any Sequence–hydrocarbon resource specification from the Genomic Standards Consortium; it integrates a user-friendly web interface and relational database model, and it enables the generation of complex custom search. MetaHCR has been tested with 36 publicly available metagenomic studies, and its modular architecture can be easily customized for other types of environmental and metagenomics studies. A customized version of MetaHCR is already in the works by a large Canadian-funded JIP consortium focused on Microbiologically Influenced Corrosion (MIC) to catalogue data from MIC-related studies.

**PLENARY**

**Title**  “Offshore Petroleum Resources - Nova Scotia and the Play Fairway Analysis”.

**Author**

1. **Adam MacDonald (Nova Scotia Department of Energy and Mines)**

Nova Scotia has been producing offshore Oil and Gas since 1992. The province has received in excess of 2 billion dollars over the last 27 years. This money went directly into the provincial budget and has been used to build roads, hospitals, schools and infrastructure for a growing population and diverse economy. Continuing our success in attracting investment of exploration and development to our offshore is the
responsibility of the Nova Scotia Department of Energy and Mines (NSDOEM). Since the release of our Play Fairway Analysis in 2011 we have managed to attract explorers who have committed to working expenditures approximating 2.18 billion dollars. This work follows a lull of activity commonly seen in this cyclic industry where global commodity prices are associated with not only supply and demand but political stability globally. Entry into and exits from an area of interest can be swift and reactive in a “herd mentality” fashion.

In the beginning of this lull we reached out to industry to gain a better understanding of the exodus of companies from our offshore. We received a common theme that exploring offshore Nova Scotia was too risky to make any continued investment under our current regulatory and expense regime. Without compromising our health, safety and environmental controls, the NSDOEM set out to manage and decrease the risk of a new entry into our offshore. By listening to the global industry we recognized a common theme of wanting “new data and access to information” to help make informed decisions. With this feedback, the NSDOEM and its partners completed a de-risking project in 2011, called the Play Fairway Analysis, which defines the fairways of opportunity. This provides the baseline data needed as a steppingstone into a frontier area, such as ours. We offer this analysis for free and in full transparency to its methodology and data. Following this success, we continue to provide new information building upon our Play Fairways Analysis and increasing our collaboration.

We have recently begun a second phase of an Offshore Growth Strategy which is a four-year initiative to continue to generate new insight about our offshore petroleum geology, attract new investment to our province, and ready our offshore regime and local supply chain to be prepared for increased offshore oil and gas activity. Our research has reached from the office to conducting satellite to sea surface to sea floor seep identification and sampling in collaboration with the Geological Survey of Canada Atlantic. This uses new and conventional remote sensing technology. The innovative approach of incorporating microbiology science by genotyping the DNA of oil eating bacteria could be a highly valuable new tool to pathfinding in the deep waters for subsea sub subsurface hydrocarbon reserves. This addition can largely help validate our deeper regional models.

Our continued applied research improves the understanding of Nova Scotia’s complex petroleum geology to remove some of the geological uncertainty associated with exploration. If exploration efforts lead to new commercial development of oil and gas in the offshore, royalties, crown share payments, and employment and contract opportunities would translate into significant economic benefit to the province.

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

INVITED SPEAKER
“Insights from microbial community analysis as part of a pipeline corrosion root cause investigation”

Authors
1. Dennis Enning - ExxonMobil Upstream Research Company
Carbon steel pipelines in upstream oil production can be susceptible to microbiologically influenced corrosion (MIC). At the same time, such pipelines also commonly undergo corrosion resulting from the acid gases CO2 and H2S, so that a distinction between biotic and abiotic corrosion as root causes to overall pipeline degradation can be challenging. In this study, we investigated the root causes of internal corrosion in two slightly sour subsea pipelines in the Arabian Gulf. Field samples were collected for the microbiological and physicochemical characterization of these pipelines. Next generation sequencing of 16S rRNA genes demonstrated that produced waters and pig debris contained Pelobacter, Halanaerobium and Geotoga species along with delta-proteobacterial sulfate-reducing bacteria (SRB). Biofilm on corrosion coupons, on the other hand, was profoundly dominated by SRB; most notably of the Desulfovibrio, Desulfonatronum and Desulfobacter genus. Measurement of in situ pH, using a portable oil-water separator with built-in pH electrodes, revealed the oil-associated, pressurized produced water to be poorly buffered, with pH ranging from pH 5.2 to pH 5.7. Under such conditions, high baseline corrosion rates (0.3 - 0.5 mm/yr) were expected based on field-specific mechanistic modeling and laboratory simulation of acid gas corrosion. In order to parse out a potential microbiological impact on pipeline degradation, corrosion reactor tests were adapted to include field-collected microorganisms. In the presence of field microorganisms, corrosion rates as high as 1.7 mm/yr were observed in a 7-week, pH-controlled corrosion reactor test (i.e. 3 – 6 times more corrosion than with acid gas alone). Steel-attached biofilms in this corrosion reactor were mainly composed of SRB of the genus Desulfovibrio, Desulfonatronum and Desulfovermiculus, and as such resembled those found on field coupons. It was concluded that the investigated pipelines experienced a combination of acid gas corrosion and severe MIC.

**Offered papers**

*77 “System-wide analysis of shale gas/liquids processing from an MIC point of view”.

**Authors**

1. Kathleen Duncan - University of Oklahoma
2. Meredith DeGarmo - University of Oklahoma
3. Ralph Tanner - University of Oklahoma
4. Mary Eid - University of Oklahoma
5. Joseph Suflita - University of Oklahoma
6. Edward Heaver - bhpBilliton
7. Jep Bracey - bhpBilliton
8. Jennifer Lilley - Nalco Champion
9. Alicia Jones - Nalco Champion
10. Renato De Paula - Nalco Champion

Samples were taken from multiple locations within a shale gas/liquids operation in order to identify the microbes and processes likely responsible for microbially influenced corrosion (MIC). The samples included drilling, fracking, production, product transmission (pipelines) and wastewater. Chemical and physical parameters of the sites were determined. Assays of the microbial communities included quantification (ATP, qPCR), identification (high-throughput 16S rRNA gene libraries), sulfate-reduction activity, and corrosion potential of selected isolates. As expected from differences among the physical and chemical parameters of the sample sites, microbial communities varied from those dominated by freshwater mesophilic aerobic bacteria (drilling and frack tank water) to thermophilic anaerobic bacteria and archaea (production), facultatively anaerobic nitrate-reducing bacteria and aerobic hydrocarbon bacteria (pipelines), and a mix of thermophilic anaerobic bacteria and facultatively anaerobic bacteria (wastewater). Corrosion potential and targets of mitigation therefore would be expected to vary with location. We emphasize that data collected from one type of location cannot be extrapolated to another. In addition, an almost exclusive focus on control of sulfate-reducing bacteria in this operation is unwarranted. Despite a relatively high abundance of sulfate-reducing taxa in production and wastewater samples, sulfate-reduction activity was well below the level of concern except for one wastewater sample. Corrosion studies of isolates from production samples found acid-producing Clostridium (APB) to be the most corrosive, more so than thiosulfate-reducing, general heterotrophic or sulfate-reducing bacteria.

*172 Predicting Sessile Microorganism Populations in Oil and Gas Gathering and Transmission Facilities.

Authors
1. Mohammed Taleb-Berrouane - Memorial University of Newfoundland
2. Faisal Khan - Memorial University of Newfoundland
3. Kelly Hawboldt - Memorial University of Newfoundland
4. Richard B. Eckert - DNV GL
5. Torben Lund Skovhus - VIA University College

Planktonic microorganisms are ubiquitous in most oil and gas gathering and transmission facilities. Though the presence of planktonic microorganisms suggests a potential for microbiologically influenced corrosion (MIC), it is challenging to directly correlate planktonic microbiological parameters with corrosion. MIC is caused by sessile microorganisms, which are part of a biofilm consortium attached to the metal surface. The numbers of sessile microorganisms on a surface in a biofilm is one parameter used to assess MIC susceptibility. Obtaining a direct count (e.g. cells/unit area or mass) of sessile microorganisms in a biofilm is expensive and time-consuming, requiring intrusive techniques that must often be carried out by third-party contractors. Conversely, fluid samples are relatively easy to collect and can be used to determine counts of planktonic microorganisms. This study aims to develop a probabilistic model to estimate counts of sessile microorganisms based upon counts of planktonic microorganisms using molecular microbiological methods (MMM), fluid flow regime (e.g. laminar or turbulent), operating conditions, water wetting and other related field parameters. The established model will provide a conceptual understanding and a
quantitative approach to estimate the count of sessile microorganisms. This model should be further calibrated by experimental data and tested and verified using field data. Once fully established, this model will provide an optimized and effective way to estimate the sessile microorganisms count given chemical, physical and microbiological data from the system.

*71 “From iron to gas: methanogen-induced microbiologically influenced corrosion (Mi-MIC) cause high corrosion in dynamic environments”

Authors
1. Annie (Biwen) An - Bundesanstalt für Materialforschung und -prüfung (BAM)/Federal Institute for Materials Research and Testing
2. Sherin Kleinbub - Bundesanstalt für Materialforschung und -prüfung (BAM)/Federal Institute for Materials Research and Testing
3. Andrea Koerdт - Bundesanstalt für Materialforschung und -prüfung (BAM)/Federal Institute for Materials Research and Testing

Microbiologically influenced corrosion (MIC) of iron is usually attributed to sulfate-reducing microorganisms (SRM) in oil and gas facilities. SRM act upon the metal by the re-activeness of hydrogen sulfide (HS-), and by withdrawal of the available electrons in electrical contact with the metal (EMIC). Methanogenic archaea (MA) can also cause MIC (Mi-MIC). Several MAs were identified to be corrosive by using elemental iron as the sole electron donor for methanogenesis, including Methanobacterium--affiliated IM1 and Methanococcus maripaludis Mic1c10. Currently, low corrosion rates were reported for MA, possibly due to the formation of siderite (4Fe + 5HCO3- + 5H+ ® 4FeCO3 + CH4 + 3H2O). Since MA do not produce HS-, withdrawal of electrons may be their main corrosive mechanism; however, mechanistic details and kinetics of the overall process are poorly understood. To investigate the corrosion potential of MA, we studied the EMIC methanogenic strains (IM1 and Mic1c10) individually or part of a syntrophic co-culture with SRM. Corrosion studies were conducted using an in-house developed flow-through system to simulate fluctuating environmental conditions. Results indicate that the rates of iron corrosion by MA (up to 0.4 mm/yr) are higher to that caused by the marine SRM Desulfovibrio alaskensis (0.15 mm/yr) and the co-culture (0.1 mm/yr). Scanning electron microscopy (SEM) images of the metal incubated with MA showed severe pitting corrosion. Genomic analysis of the EMIC MA was conducted to provide an insight on the possible cellular mechanisms that could be involved. Furthermore, low concentrations of MA-targeting biocides will be applied to EMIC MA in static and flow conditions to gain insights for possible mitigation strategies. Such knowledge and deeper understanding also from an electrokinetic point of view may not only provide further models in microbial electrophysiology, but also contribute to mitigation strategies in MIC.

*84 “Corrosion inhibitors influence the occurrence of highly localized MIC features”.

Authors
1. Jaspreet Mand - ExxonMobil
2. Ramsey Smith - ExxonMobil
3. John Longwell - ExxonMobil
4. Dennis Enning – ExxonMobil
There are numerous tactics employed in the oil and gas industry to limit or inhibit damage caused by corrosion, including the use of film-forming corrosion inhibitor chemistries. Corrosion inhibitors (CI), generally surfactant-like chemicals, are commonly injected into pipelines on a continuous basis to ward against acid gas (H2S and CO2) corrosion. However, the effect of these chemicals on pipelines where microbiologically influenced corrosion (MIC) is a greater integrity threat than acid gas corrosion remains unknown. We hypothesized that CI may be influencing the highly localized corrosion morphology that is often associated with MIC in pipelines. To test this hypothesis, controlled bioreactor experiments were set up in the laboratory using both CI-containing field produced water and synthetic produced water brine with the addition of exogenous CI. In all cases, we examined the effect of the presence of CI on microbial community composition, microbial physiology, and the extent and morphology of MIC. Our results show that the presence of CI has a profound impact on MIC, as it appears to retard microbial activity (by sulfate-reducing bacteria and methanogenic archaea) and ultimately lead to highly localized corrosion features surrounded by otherwise unaffected metal surfaces. This was in contrast to the corrosion morphology witnessed in bioreactor experiments conducted in the absence of CI. Without CI, an uneven corrosion morphology was evident, but the corrosion features were not as highly localized as in the cases where CI were present. The implications of this comprehensive study looking at the effect of CI on MIC are far-reaching, as CI are present in a majority of produced waters from upstream and midstream oil and gas operations. Therefore, the influence of CI on the highly localized nature of MIC failures in oil and gas pipelines may be widespread.

*138 Characterization of a highly corrosive sample enriched from nitrate injected produced waters after long-term incubation in nutrient limited conditions using metagenomic, electrochemical, and surface analyses.

Authors
1. Mohita Sharma - University of Calgary
2. Joshua Handy - University of Calgary
3. Dongshan An - University of Calgary
4. Gerrit Voordouw - University of Calgary
5. Nicolas Tsesmetzis - Shell Technology Center
6. Lisa Gieg - University of Calgary

Microorganisms can participate in the damage of metal infrastructure, known as microbiologically influenced corrosion (MIC). Long term incubations (104 weeks) with carbon steel beads were established using produced water collected from a Canadian oilfield where nitrate was routinely used for souring mitigation. Experiments were conducted under methanogenic, sulfate-reducing, and nitrate-reducing conditions to stimulate electrical MIC (EMIC) with iron present as the sole electron donor. The samples were transferred for a second enrichment under similar conditions, and the most corrosive samples after 18 weeks of incubation were transferred again in the presence of C1018 coupons for an additional 4 weeks (3rd enrichment). Microbial community analysis, metagenomics analysis, electrochemical studies, chemical measurements, metal weight loss, and surface analyses were conducted to assess EMIC under these different conditions. The incubations conducted under nitrate-reducing and methanogenic conditions did not yield substantial metal weight loss. In
contrast, incubations under sulfate-reducing conditions showed a 10-16% weight loss compared to controls, with severe pitting observed on the carbon steel beads and coupon surfaces. 16S rRNA gene sequencing revealed the predominance of known sulfate reducers (SRM) (Desulfobulbus and Desulfovibrio), acid producers (Mesotoga and Acetobacterium), Actinobacteria, and methanogens (Methanosaeta). SEM analyses of the most corrosive SRM enrichment revealed the presence of filamentous chains of bacteria, possibly contributing towards a mechanism of extracting electrons from solid metal surfaces across long distances. Electrochemical studies showed that this corrosive SRM enrichment was capable of extracting electrons directly from graphite poised at -600 mV vs. SHE. Comparative metagenome analysis of the planktonic and sessile populations against the genome of the EMIC organism Desulfovibrio ferrophilus IS5 has led to the identification of several genes potentially involved in EMIC processes. In all, our results highlight the potential for oilfield produced waters to harbour EMIC-type SRM.

*166 “Contrasting pathways for anaerobic methane oxidation in Gulf of Mexico cold seep sediments”.

Authors
1. Adrien Vigneron - Newcastle university
2. Eric Alsop - Joint Genome Institute
3. Ian Head - Newcastle university
4. Nicolas Tsesmetzis - Shell Technology Center

Gulf of Mexico sediments harbor numerous hydrocarbon seeps associated with high sedimentation rates and thermal maturation of organic matter. These ecosystems host abundant and diverse microbial communities that directly or indirectly metabolize components of the emitted fluid. To investigate microbial function and activities in these ecosystems, metabolic potential (metagenomic) and gene expression (metatranscriptomic) analyses of two cold seep areas of the Gulf of Mexico were carried out. Seeps emitting biogenic methane harboured microbial communities dominated by ANME-1, whereas seeps producing fluids containing a complex mixture of thermogenic hydrocarbons were dominated by ANME-2 lineages. Metatranscriptome measurements in both communities indicated high levels of expression of genes for methane metabolism despite their distinct microbial communities and hydrocarbon composition. By contrast, the transcription level of sulfur cycle genes was quite different. In the thermogenic seep community, high levels of transcripts indicative of syntrophic AOM coupled to sulfate reduction were detected. This syntrophic partnership between the dominant ANME-2 and sulfate reducers potentially involves direct electron transfer through multiheme cytochromes. In the biogenic methane seep, genes from an ANME-1 lineage that are potentially involved in polysulfide reduction were highly expressed suggesting a novel bacteria-independent anaerobic methane oxidation pathway coupled to polysulfide reduction. The observed divergence in AOM activities provides a new model for bacteria-independent AOM and emphasizes the variation that exists in AOM pathways between different ANME lineages.

*151 “Highly Resolved Sampling and Analysis of a Hydraulic Fracturing Pad Reveal Insights into Effective Microbial Control”.

Authors
Hydraulic fracturing uses large volumes of water that are formulated into fracturing fluids. These fluids, often rich in nutrients, are an ideal breeding ground for microbial proliferation, even after entering the reservoir. When left uncontrolled, bacteria and/or archaea can cause souring and devaluation of produced hydrocarbons, biofouling, microbiologically influenced corrosion (MIC), produced fluid separation issues, and HS&E risks. Mitigating these risks requires effective selection and application of biocides during drilling, completion, and production. Dow Microbial Control (DMC), Colorado State University, and Devon Energy collaborated to conduct an advanced microbiological audit of a well completion operation in the Anadarko Basin with the objectives of (1) determining the effectiveness of a tandem chlorine dioxide (ClO2) and glutaraldehyde/quaternary ammonium (glut/quat) microbial control program; and (2) pursuing a deeper dive into microbial community characteristics and dynamics in fractured shale. Topside, the highly resolved sampling process and critical analysis of biocide efficacy in the field using molecular assays (qPCR, ATP) and complementary culturing techniques (bug bottles, microtiter MPN) allowed Devon to improve the efficiency of their operation through more effective biocide use. Downhole, source tracking and 16S iTag community profiling provided deeper understanding of the influences of fracturing operations on longer-term reservoir performance, the results of which could be applied to many aspects of the industry. Results showed that multiple microbial contamination sources—including source waters, working tanks, hydration units, and guar—existed topside. Additionally, critical analysis of biocide efficacy revealed that ClO2 treatment of source water was short-lived and ineffective for operational control, whereas glut/quat treatment of fracturing fluids at the blender was effective both topside and downhole. This overview of sampling/analysis protocols and biocide efficacies in the field will facilitate recommendations for both immediate and long-term microbial control in fractured shale reservoirs.

Session 03: Hydrocarbon Biodegradation

INVITED SPEAKER

“Controls on anaerobic biodegradation of crude oil hydrocarbons”

Authors

Ian M. Head\(^1\), Angela Sherry\(^1\), Ana Suarez-Suarez\(^1\), Julia de Rezende\(^6\), Luiza Lessa Andrade\(^1\), Adrien Vigneron\(^1\), Sven Lahme\(^1\), Eric B. Alsop\(^3\), Casey Hubert\(^5\), Tetyana Korin\(^1\), Emma Bell\(^7\), Carolyn M. Aitken\(^1\), D. Martin Jones\(^1\), Juliane Bischoff\(^8\), Bernard F.J. Bowler\(^1\), Obioma Mejeha\(^1\), Stephen R. Larter\(^6\), Bart Lomans\(^4\), Nicolas Tsesmetzis\(^2\) Júlia R. de Rezende\(^1,8\), Thomas B. P. Oldenburg\(^6\), William D. L. Richardson\(^5\), Milovan Fustic\(^3\), Aleksandr Grigoryan\(^3\), Gerrit Voordouw\(^5\), Neil D. Gray\(^1\) and Casey R. J. Hubert\(^1,5\)
Petroleum reservoirs harbor a diverse array of microorganisms which on production and geological timescales, influence the properties and quality of emplaced oil, affect its production and ultimately its value. Heavy oil formation over tens of millions of years, more rapid oil-driven sulfide formation leading to souring, microbially influenced corrosion and the potential to enhance oil recovery, all have a central microbiological component and in some cases there may be microbiological solutions to detrimental processes that occur during petroleum production. When petroleum enters surface environments from petroleum reservoirs, either through natural seepage or anthropogenic activity, the fate of the petroleum is also influenced significantly by the microbial communities present. Aerobic biodegradation of crude oil is generally considered to be the most important route for microbial removal of crude oil from the environment, however there is a range of situations where oil contamination of anoxic sediments occurs. The fate of contaminant hydrocarbons in anoxic environments, and the behaviour of the microbial communities responsible for their transformation sometimes defy conventional wisdom. The reasons for some of the unexpected behaviour of crude oil-degrading microbial communities in anoxic systems will be explored and factors controlling the rate and extent of crude oil biodegradation in anoxic environments will be discussed in the context of both subsurface petroleum reservoirs and oil-contaminated surface sediments.

Offered papers

*46 Anaerobic BTEX Bioremediation is a Number’s Game

Authors
1. Courtney Toth - University of Toronto
2. Sandra Dworatzek - SiREM
3. Jennifer Webb - SiREM
4. Fei Luo - University of Toronto
5. Shen Guo - University of Toronto
6. Nancy Bawa - University of Toronto
7. Charlie Chen - University of Toronto
8. Johnny Xiao - University of Toronto
9. Chris Shyi - University of Toronto
10. Olivia Molenda - University of Toronto
11. Yawen Guo - University of Toronto
12. Kris Bradshaw - Federated Co-Operatives Limited
**13. Elizabeth Edwards - University of Toronto**

Benzene, Toluene, Ethylbenzene and Xylene (BTEX) are widespread groundwater pollutants arising from releases of petroleum products. The ability of microorganisms to degrade these compounds anaerobically gained bioremediation attention as prospective remediation tool in the 1980’s. However, slow and sometimes undetectable rates of anaerobic BTEX biodegradation in situ led many end-users questioning the efficacy of this technology for hydrocarbon contaminants. Here, we present new experimental and molecular evidence that suggests active benzene biodegradation in anoxic environments is indeed possible, but is largely controlled by the abundance of active anaerobic benzene degraders. Soil and groundwater materials from 10 global BTEX-polluted sites were probed for anaerobic benzene degraders using quantitative PCR and 16S rRNA amplicon sequencing. Most sites harboured low concentrations (< 10^2 copies/L) of predicted benzene degraders (e.g., Deltaproteobacterium ORM-2, Peptococcaceae spp.) and benzene-degrading functional genes (abcA). This is well below the predicted threshold of benzene degraders required for active biodegradation (10^6 – 10^8 copies/L), based on independent enrichment culture studies. The good news is that when cultured anaerobic benzene degraders were bioaugmented into site materials (> 10^8 copies/L), we observed a 70% increased probability of benzene bioremediation success in microcosm-based treatability studies versus intrinsic bioremediation controls. We are currently conducting qPCR studies of predicted TEX degraders and functional genes (bssA) to see their abundance is also correlated to active bioremediation. Further, we are conducting bioaugmentation pilot studies at two anoxic benzene-contaminated sites to validate our laboratory results. It remains unclear why intrinsic anaerobic benzene degraders rarely proliferate in situ, but we are encouraged that bioaugmentation has the potential to provide effective BTEX cleanup in anoxic environments where conventional physical and chemical remediation approaches may not be feasible or economically viable.

*129 “Microbial degradation of residual recalcitrant hydrocarbon in mined tailings under different redox conditions”*

**Authors**

1. Gloria Okpala - University of Alberta  
2. Luke Gjini - University of Alberta  
3. Alsu Kuznetsova - University of Alberta  
4. Ania Ulrich - University of Alberta  
5. Phillip Choi - University of Alberta  
6. Tariq Siddique - University of Alberta  

Current extraction technology used in bitumen recovery from surface mined oil sands in Alberta results in consumption of significant volumes of fresh water and subsequent generation of waste fluid fine tailings (FFT). To address this problem, non-aqueous extraction (NAE), an alternative process using solvents (e.g. cyclohexane) is employed. NAE is efficient since most of the solvents used is recoverable post-extraction and smaller volumes of dry waste are produced. However, NAE wastes contain residual solvents that could negatively impact the environment upon disposal. Hence, an in-depth study of biodegradability of NAE solvents under upland (aerobic) and wetland (anaerobic) reclamation scenarios is necessary before commercialization.
of this process. Small-scale microcosm experiments containing FFT or OSPW amended with cyclohexane and/or cyclopentane were incubated under aerobic conditions. Microcosms inoculated with OSPW were used to evaluate co-metabolic degradation of cyclohexane by monooxygenases produced during growth of ammonia or methane oxidizing bacteria. But for anaerobic (sulfate-reducing, nitrate-reducing, iron-reducing and methanogenic) incubations, only FFT was used as source of inoculum. The effect of nutrients and supplementation with n-heptane on degradation of cyclohexane and cyclopentane was also evaluated under methanogenic degradation. DNA-SIP was applied identify key cyclohexane degrading communities in aerobic and methanogenic incubations. Aerobic FFT microcosms showed rapid degradation of cyclohexane degradation with subsequent CO2 generation. Also, enrichment of ammonia oxidizing bacteria in microcosms inoculated with concentrated OSPW led to 99% degradation of cyclohexane with concomitant oxidation of the added ammonia to nitrite. Similarly, co-metabolic degradation of cyclohexane was observed in OSPW inoculated microcosms supplied with labeled methane and oxygen. Biodegradation was accompanied with methane consumption. In methanogenic incubations, onset of cyclohexane degradation occurred once heptane was degraded and in some instances alongside heptane. Nutrient addition enhanced degradation of cyclohexane and cyclopentane with heptane. These findings will enable appropriate designing/tailoring of reclamation strategies for NAE solids management.

*174 “Genomics-based analyses to evaluate natural microbial assemblages and their response to petroleum hydrocarbons in Canadian marine environments”.

Authors
1. Charles Greer - National Research Council
2. Julien Tremblay - National Research Council Canada
3. Lars Schreiber - National Research Council Canada
4. Nathalie Fortin - National Research Council Canada
5. Jessica Wasserscheid - National Research Council
6. Susan Cobanli - Fisheries and Oceans Canada
7. Thomas King - Fisheries and Oceans Canada
8. Brian Robinson - Fisheries and Oceans Canada
9. Kenneth Lee - Fisheries and Oceans Canada

A genomics-based approach, combined with microcosm studies, is being used to characterize the structure and function of natural marine microbial communities and their response to petroleum hydrocarbons. The natural attenuation potential for oil hydrocarbons was assessed on the east and west coasts of Canada and in the Arctic marine environment. Although natural marine microbial communities possess the ability to degrade petroleum hydrocarbons, the rate at which this occurs as well as local environmental conditions will influence the efficiency of the process and the final outcome of microbial degradation activity. All the oceans surrounding Canada possess natural hydrocarbon degrading bacterial communities, very often dominated by taxa such as Colwellia, Alcanivorax, Thalassolituus, Oleispira and Flavobacterium and other Oceanospirillales and Alteromonadales. Microcosm studies have shown results for the biodegradation half-life for alkanes to be in the range of 1-2 weeks in all systems studied including the Arctic. The inclusion of dispersant had a variable effect, but more positively influenced winter biodegradation activity. The inclusion of dispersant with oil also had significant effects on the microbial community structure, with a dominance of
Thalassolituus on the east coast in the summer, while Oleispira dominated on the west coast in dispersant with oil in the winter. In situ microcosms, an experimental system that allows a more real-time assessment of natural hydrocarbon degradation activity is providing valuable data on how the microbial community responds to oil and how its’ structure changes over time. Bacterial communities on in situ microcosms incubated for three months were the most active in alkane biodegradation and differed significantly from those that were present after one year of incubation. Incorporating various genomic methods of community analysis, such as high-throughput amplicon and shotgun metagenomic sequencing, are proving to be extremely valuable in identifying key hydrocarbon degrading bacteria and community-wide responses to petroleum hydrocarbons.

*127 “Thermochemical Oil Formation in Hydrothermal Vent Sediments at Guaymas Basin, Gulf of California and the Search for a Deep Biosphere Hydrocarbon Fingerprint”.

Authors
1. Connor J. Dalzell - Saint Mary’s University
2. G. Todd Ventura - Saint Mary’s University
3. Stefan M. Sievert - Woods Hole Oceanographic Institution
5. Christopher M. Reddy - Woods Hole Oceanographic Institution
6. Jeffrey S. Seewald - Woods Hole Oceanographic Institution

We are examining the accelerated petroleum production that naturally occurs at Cathedral Hill, a hydrothermal vent site in Guaymas Basin, Gulf of California. At this site, high temperature fluids projected to reach up to 155°C by 21cm sediment depths should be capable of pyrolyzing the organic-rich sediments. Under these conditions, the extractable organic matter may be holistically transforming into petroleum forming hydrocarbons. We have performed a multi-molecular, chemometric survey using comprehensive two-dimensional gas chromatography (GC×GC) on four push cores taken along a transect stretching from the center of the vent complex to the exterior of a microbial mat formed by Beggiatoa spp. Through this, we aim to elucidate how the hydrocarbon matrix is attenuated or added to by biodegradation, thermochemical degradation, in-situ hydrocarbon production, and migration. Preliminary extract data and matrix compositional patterns (such as hydrocarbon variety/quantity) initially supported the existence of a peak oil generation window at ~8-10cm (marking fluid temperatures of 125°C) sediment depth. However, the presence of abundant archaeal GDGT core lipids at the base of the push cores and the lack of significant biphytanes is indicative of an incomplete ether cleavage of core lipids implying that production of hydrocarbons occurs at deeper sediment depths. Ratios of low to intermediate molecular weight n-alkanes and acyclic isoprenoids show moderate levels (reaching 2-3) of biodegradation that increase down-core at variable degrees across the transect. Evidence of subsurface microbial inputs may also be present in the form of an unknown pseudohomologous series of tetracyclic compounds, along with decoupled ratios of bacterial-sourced lipids C30 hopene and C30 hopane. Finally, hierarchical cluster analysis and multi-way principal components analysis scores plots show extreme differences in bulk matrix composition between certain sections of the transect, likely due to staining from deeper sourced migrating oil.
An estimated 1.3 million tonnes of petroleum enter the marine environment annually, from a variety of sources, including large spills of crude oil from tankers and drilling accidents such as the Deepwater Horizon blowout. One widely practised remediation strategy to clean up oil spills is the application of dispersants containing surfactants that reduce the interfacial tension between oil and water and disperse oil droplets into the water column, thereby increasing bioavailability. However, little is known about the effects of surfactants on the development of marine oil-degrading microbial communities. The effects of three biosurfactants (rhamnolipid, trehalolipid, and sophorolipid) and three commercial dispersants (Finasol OSR 52, Slickgone NS, and Superdispersant 25) on bacterial growth and community structure were quantified in North Sea microcosms contaminated with Norwegian Geochemical Standard, North Sea Oil (NSO-1), using 16S rRNA qPCR and amplicon sequencing. All surfactants, except sophorolipid, significantly (P<0.001) reduced the surface tension of the seawater from 70 mN/m to below 30 - 35 mN/m. After 24 hours, all surfactants (excluding sophorolipid) significantly stimulated the growth of bacteria, evidenced by up to a 17-fold increase in bacterial 16S rRNA gene abundance, in comparison to the oil-only controls (P<0.05). Generally, bacteria from the genera Marinomonas, Pseudoalteromonas and the obligate alkane-degrading Oleispira became dominant during the initial three days. This was followed by large increases in the relative abundance of alkane-degrading Alcanivorax and PAH-degrading Cycloclasticus after seven days. Some surfactant-specific responses were also observed, such as the enhanced growth of Arcobacter, Marinobacter, and Zhongshania that was stimulated by rhamnolipid addition. The results of this study suggest that most surfactants significantly decrease the lag-phase of bacterial growth following oil contamination, as they increase hydrocarbon bioavailability and thus allow bacteria to invest more energy into growth and hydrocarbon degradation, rather than the production of their own biosurfactants.

In nutrient-limited environments, microbial communities employ a variety of mechanisms to cope with nutrient limitations. Evidence of recycling and remineralization of nutrients locked up in necromass in nutrient-limited pelagic environments has previously been reported. However, the significance and extent of
the process in subsurface terrestrial environments is unknown. This study aims at elucidating the carbon and nitrogen flow in an anoxic hydrocarbon-degrading enrichment culture amended with naphthalene. The culture consisted of the hydrocarbon-degrading, sulfate-reducing Desulfobacterium N47 and the spirochaete Rectinema cohabitans. Stable isotope-labeling and Raman microscopy were used to determine the carbon and nitrogen incorporation at the single-cell level. An in-house code was developed for analysis of spectra including baseline correction, denoising, statistical and ordination analyses. Distinct Raman spectra for Desulfobacterium N47 and R. cohabitans cells allowed for linking the identity and function. Amendment with 13C-naphthalene compared to non-labelled systems resulted in a clear shift in Raman band of phenylalanine as a marker of 13C incorporation. The ratio of 1003 to 991, 981, 968 cm⁻¹ bands of imaged cells was significantly decreased from 1.4±0.5 in non-labelled systems to 0.37±0.1 in labelled ones. The outcomes of this study will provide insights on the extent of necromass degradation leading to carbon and nitrogen recycling in environments with elevated concentrations of oil compounds.

*150 “Use of transcriptomics and genomics to understand the underlying mechanisms of bacterial adaptation to jet-fuel”.

Authors
1. Thusitha Gunasekera - Air Force Research Laboratory
2. Loryn Bowen - University of Dayton Research Institute
3. Oscar Ruiz - Air Force Research Laboratory

Microorganisms that have the ability to thrive in a hydrocarbon rich environment often have specialized adaptive mechanisms. Fuel contains toxic compounds including aromatic hydrocarbons. Interplay between the ability to degrade hydrocarbons and having efficient vital cell adaptive mechanisms are crucial for cells to proliferate in jet fuel. These adaptive mechanisms include impermeabilization of the cell to prevent entrance of toxic compounds, activation of efflux pumps to extrude toxic solvents, and general stress responses. Additionally, bacteria can increase the production of exopolysaccharides and formation of biofilms along with secretion of surfactants and emulsifiers to facilitate uptake of hydrocarbons. These responses are primarily controlled at the transcriptional level by global and local regulatory proteins and sRNAs. We have used transcriptomic and genomic approaches to discover novel genes and pathways and gain mechanistic insights into how bacteria respond to jet fuel. P. aeruginosa activates multiple metabolic pathways and adaptations to overcome fuel stress. We found fuel down regulates major porins such as oprF and oprG required for uptake of toxic aromatics including toluene and naphthalene. In addition to preventing internalization, upregulations of efflux pumps such as mexCD-oprJ, mexEF-oprN, mexGHI-opmD indicates extrusion of toxic hydrocarbons. Our data suggests porin regulation and efflux pumps are required for fuel tolerance. In addition, we found fuel adapted strains have better stress response mechanisms. A fuel adapted strain employs a rapid heat shock response as a mechanism to tolerate fuel stress compared to strains less adapted to fuel. In addition, cells produce surfactants and extracellular polysaccharides that facilitate bacterial colonization at the water-fuel interface. Additionally, genes encoding alginate and Pel protein biosynthesis which play a role in cell aggregation and biofilm formation in the fuel environment were upregulated. Our results collectively suggest multiple metabolic pathways and adaptations are crucial for P. aeruginosa proliferation in fuel.
Session 04: Oil and gas microbiome: Problems, control and opportunities

INVITED SPEAKER
“Long-term succession in a gas and oil microbiome during in situ stimulation of methane using conductive Neutral Red Crystals”.

Author

1. Sabrina Beckmann (University of Delaware)

With one billion tons of methane produced annually by microorganisms, biogas production can be appreciated both for its role in organic matter turnover and as an energy source for humankind. Methanogenic degradation of crude-oil hydrocarbons is a major process in conventional and unconventional subsurface oil-reservoirs and usually limited by the availability of electron acceptors and a restricted oil-water transition zone leading to the remains of non-degradable heavy oil. We accelerated and enhanced methane formation from coal and crude-oil through the application of the synthetic phenazine Neutral Red. Neutral Red (2-amino-8-dimethylamino-3-methylphenazine) is a redox active dye shown to stimulate in vitro and in situ biogas production from anaerobic coal- and oil-fed cultures through the formation of crystals1-3. In situ long term field trials over 1.5 years demonstrate the crucial role of the recently discovered Neutral Red Crystals that channel electrons as catalysts directly to methanogenic archaea. The phenazine favours acetoclastic methanogenic archaea by delivering electrons directly to the terminal respiratory enzyme heterodisulfide reductase2. The application of Neutral Red to a non-gassy sulfate-rich coal seam resulted in spontaneous crystal formation and a 5-10 fold increase in methane production outperforming other enhancement methods (e.g. nutrient amendments)4. Neutral Red can also stimulate methanogenesis with other hydrocarbon inputs. For example, crude oil cultures amended with Neutral Red Crystals showed an increase in relative abundance of methanogenic archaea. Interestingly, sulfate reducing bacteria (SRB) were relatively abundant despite the activity of methanogens and low sulfate concentrations. The majority of cells were attached to the Neutral Red Crystals that nucleated from the oil layer into the water phase adding additional surface area and potentially an oil-water contact extension for enhanced microbial activity. Neutral Red Crystal amendments, by enhancing electron flow, may be helpful in energy recovery processes across a wide range of systems.

1Beckmann et al., Enhanced biogas production. NewSouth Innovations Pty Limited. APO2013901758.

Offered papers
*69 “Tiny microbial ecosystems isolated from oil help understanding microbial community assembly”.

Authors
1. Lisa Kroll - University of Duisburg Essen

The world’s largest natural asphalt seep, the Pitch Lake in Trinidad and Tobago, harbors water droplets in microliter size (1-3 µL) originating from the oil reservoir deep below. These water droplets contain diverse and metabolically active microbiomes [1]. In this study we make use of this system to investigate the impact of chemo diversity on microbial communities. Chemo diversity is here understood as the concentrations of the anions (F-,HCOO-,CI-,PO42-,SO42-) and cations (Li+,Na+,NH4+,K+,Mg2+,Fe2+) measured in each droplet with ion chromatography. 24 water droplets were isolated from different sampling sites of the Pitch Lake and analyzed in technical duplicates for their 16S rRNA gene composition by amplicon sequencing and the mothur bioinformatics pipeline. Multivariate statistics were applied to examine the correlation between sampling site, chemo diversity and single OTUs as well as the whole droplet community. For all of the 24 water droplets, more or less different patterns of taxonomic and ion composition were detected. Statistically significant correlation between the variables and OTUs was found, e.g. the relative abundance of OTU1 (family Clostridiales Incertae Sedis XI from the phylum Firmicutes) was correlated with sulfate concentration (coefficient= -0.3, p < 0.05). Furthermore, the relative abundances of 60 % of OTUs (with relative abundances > 0.1 %) was significantly correlated with the measured chloride level in the droplets. Our findings provide novel insights into these isolated ecosystems and into the role of chemo diversity on microbial community assembly.


*92 “Phylogenomic and Metagenomic analyses of oilfield microbial communities shows they genetically differ and align with the differences in the chemical engineering parameters of top side asset elements”.

Authors
1. Geert van der Kraan - The Dow Chemical Company

An oilfield in Europe was sampled at six locations across its top-side facility (producer to injector). 16S RNA gene phylogenomics was first carried out to determine microbial population distributions. Also, qPCR analyses were done. This revealed that the top-side facility consisted of four zones. Three of the samples were subjected to a metagenomic analysis. Differences between the oil-water separator and the oil storage tank were profound. The oil storage tank held predominantly Archaea, and the metabolism was, acetate to methane centered. The oil-water separator, contained predominantly sulfate reducing bacteria and organic acid related metabolism. From the shotgun sequencing, obtained sequences were compared to the KEGG and SEED databases. (The gathering pipeline, oil-water separator and storage tank). It was shown that their communities are equipped differently from each other on a genetic level. In the gathering line, including chemical addition, three times more genes associated to sensing changes in environments and motility were found, showing that chemical addition linked with engineering parameters dictated the community
established, its potential metabolic profile and level of adaptability. Interestingly, it confirmed previous studies regarding the presence of amino acid metabolism in similar systems. What can be observed is that, the ecosystems and thereby belonging microbial populations are different and that in some locations the population is better equipped to, for example handle a dynamic system. This type of information proved to be relevant when designing a microbial control program for top side systems. Based on the metabolic profile, added biocide levels may need to be adjusted and tuned to the different top side elements. This subsequently calls out for better system monitoring allowing the development of tailor made microbial control solutions aimed at maximizing the potential of biocides for the system. Engineering solutions were proposed on the water handling side to aid the biocide recommended.

*99 “Microbiological assessment of 3 hydraulically fractured wells at the HFTS (Hydraulic Fracture Test Site), Permian”

Authors
1. Sarah Eisenlord - Gas Technology Institute
2. Djuna Gulliver - NETL
3. Kara Tinker - NETL

The Hydraulic Fracture Test Site (HFTS) experiment, co-funded by the oil and gas industry and NETL-DOE, tested advanced fracturing and completions technologies and techniques to increase production efficiency and limit the environmental impact of future shale hydrocarbon development. Oilfield microbiology was assessed in water sourced for hydraulic fracturing (fresh and recycled), drilling fluids, and produced water from three hydraulically fractured oil and gas wells over 550 days of production using qPCR of functional genes, 16S rRNA gene sequencing, metagenomic analysis, and chemical analysis. Our results demonstrate after 36 days of production, the microbial community for each well converged to a population primarily composed of methane producing Methanothermococcus and fermentative, thiosulfate reducing Halanaerobium. Also present was a low relative abundance of common sulfur-reducing bacteria and Archaeoglobus, which is known for induced oil souring and biofilm production. Biocide application after microbiologically influenced corrosion occurred lead to a shift in microbial populations and a temporary decrease in microbial concentrations, which shortly rebounded to pre-treatment levels. A metagenomic analysis additionally reveals complete assimilatory and dissimilatory sulfate reduction pathways, which further contributes to well souring and corrosion, as well as the presence of oxidative and osmotic stress genes associated with biofilm formation. As the practice of supplementing fresh water with recycled produced water for hydraulic fracturing increases, so does the likelihood of introducing unwanted microbiological activity in the subsurface. A deeper understanding on the chemistry and biology and response to biocide application will lead to improved bio-control efforts while increasing water re-use potential.

*156 “De-risking off-shore reservoir exploration through DNA sequencing and Machine Learning”.

Authors
1. Jose Miguel Seoane - Repsol
2. Anirban Chakraborty - University of Calgary
Microbial Exploration Techniques (MET) is an oil and gas DNA-based exploration approach that identifies microbial diversity anomalies in surface soil overlying subsurface reservoirs and uses them as an indicator of hydrocarbon presence. MET applications on several on-shore fields have reported a positive correlation between the presence of certain taxonomic groups and hydrocarbon deposits, achieving up to 78% success rate on exploratory wells drilled on MET. However, despite the high cost of drilling offshore wells (100-600 M$ per unit) and the ultra-high risks associated with O&G exploration (21% success rate), few studies have demonstrated the validity of MET off-shore. Our research aims to evaluate the feasibility and potential of MET for offshore exploratory campaigns. To this end, we performed taxonomic characterization of Bacteria and Archaea in 344 piston cores extracted for standard geochemical analysis during several different offshore exploratory campaigns. 80% of the obtained dataset was randomly split to train a machine-learning algorithm to automatically search for correlations between geochemical data (i.e. oil or gas presence or absence) and the presence or absence of specific bacteria (called biomarkers) that were not known in advance (unsupervised learning). “Trained” models were then used to predict geochemical features on the remaining samples solely based on their microbial DNA signature. Hydrocarbon presence was predicted with 86 and 97.5 % accuracy, and hydrocarbon absence with 90 and 69 % accuracy, for Archaea and Bacteria, respectively, without any further adjustments. Using model residuals, we were able to generate a probability heat map compatible with standard exploration software indicating the most promising areas for prospects (“sweet spots”). Future developments will aim to perform MET directly on board, allowing for the first time real-time data-driven decision-making during prospection campaigns.

*108 “Influence of water quality on biofilm growth and injectivity”.

Authors
1. Gunhild Bødtker - Norce Research
2. Janiche Beeder - Equinor ASA
3. Edin Alagic - Norce Research
4. Beate Hovland - Norce Research
5. Bartek Vik - Norce Research
6. Dag Chun Standnes - Equinor ASA
7. Espen Kowalewski - Equinor ASA

The aim of the current work is to improve understanding of factors that determine biofilm growth in porous media and how microbial growth may influence the water injectivity of oil reservoirs. Significant factors that are considered are injection water quality, nutrient load and microorganisms present. Results from screening of plug-potential in serial linked core floods (2 x 5 cm Bentheimer cores) show that the biofilm plug-potential of seawater microorganisms varies significantly with brine type and nutrient composition. Seawater bacteria flooded with synthetic produced water brine (sPW) show a higher plug potential (75% and 11%; refers to permeability reduction in first and second core in line, respectively) compared to synthetic sulphate-reduced
seawater brine (sSRP) (51% and 8%). The combination of seawater bacteria and produced water bacteria flooded with sPW show a significantly higher plug potential in the second core (73% and 43%), which suggests potential for forming longer biofilm plugs. DNA analysis of core material revealed significant differences in species composition, where a dominance of phylotypes affiliated to the genus Vibrio was associated with high plug-potential. The plug potential of Vibrio was confirmed in single species floods where V. ruber formed significant plugging when flooded with seawater brine (96% and 58%). Another core experiment with V. ruber, flooded with sPW added iron, showed modest plugging (14% and 20%). Results from a parallel filter-test suggested high plug potential of V. ruber when grown in sPW batch added iron. The study has revealed a complex cause of effects related to the plug potential of biofilm in porous media. The plug potential by single cells as well as biofilm are influenced by brine and nutrient composition, species specific characters, growth medium (solid or liquid) and metabolic activity. The results presented could have consequences when evaluating e.g. injection of different water qualities into oil reservoirs.

*110 “Microfluidic studies on mechanisms of biofilm formation and its effect on flow properties in porous media”.

Authors
1. Na Liu – Norce Research
2. Bartek Vik - Norce Research
3. Beate Hovland - Norce Research
4. Thomas Baumann - Technische Universität München
5. David Landa-marbán - University of Bergen
6. Florin Adrian Radu - University of Bergen
7. Gunhild Bødtker - Norce Research

Biofilm accumulation in porous media can cause pore plugging (bioplugging), leading to significant changes in the physical properties of porous media by reduction of porosity and permeability, which influence the local flow pattern. Up to now, applications of desired biofilm growth in porous media have been attempted for various practices, such as bioremediation, water purification and microbial enhanced oil recovery (MEOR). A deeper understanding of mechanisms involved in biofilm formation and its effects on flow properties in porous media is necessary to evaluate and optimize the efficiency of selective bioplugging strategy in MEOR trials. Herein, we performed experiments in microfluidic devices to study biofilm growth, detachment, and distribution in porous media at different environmental conditions including nutrients, flow shear stress, temperature, pressure, and reservoir wettability and heterogeneity. Microscope imaging was applied to quantify the biofilm growth while the pressure drop across the micromodel was monitored during flow to evaluate the permeability reduction in the micromodel. Experiments in a T-shape microchannel were used to assess the processes governing the interactions between water flow and biofilm development. The results showed that increase in fluid velocity could facilitate biofilm growth, but that above a velocity threshold, biofilm detachment and inhibition of biofilm formation due to high shear stress was observed. High nutrient concentration prompts the biofilm growth, however the generated biofilm displayed weak adhesive
strength. Biofilm growth in micromodels occurred at a faster rate in high permeability zones, and caused the decrease in permeability of micromodels. Particle tracking demonstrated that biofilm accumulation in the pore space could divert water flow into other pores. This research developed a microscale simulator to investigate mechanisms involved in biofilm development in porous media at porescale, which could aid evaluations of bioplugging strategy, and for better prediction and modelling for petroleum industry, hydrogeology, and water purification.

*133 “Dissecting the inhibition of sulfidogenic communities by the specific inhibitor perchlorate”.

Authors
1. Magdalena Stoeva - UC Berkeley
2. John Coates - UC Berkeley

The sulfate analog perchlorate is emerging as a promising inhibitor of sulfate reducing microorganisms (SRM across sulfidogenic systems and its applicability has previously been investigated in batch and packed column systems. As a continuation of our studies into this inhibitor we quantitatively dissected the inhibitory dynamics of perchlorate in a chemostat system using a complex sulfidogenic community. Sulfidogenic mixed continuous-flow systems were treated with perchlorate. SRM number, sulfide production and community structure were monitored pre-, during and post- treatment. The data generated was compared to a simple mathematical model, where SRM growth slows as a result of inhibition. The experimental data supports the model that perchlorate acts largely to decrease SRM growth rates, rendering planktonic SRM increasingly susceptible to wash-out. Surface-attachment was identified as an important parameter preventing SRM wash-out and thus governing inhibitory dynamics. A subsequent experiment confirmed the lesser depletion of surface-attached SRM as compared to planktonic SRM during perchlorate treatment. Indirect effects of perchlorate (bio-competitive exclusion of SRM by dissimilatory perchlorate-reducing bacteria, DPRB) were also assayed by amending reactors with DPRB. Indeed, low concentrations of perchlorate coupled with DRPB amendment can drive sulfide concentrations to zero. Further, inhibition in a complex community was compared to that in a pure culture, highlighting similarities and differences between the two scenarios. Finally, we quantified susceptibility to perchlorate across SRM in various culture conditions, showing that prediction of complex behavior in continuous systems from batch results is possible. This study thus provides an overview of the sensitivity of sulfidogenic communities to perchlorate, as well as mechanisms underlying these patterns.

Session 05: Emerging Innovation and Applications in petroleum microbiology

INVITED SPEAKER

“Detecting the Signal through the Noise: Identifying Subsurface Organisms through Machine Learning”.

1. Zarath Summers (ExxonMobil)
In the age of big data, discerning signal from noise has become paramount. With the rapid acquisition of DNA sequence data being utilized to interpret environments, faith is placed in the fact that such data is representative of the intended environment. Collection of environmental microbial data follows two routes: 1) direct collection: scooping up soil, or filtering water onsite and extracting DNA, or 2) in the case of oil reservoir samples, a more indirect collection, sampling production wells, separators, or holding tanks. This indirect method introduces additional microbial communities, by the time the researcher accesses a sample, many microbial community signals are present. Additionally, the difficulty in collection from these production sites leads to the potential for small sample set size. Interpretations of the microbial community structure, function, and community interactions can be clouded by the contaminating communities, and inappropriate emphasis on outliers can be leveraged to promote false claims. We propose a method by which, with appropriate sample set size, we can begin to separate out the true signal of the environment of interest from the contaminating microbial “noise”. Production wells and topside separators were sampled from multiple reservoirs and the microbial communities were analyzed through Illumina sequencing of the 16S rRNA gene. We then constructed a co-occurrence network, removed weak correlations, identified groups of highly-interactive organisms and assigned value to each. Groups of organisms were then related back to the environmental parameters for each sample. Using this technique we were able to discern that a large proportion of our “oil reservoir” community was not, in fact, from the subsurface. Additionally, we were able to assign species of unknown origin to an environment. Not taking microbial community samples at face value when working with these types of samples is integral in elucidating microbial communities from hard to reach environments.

Offered papers

*116 “Marine bacterial diversity in seafloor sediments for environmental baseline monitoring prior to deep sea offshore oil exploration”.

Authors

1. Deidra Ferguson - University of Calgary
2. Carmen Li - University of Calgary
3. Anirban Chakraborty - University of Calgary
5. Martin Fowler - Applied Petroleum Technology
6. Adam MacDonald - Nova Scotia Department of Energy
7. Calvin Campbell - Natural Resources Canada
8. Casey Hubert - University of Calgary

Oil exploration is extending into ultra-deep-water frontier areas including offshore Nova Scotia in Atlantic Canada. Baseline data for these marine ecosystems is limited, pointing to a need for information that will enable monitoring ecosystem recovery in the event of uncontrolled pollution. Traditional baseline studies monitor invertebrates, but this practice is labor intensive and can be fraught with error. Since microorganisms are the first responders to marine pollution events, microbial population surveys offer a highly sensitive, time saving and cost effective approach to baselines and environmental effects monitoring. Over 200 amplicon libraries of bacterial 16S rRNA
genes from 31 marine sediments offshore Nova Scotia (>2500 m water depth) were generated to establish the marine baseline community composition in an area planned for offshore oil and gas development. In most locations OTUs affiliated with the Woeseiaceae/JTB255 lineage within the Gammaproteobacteria were dominant (3-10% relative sequence abundance) in uppermost surface sediments (0-1 cm). The relative abundances of this group were significantly lower at 20 cm depth (p<0.001). This is consistent with recent observations that Woeseiaceae/JTB255 forms part of the core microbiome in surficial sediments in the deep sea. In a mock oil spill experiment that exposed Nova Scotia sediment to light crude oil, DNA- and RNA-based 16S amplicon libraries revealed that OTUs related to Woeseiaceae/JTB255 (including the recently isolated Woeseia oceani XK5) were present initially, but then detected in significantly lower abundance after 30 days (p<0.001), and never re-emerged during 300 days of incubation. Additionally, alpha diversity decreased after 30 days (p<0.001) of exposure to oil and remained low for 300 days. Core microbiome baseline taxa becoming undetectable, and significant changes in diversity in response to oil, support the use of amplicon-based microbial community assessment for establishing baselines and performing environmental effects monitoring before and after hydrocarbon contamination in offshore settings.

*104 “Dipicolinic acid as a tracer for thermospores and oil seeps in marine sediments”.

Authors
1. Jayne Rattray - University of Calgary
2. Gretta Elizondo - University of Calgary
3. Anirban Chakraborty - University of Calgary
4. Casey Hubert - University of Calgary

Understanding the sediment biogeography of dormant marine thermophilic bacterial endospores (thermospores) has the potential to assist locating and characterising working petroleum systems. The presence of thermospores in cold ocean environments suggests that distribution occurs via hydrocarbon seepage from thermally active reservoirs. Low abundance and endospore coat physiology mean nucleic acid based techniques have limited success for in situ detection of thermospores. The biomarker 2,6-pyridine dicarboxylic acid (dipicolinic acid or DPA) is specific to endospore-forming bacteria from the phylum Firmicutes, and constitutes a significant percentage of endospore dry weight. DPA is therefore a potential biomarker for sediment dwelling thermospores and hydrocarbon rich thermal seeps however its suitability for seabed screening surveys has so far not been tested. To address the current lack of data, we set up a modified Tb3+ chelation method for the analysis of DPA (Lomstein and Jørgensen, 2012) using HPLC-FLD measuring at 270 nm emission and 545 nm excitation. Samples were extracted using complete digestion with acid hydrolysis. DPA distribution was assessed in surface sediment samples at 97 locations in the Eastern Gulf of Mexico and results from both oil positive and oil negative sites were compared. Recent expeditions in Canadian waters to the Scotian shelf and Laurentian channel provided both oil positive and negative sediment cores, and enabled higher resolution down-core DPA depth profiles. Deep water sediment cores showed a higher and more variable concentration of DPA down core at positive
stations in comparison to negative stations. The efficacy of DPA for tracing thermospores in oil seeps in marine sediments is further assessed and we propose that DPA has potential as a biomarker for assisting the location of hydrocarbon systems in deep water.

References

*115 “MICON: an ontology to unify the disparate field of Microbiologically Influenced Corrosion”.

Authors
1. Elvira Mitraka - Dalhousie University
2. Robert Beiko - Dalhousie University

Big-data initiatives, such as the geno-MIC project (https://bio.ucalgary.ca/microbial-corrosion/research), generate constantly increasing amounts of important data, but without a way to organize and make logical connections, the data will become less useful. Ontologies represent knowledge of a specific domain in a manner that is understandable by machines and humans alike, and are used as backbones for databases and data management. The Microbiologically Influenced Corrosion Ontology (MICON) is a connecting point among standard operational protocols in on- and off-shore oil pipelines, pipeline maintenance, sample extractions, metagenomics analysis, and various other related wet lab and computational procedures. It is already interoperable with other well established ontologies in different domains and will further expand to cover all the aspects of microbiologically influenced corrosion. MICON includes both unique terms and imported terms from other established and curated ontologies. It also includes logical definitions and axioms, which will enable for smarter searches in the database, as well as automatic categorization of samples and analysis outputs. Standard Operational Protocols have been translated into ontological processes, so that every step has a machine-readable representation in the ontology. The metadata fields are in place, according to the minimum information requirements for the samples. We have also established textual definitions for a variety of corrosion specific terms, standardizing the exact meaning and facilitating communication between different experts. The database that we built (QUOR’em DB) will use the logical modelling in MICON to enable smarter searches, interoperability between data sources and precisely annotated and curated data. Additionally, MICON will provide a quality-control check when uploading user data for completion and logical consistency. It is also built in parallel with a comprehensive ontology for Omics data in general, allowing for even greater interoperability between large research and industry projects.

*137 “Enhancement of methanogenic crude oil degradation using organic nutrients toward biotechnology applications in oil reservoirs”.

Authors
1. Daisuke Mayumi - aist
2. Konomi Suda - aist
Methanogenic crude oil degradation is currently attracting much attention in biotechnology, i.e., microbial enhanced energy recovery (MEER) technology, converting the humanly inaccessible crude oil into recoverable biogas. In previous study, we reported on a unique methanogenic microbial community degrading toluene in crude oil recovered from a high-temperature oil reservoir. However, commercially practicable MEER technology using this microbial community is still challenging because toluene is a small component in crude oil hydrocarbons, and methane produced from only toluene was insufficient for the field application. Therefore, we focused on biostimulation for the microbial community aiming to degrade n-alkanes, major components in crude oil hydrocarbons. We conducted incubation experiments of the oil reservoir production water amended with inorganic (nitrogen and phosphorus sources, trace metals and vitamins) or organic (four types of commercially available yeast extracts) nutrients under the in situ oil reservoir condition, i.e., 55°C and 5 MPa. Methane production was drastically accelerated up to three to five times in incubations amended with two types of yeast extracts (1 to 1.6 mmol/ml-oil) compared with those amended with inorganic nutrients (0.33 mmol/ml-oil). This result was supported by hydrocarbon composition analysis showing that cultures amended with their yeast extracts degraded high-molecular weight n-alkane (C20 to C32) as well as toluene. Microbial community compositions in cultures amended with inorganic nutrients were dominated by uncultured Ca. Atribacteria (class JS1) which has been known to encode a bss gene, the initial activating enzyme for toluene, through our metagenomic analysis. Meanwhile, uncultured Ca. Acetothermia (class OPB14) and class JS1 dominated in cultures amended with yeast extracts, suggesting that these uncultured bacteria may play an important role in n-alkane degradation. This study demonstrates that the biostimulation strategy using our organic nutrients is effective for enhancement of methane production from crude oil, promoting the field application of commercially feasible MEER technology.

*160 “Microbial metal dependent conversions of hydrocarbons for zero CO₂ emission energy extraction”.

Authors
1. Breda Novotnik - University of Calgary
2. Jagos Radovic - University of Calgary
3. Arpita Nandy - University of Calgary
4. Stephen Larter - University of Calgary
5. Marc Strous - University of Calgary

Green energy recovery from petroleum reservoirs via microbial routes by generating reduced energy vectors was explored in our work. These energy vectors would be
produced underground and re-oxidized above ground generating green electricity. Our work focused on microbial anaerobic crude oil oxidation with manganese oxide (birnessite) and AQS reduction, both serving as energy vectors. Hydrocarbon degradation was determined with GC-MS and FTICR-MS analysis and substantial degradation of alkanes as well as polycyclic aromatic hydrocarbons (PAHs) was detected. PAHs degradation decreased with increased alkylation and FTICR-MS analysis confirmed the presence of oxygenated species – putative acidic products. Manganese oxide reduction was determined by the presence of reduced Mn, Mn(II), that was measured by ICP-MS. Mn(II) also forms precipitates with carbonates additionally lowering emitted CO2. AQS reduction was also achieved, independently of birnessite reduction. Anaerobic hydrocarbon degradation was confirmed by oxidation of 13C enriched naphthalene and hexadecane. Carbon dioxide that was enriched in 13C, derived from the isotopically enriched label, was detected. Corresponding metabolites of crude oil and the label degradation were investigated by high resolution mass spectrometry. The source of microbial biomass for these experiments was activated sludge from a local waste water treatment plant. During the incubation an enriched microbial community was established comprised of Bacteroidetes, Proteobacteria and Firmicutes, with several species without Family/Genus assignment. The application of manganese oxide as an electron acceptor was confirmed in a microbial fuel cell where the generation of current was increased with anode sputtered with the oxide in comparison to anode alone. Power densities of around 50 mW m^-2 were achieved.*

*158 “Assessing wettability alteration potential and rheological properties of novel polymer for development of biochemical enhanced oil recovery (BcEOR) technology”.

Authors
1. Akhil Agrawal - Central University of Rajasthan
2. Sandeep Rellegadla - Central University of Rajasthan
3. Shikha Jain - Manipal University Jaipur

At present, the discovery of new oil reserves are decreasing and the recovery of crude from marginal wells is becoming increasingly important. Hence, new materials are needed for extraction of crude from these challenging reservoirs and harsh environments. Many Chemical Enhanced Oil Recovery (CEOR) methods, in particular polymer flooding offers solution to such limitations and has become critical and much effective technique for EOR. There are a number of polymers and copolymers that have been developed and used in field studies but are sensitive to changes in the reservoir environment. Therefore in the present study changes in intrinsic viscosity [h] of different polymer solutions and the potential of using natural polymers for altering wettability of reservoir rocks were evaluated under reservoir conditions. The [h] values of all the samples, except for Galactomannan polymer (~21 gm/dl at 65 °C) showed significant decrease in their viscosity characteristics under reservoir conditions. There was very low changes observed in its molecular weight compared to other natural derived polymers and EOR polymers. Contact angle measurements were also performed on oil wet reservoir rocks (XRD peaks showed calcite and quartz SiO2 sandstone) treated with different polymers. The change in the contact angle was maximally noted in the case of Galactomannan polymer, which was found to be 81° near to that of Triton-X-100 surfactant (79°) taken as control. Qualitative wettability
studies (floatation based and two phase separation tests) had shown the tendency of Galactomannan polymer solutions to alter wettability of oil-wet surfaces towards water wet. FTIR of the oil wet powdered rock samples treated with polymer solutions were performed and the absorption spectra revealed reduction in peaks of methyl and methylene indicating altering wettability. This changes indicates a stable nature of Galactomannan polymer, making it a more suitable biopolymer to be used as EOR polymer.

*111 “Seven years of Microbial Enhanced Oil Recovery (MEOR): Lessons learned on the way to application”.

**Authors**

1. Nicole dopfelf - BASF SE
2. Hakan Alkan - Wintershall

In the last seven years Wintershall and BASF jointly developed a roadmap for the use of microbial enhanced oil recovery (MEOR) for mature oil fields. Having started in 2011 with a field screening and the establishment of basic oilfield microbiology methods in the lab, we gained a broad knowledge and lot of experience not only in microbiology but also engineering, modelling and field work. This talk will give an overview of the highlights and the major findings and also learnings of the last years of the MEOR project, which include the use of nitrate and molybdate as SRB (sulfate-reducing bacteria) inhibitor, the results of our first field test, the uncertainties and challenges working with original field water, the isolation of a new microbial species from our field, pressure effects and others. Focusing on the single MEOR effects, for example microbially induced wettability change, it is now possible to calculate and predict incremental oil for our field of interest. Our work shows that MEOR is not necessarily a black-box and can be applied as feasible EOR technology as long as a multi-disciplinary team is cooperating.

**KEYNOTE:**

“Microbes will Influence the Effectiveness of Future Operational Oil Spill Countermeasures”

1. Kenneth Lee (Fisheries and Oceans Canada)

Despite advances in technology and safety protocols used by the offshore oil and gas and marine shipping industry, accidental oil spills can and will occur in the future. In terms of oil spill response, to complement “physical recovery” of oil from the sea surface by booming and skimming, there are a number of alternative oil spill countermeasures under study within Canada under the Multi-Partner Research Initiative of Canada’s Oceans Plan, which aims to provide the science-based evidence required for their regulatory approval and selection for use. These include: in-situ burning, the use of spill treating agents, oil translocation, monitored natural attenuation, and bioremediation. With the exception of in-situ burning the effectiveness of all of the above oil spill response strategies are largely dependent on the activity of oil degrading bacteria. While their remains some controversy, the
premise of oil dispersant use was based on the dispersion and dilution of surface oil slicks into the water column to concentrations below toxicity threshold limits in the form of oil droplets more open to microbial attack. Academic and applied research programs are now being focused on oil translocation, based on the transport of oil from one environment to another where it is more amenable to physical recovery strategies or microbial degradation. Monitored natural attenuation, the degradation of oil by indigenous bacteria is now recognized as an operational response. There is now a major effort applying molecular and microbiological tools to determine the capacity of microbes to degrade petroleum hydrocarbons and their refined products in Canada’s three oceans and its freshwater systems under both natural and enhanced environmental conditions.

POSTER PRESENTIONS

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

*113 “Indole-mediated regulation of anaerobic biofilm formation in Desulfovibrio vulgaris Hildenborough: Implications in Microbiologically Influenced Corrosion”.

Authors
1. Mohor Chatterjee - ExxonMobil Research and Engineering
2. Kuang He - ExxonMobil Research and Engineering
3. Antonio Paiva - ExxonMobil Research and Engineering
4. Zarath Summers - ExxonMobil Research and Engineering
5. Giovanni Pilioni - ExxonMobil Research and Engineering

Sulfate-reducing bacteria (SRB) are frequently found adhering to the inside walls of anaerobic oil pipelines in heterogeneous biofilms. Using sulfate as the terminal electron acceptor, they produce hydrogen sulfide leading to potential for corrosion and pipeline failure of steel infrastructures. Understanding of this phenomena of anaerobic biofilm development by SRB’s leading to microbial corrosion is limited to this date. Here we show that the microbial metabolite indole is involved in mediating surface attachment during biofilm formation by modulating the expression of prophages in the model SRB Desulfovibrio vulgaris Hildenborough (DvH). Indole is a signalling molecule that mediates biofilm formation in a variety of microbes. Indole repressed surface attachment during initial biofilm formation by DvH in our lab. When we RNA-sequenced planktonic and biofilm samples of DvH harvested from the same test bottles growing with and without indole, our results revealed an exceptionally high amount of prophages up-regulated only in biofilm samples growing without indole, despite the highly comparable metabolisms observed in both the cultures. Prophages are genetic material of viruses that are integrated within host bacterial genome. They can cause lysis of the host cell upon certain environmental triggers. Due to their ubiquitous presence, the specific role of integrated prophages in bacteria is very much debated. Our observations show that prophages are important during biofilm formation in DvH and indole regulates biofilm formation by possibly repressing these genes. Based on our results, we hypothesize that prophage mediated lysis of microbes cause
release of cellular materials that can serve as a matrix for further biofilm attachment, eventually leading to the cascade of hydrogen sulfide production. Thus, our work gives unique insights into anaerobic biofilm formation and emphasizes the importance of basic research to provide support to relevant business operations.

*82 “The role of ferric-iron complexing ligands in the microbial influenced corrosion of oil field production water tanks”.

Authors
1. Mark Nanny - University of Oklahoma
2. Joseph Suflita - University of Oklahoma
3. Kathleen Duncan - University of Oklahoma
4. Meredith DeGarmo - University of Oklahoma
5. Sylvie Le Borgne - University of Oklahoma
6. Yan Li - University of Oklahoma

Despite lower thermodynamic gains in free energy, sulfate reduction typically occurs instead of ferric iron reduction during microbial influenced corrosion (MIC) because of kinetic barriers imposed by dissolution of solid-phase ferric oxides. This kinetic barrier can be overcome by adsorption of organic ligands to ferric oxide surfaces, with the subsequent dissolution of soluble ferric iron-ligand complexes. The apparent control of organic ligands governing sulfate vs. ferric iron reducing MIC mechanisms was demonstrated in five produced water tanks located in the natural gas producing region of the Barnett Shale. Three tanks were identified as having MIC problems based on SRB/APB dilution measurements, even though produced water from all five tanks had elevated dissolved manganese concentrations and Mn/Fe ratios indicating steel corrosion. Targeted metabolomic analysis showed a positive linear correlation between dissolved iron and metabolites consisting of dioic acids and a-hydroxycarboxylic acids, both of which form soluble ferric iron-ligand complexes. Tanks exhibiting a definitive sulfate reducing MIC mechanism (e.g. high abundance of SRBs, high sulfate reducing activity, and ferrous sulfide corrosion products) had lower amounts of metabolite ligands relative to tanks with lower values for microbial and chemical indicators of sulfate reducing MIC. Furthermore, this later set of tanks had a higher abundance of aerobic heterotrophs, suggesting aerobic biodegradation of condensate hydrocarbons as the initiator of the metabolite ligands. Periodic draining and filling of produced water tanks creates cyclic aerobic/anaerobic conditions. If metabolite ligands are produced by aerobic heterotrophs and lead to soluble ferric iron complexes, this can preferentially favor a ferric iron MIC mechanism and delay the onset to a sulfate-reducing MIC mechanism despite anaerobic conditions. For steel infrastructure experiencing cyclic aerobic/anaerobic conditions, it’s critical to identify the environmental “tipping points” for anaerobic microbial communities and the resulting predominant MIC mechanism so to effectively monitor and manage MIC.

*74 Desulfovibrio giganteus C2, a novel isolate from a paraffin-degrading methanogenic consortium capable of electrical microbial induced corrosion (EMIC)

Authors
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2. Shin Ichi Hirano - University of Oklahoma
We explored the role of sulfate reducing bacteria (SRB) that persisted in a paraffin-degrading methanogenic consortium in the absence of sulfate. Upon sulfate amendment and repeated transfers, the consortium was able to biodegrade octacosane and to accelerate steel corrosion. Metagenomic analysis suggested that the SRBs became predominant organisms. A SRB was isolated from the consortium with H2:CO2 and identified as *Desulfovibrio giganteus* (99% 16S rRNA gene similarity). Quantitative analysis of sulfate and H2 consumed in live, sterile and abiotic incubations with Fe0 demonstrated that live cultures reduced 57% more sulfate that would be expected based on the amount of H2 produced. Iron dissolution (measured as Fe2+) was twice as high in live cultures than with abiotic or sterile controls. Thus, the isolate grew by sulfate-reduction using elemental iron as a sole source of electrons. The corrosion of carbon steel in the presence and the absence of *D. giganteus* C2 was evaluated by electrochemical procedures including linear polarization resistance (LPR), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). These analyses were facilitated in a bio-electrochemical cell using a carbon steel coupon as a working electrode. LPR measurements demonstrated a decrease in polarization resistance and a 4 fold increase in the corrosion rate in the presence of *D. giganteus* C2 relative to the abiotic control. Tafel plots indicated that the isolate accelerated the cathodic reaction of carbon steel corrosion. EIS experiments showed that the charge transfer resistance increased with time in abiotic controls, but remained stable in the presence of the isolate. CV confirmed the transfer of electrons between *D. giganteus* C2 and carbon steel. These findings suggest that *D. giganteus* C2 persisted in the consortium probably as hydrogenotrophs, but could also promote corrosion in the presence of iron and sulfate by forming a conductive biofilm that extracted electrons from carbon steel surfaces.

*70 “Microbial modelling of sulfate-reducing bacteria (SRB) and methanogenic archaea (MA) using elemental iron”*

**Authors**

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Sulfate reducing bacteria (SRB) and methanogenic archaea (MA) are commonly found in the oil and gas environments. The formation of hydrogen sulfide (HS-) is particularly concerning for the petroleum industry due to its corrosiveness. However, the activities of SRB are limited to the concentration of sulfate present in the environment, whereas methanogens can utilize substrates such as H2 for methanogenesis. MA is commonly found in sulfate-free environments, such as deep sediments, and are known to form interspecies electron transfer relationships with SRB. Recently, SRB and MA capable of microbiologically influenced corrosion (MIC) by using elemental iron as a direct electron source (EMIC) have gained increased attention. On the iron surface, EMIC-SRB can outcompete EMIC-MA in the presence of sulfate, but this changes as sulfate depletes. The formation of FeS on the metal surface can be further utilized by MA for methanogenesis as it provides a conductive path. However, the possible kinetics involved of the overall process are currently unknown. We obtained a co-culture of
EMIC-SRB and EMIC-MA to investigate the growth rates and electrical potential changes under different environmental conditions, including changes in pH, temperature and salinity. Results indicate that under neutral conditions and using iron as the sole electron donor, methane production (up to 5 mM) starts after sulfate was depleted. Electrochemical measurements will be conducted on the co-culture under different conditions to determine the changes in the electrical potential in correlation with the sulfate and methane concentration. Fluorescence and electron microscope images of the biofilm structure will be used to visualize cell distribution and morphology. This study embarks the first step of understanding the relationship between EMIC-SRB and EMIC-MA. Such knowledge is important for the field of microbial electrophysiology and can be further explored for industrial applications.

*36 “Modeling of Microbiologically Influenced Corrosion (MIC) for Risk-Based Inspection (RBI) in the Oil and Gas Industry: Screening Influential Parameters”

Authors
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Microbiologically Influenced Corrosion (MIC) causes considerable losses to the oil and gas industry annually. Although studies on MIC have become more frequent over the last few years, it is still not fully understood. This poses a significant challenge to operators of oil and gas facilities since methodologies and guidelines for MIC assessment and mitigation are still evolving. The objective of this study is to develop a practical risk-based inspection (RBI) methodology for industry that specifically incorporates MIC threats. The RBI approach is based on semi-quantitative methods, which aim to 1) identify localized "hot spots" for possible MIC threats (screening), and 2) help establish maintenance priorities by ranking both facilities and pipelines based on MIC threat susceptibility. This new RBI approach could be used for both onshore and offshore facilities, and will incorporate modern techniques to quantify microbiological abundance, diversity, activity and function. Furthermore, the assessment method will be tailored for certain archetypes of the oil and gas industry (e.g. crude gathering systems, seawater injection systems and produced water systems) and account for various levels of data availability. Potential MIC threats are quantified by determining the probability of failure (PoF) which systematically considers metallurgical, chemical, biological and physical/operating parameters based on information from operations (e.g. inspection data) and the literature. To implement the model, a decision tree (flow-chart) has been developed, which provides end users an easy tool to assess and screen assets for potential MIC threats.

*102 “Sulfur and Nitrogen Biotransformations and Microbiologically Influenced Corrosion in Offshore Oil Production Operations”.

Authors
1. Danika Nicoletti - University of Calgary
2. Mohita Sharma - University of Calgary
3. Lisa Gieg - University of Calgary
Microbiologically influenced corrosion (MIC) is an underestimated cause of corrosion in oil and gas infrastructure. In sour oil production operations, nitrate or nitrite have often been applied to help control souring. However, recent reports have indicated that sulfur (S) and nitrogen (N) biotransformations that may occur as a result of these treatments can yield corrosive products. In this research, we are investigating the key microbial taxa implicated in MIC and the S and N biotransformations that occur on the topsides of offshore oil producing platforms. Incubations at 54°C were prepared from produced water samples collected from various locations along topside facilities that process fluids recovered from a soured and a non-soured crude oil reservoir. The microcosms contained iron alloy coupons, nitrate or nitrite (at concentrations that span those typically used in the field) and were conducted in the absence or presence of sulfide. Appropriate controls were also established. Incubations were sampled over time to measure concentrations of S and N species including sulfate, sulfite, nitrite, nitrate, and ammonium, among others. Notable results from the platform processing fluids from the non-soured reservoir have included a 48% decrease in sulfate concentration in an unamended control (free of nitrate, nitrite and sulfide) indicating the presence of sulfate-reducing taxa, and sulfate production in a 5 mM nitrite plus sulfide amendment which indicated the presence of sulfide-oxidizing taxa. The time zero 16S rRNA gene sequencing results for this location sample showed the presence of *Desulfacinum* spp., a genus that contains sulfate-reducing strains, as well as *Caminicella* spp., a genus containing anaerobic and thermophilic strains. Such measurements, coupled with corrosion rates, microscopy, and profilometry of the corrosion coupons, are ongoing and will indicate the MIC potential resulting from N and S biotransformations in offshore oil processing operations.

*132 “Linking Metabolism to Microbiologically Influenced Corrosion”*

**Authors**
1. Natalie Rachel - University of Calgary
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3. Gloria Okpala - University of Calgary
4. Mohita Sharma - University of Calgary
5. Lisa Gieg - University of Calgary

Microbiologically influenced corrosion (MIC) is one of the root causes of iron corrosion, posing a serious economic problem. In recent years, an increasing amount of research has been devoted towards better understanding the mechanisms of MIC, so that effective detection and mitigation strategies may be developed. The vast biological diversity that exists amongst microorganisms complicates the creation of broadly applicable strategies, as not all organisms are created equally: the severity of corrosion risk posed by microorganisms varies significantly depending on their biochemistry. The hydrogen sulfide produced by sulfate-reducing microorganisms (SRM) has been previously established as their defining corrosive characteristic (e.g., wherein the sulfide reacts with Fe2+ to produce corrosive FeS). However, there are SRM with the capacity to strip electrons directly from metallic iron to power their metabolism. Microorganisms using this direct-iron electron uptake pathway are substantially more corrosive, as reported both by others and in our own studies. While current approaches that involve the surveying of microbial populations are helpful in assessing MIC potential, these are indirect methods as the mere presence of microorganisms such as SRM does not necessarily indicate that corrosion will occur.
This fact highlights the value for concretely identifying the metabolic processes responsible for MIC; enzymes that are specifically responsible for oxidizing metallic iron may serve both as genetic diagnostic markers, as well as inhibition targets. To this end, using the highly corrosive model organism Desulfovibrio ferrophilus IS5, we have performed genomic and transcriptomic analyses of various incubations in the presence and absence of metallic iron. We present and investigate putative diagnostic enzymatic (gene) candidates, which we hypothesize will provide valuable insight into the metabolisms leading to MIC.

*168 “The use of pressurised, sand-packed bioreactors to improve nitrate treatment calculations within a souring forecasting model”.

Authors
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4. Leanne Walker - Rawwater Engineering
5. Bob Eden - Rawwater Engineering

Pressurised, sand-packed, oil-saturated bioreactors have been used to simulate realistic downhole oilfield conditions in order to investigate the impact of extended shut-in periods in the presence and absence of a competitive exclusion treatment. During the simulation study, Molecular Microbiological Methods (MMM) were used to demonstrate the growth of both mesophilic sulfate-reducing microorganisms (SRM) and mesophilic nitrate-reducing microorganisms (NRM) at 1,000psig, 30°C (69barg, 86°F). Twelve pressurised bioreactors were packed and inoculated prior to oil-saturation with a pre-established sand matrix known to support microbiological sulfide production and nitrate reduction from crude oil biodegradation. The crude oil selected for this study was also known to support the growth of mesophilic SRM and NRM. At the start of the study, all of the pressurised bioreactors were operated under daily batch injection, and effluent water analysis included [total sulfide], [nitrate], [nitrite], [VFA] and pH. Effluent water samples were also taken for MPN, qPCR and 16S genomic analysis, and compared to the original inoculation community. Upon recording either stable microbiological sulfide production or nitrate reduction for each experimental condition, the shut-in period of the test bioreactors was doubled to two, four, eight, 16, and finally 32 days. Throughout the project, the rate of microbiological sulfide production and nitrate reduction was monitored to determine to what extent shut-in periods stimulated microbiological utilisation of sulfate and nitrate. Particular attention was given to quantifying the differences in metabolic activity between NRM and SRM during the extended shut-in periods. The outputs from the pressurised bioreactors have been used to improve the nitrate treatment calculator for the DynamicTVS© souring forecasting model, a bolt-on module to industry-standard reservoir simulators (e.g. Eclipse, Petrel). The revised souring forecasts reflect field observations that longer communication times between injector/producer pairs, and extended shut-in, result in a stimulation of microbiological nitrate utilisation and reservoir souring.

*98 “Investigation of natural antimicrobial compounds for prevention of microbiologically influenced corrosion (MIC)”.
Offshore oil production facilities are subjectable to internal corrosion, which can occur through microbiologically influenced corrosion (MIC). A consortium of surface living microorganisms (often including sulfate reducing prokaryotes, SRP and methanogens) facilitates the corrosion attack as an embedded matrix referred to as biofilms. To mitigate MIC, the oil and gas industry relies mainly on biocides and corrosion inhibitors. Active phytochemicals including strong antimicrobial compounds from halophytes were applied in this study to combat microorganisms responsible of MIC such as methanogens and SRP. A bioreactor system for biofilm production on metal coupons was setup and inoculated with anaerobic sediment from the Wadden Sea (Denmark) to mimic MIC from local oil production facilities in the Danish sector of the North Sea. The inhibitory effect of using selected halophyte plant extracts on microorganisms and corrosion was studied with both chemical, corrosion and molecular microbiological methods. Significant inhibition of microorganisms was observed in liquid samples from the reactors treated with halophyte plant extracts. Biofilm formation (measured as ATP levels on coupon surfaces) was reduced by three times on carbon steel coupons and four times on stainless steel coupons in biofilm reactors treated with halophyte plant extracts. Visible and measurable reduction in pitting and corrosion damages on carbon steel coupons was observed with 3D surface scanning of the metal coupons. Next generation 16S rRNA amplicon sequencing of DNA from Bacteria and Archaea will help identify the surface living microbial populations and study the effects of antimicrobial compounds on these populations and relate to the observed reduction in pitting corrosion. Long-term solutions to prevent MIC using natural antimicrobial compounds from halophyte plants are discussed and proposed in this study.

*91 “Suspected microbiologically-influenced corrosion of an offshore pipeline”*

Microbiologically-influenced corrosion (MIC) is estimated to be responsible for billions of dollars of damage annually to oil and gas facilities worldwide. An offshore pipeline with multiphase flow is experiencing corrosion, suspected to be primarily due to MIC. Methanol is being injected for gas dehydration. In this study, we characterised water and solids collected during routine pigging operations to determine whether the corrosion observed is attributable to the action of microorganisms. A suite of complimentary analytical techniques was used including, inductively coupled plasma mass spectrometry (ICP-MS), Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDS), Fourier-Transform Infrared Spectroscopy (FT-IR), high temperature-gas chromatography (HT-GC) and molecular microbiology method (MMM) analyses. The total iron measured in the water fractions increased from 2.55 mg/L in fraction 3 to 8.2 mg/L in fraction 8. The total suspended solids (TSS) of the samples ranged between 101–133 mg/L with an oil-free TSS of 32.1 mg/L. The major
component (~80%) of the solids were predominantly aliphatic hydrocarbons, with typical wax characteristics. HT-GC analysis identified n-alkanes with 10–89 carbon atoms, with most compounds in the range C40–C70. Approximately half of the remaining inorganic fraction was due to bead-like structures, thought to be proppant. The remaining ~10% comprised mostly of a mixture of carbonate and sulphate species. In the aqueous phase, the total number of bacteria ranged between $1.18 \times 10^5$ and $1.72 \times 10^5$ cells/mL, and SRP numbers were between $3.60 \times 10^2$ and $5.59 \times 10^2$ cells/mL. Within the pigging solids, the total number of bacteria was $2.91 \times 10^5$ cells per gram and total SRP $1.63 \times 10^4$ cells per gram. Methanogens were measured at $3.37 \times 10^8$ cells per gram. This number of methanogens, together with the SRP numbers represents a high risk of MIC to the pipeline. The above data support the hypothesis that MIC is contributing to the corrosion observed in the pipeline.

*59 “Assessment of microbially influenced corrosion threats on water handling systems in Kuwait”

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2. Samundraga Surya Prakash - Kuwait Oil Company
3. Abdullah Fadel Jamsheer - Kuwait Oil Company
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Microorganisms can cause localized pitting and crevice corrosion by consuming electrons directly from steel, accelerate chemical reactions and produce corrosive metabolic such as H2S. This process, termed microbially influenced corrosion (MIC), pose a risk to system integrity, ultimately leading to unexpected production shutdown with extensive need for repair. The presence of microorganisms is unavoidable in complex water handling systems, but detailed information about hotspots for microbial presence and activity enables operators to focus mitigation activities towards high-threat parts of the system. In this case study, we used molecular microbiology methods to map microbial threat in Kuwaiti injection and produced water systems to demonstrate the operational value of microbial hotspot identification. Quantitative (qPCR) methods were applied to identify sites with elevated numbers of known culprit microorganisms (e.g. sulfate-reducing prokaryotes and methanogens), and specific high-risk microbial groups were mapped using next-generation amplicon sequencing (NGS). The results revealed that microbial communities, the presence of detrimental organisms and number of microorganisms varied significantly between systems, and even from sampling point to sampling point in each system. Specifically, the detrimental microorganisms found in high salinity parts of the system deviated from the typical oil and gas culprit microorganisms. Following this, chemical mitigation strategies could be optimized to target the main culprit organisms present in each of the concerned system, and the acquired knowledge may be integrated into risk-based inspection programs, as for chemical corrosion threats. Ultimately, this will enable full overview of system integrity allowing for timely actions and eventually a reduction in operating (OPEX) and capital (CAPEX) expenditure.
**An Online Electrochemical Based Microbial Detection System**

Authors
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In an effort to create an online, real time microbial detection system for Microbiologically Influenced Corrosion (MIC), a two-electrode system was constructed which characterizes biofilm activity using the Electrochemical Impedance Spectroscopy (EIS) technique. EIS is a highly sensitive and advanced method used in corrosion science to characterize electrochemical reactions and the characteristics of an electrochemical system (i.e. impedance and capacitance behavior). The pattern of the recorded response from an EIS signal can be related to the structure of liquid-solid interfaces, such as a biofilm on a substrate. In this study, a biofilm monitoring system consisting of parallel plate electrodes was tested to determine its effectiveness in detecting Pseudomonas.putida, an aerobic fermentative bacterium. The bacteria were first inoculated in Lysogeny Broth (LB) medium to grow biotic material for testing. Tests were conducted using parallel plate electrodes made from 316 stainless steel connected to an EIS system. Exposure tests were performed with biofilm and without biofilm. For biofilm system with planktonic cells, test was conducted in three conditions: 1). sterile LB medium (control group), 2). LB medium with inactive cells, and 3). LB medium with living cells. The tests with the inactive cells were used to study the effect of metabolites on the EIS response, while the active cells were used to study the response of active cell growth with biofilm present. For non-biofilm system, concentrated cells were added into NaCl buffer with titration to only study the effect of planktonic cells on EIS. Serial dilution and plate counting were also performed during the test to quantify the bacterial count. This value was then correlated with the EIS response to determine calibration curves. Results from these tests are shown in the poster, and potential future modifications to the system are discussed.

**Modeling of the Chemical Environment in Offshore Facilities**

Authors
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The nature of the chemical environment in oil and gas fluids such as produced water, soured oil, or low oxygen environments plays an important role in Microbiologically Influenced Corrosion (MIC). H2S and/or other forms of sulfur species in soured oils and produced water are key causes of corrosion and are involved in the growth of microbes. To mitigate reservoir souring and subsequent corrosion, nitrate is sometimes injected to displace sulfur reducing bacteria with nitrogen reducers. However, nitrates and the associated nitrogen species also impact the chemistry and microbial activity and hence the corrosion potential in the system. This work will investigate the behavior of the chemical environment as a function of temperature,
pressure, nitrate level, oxygen availability, oil-water ratio and time. Computer modeling will be conducted using the kinetic-equilibrium approach, and then a wet lab experiment. The reactivity and partitioning behavior (in oil-water system) of the identified important chemical species will be studied. The current studies will describe the chemical environment in the light of S and N chemistry and provide information towards understanding the chemical-microbial relationships under different conditions of the oil and gas operations.

*124 “High-throughput solutions for the oil and gas industry: Identifying and developing next generation treatments for biosouring”.

Authors
1. Hans Carlson - Ogma Scientific
2. John Coates - UC Berkeley
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Microbial sulfidogenesis (biosouring) in oil recovery operations is economically and environmentally costly with a global cost of ~120 billion US dollars a year. Despite this cost, there has been relatively little exploration of the chemical space of possible inhibitory compounds, and very little work has quantitatively assessed the selectivity of various souring treatments. We have developed the first high-throughput screening method to identify potent and selective inhibitors of SRM. We have screened >30,000 small molecules to identify potent inhibitors of SRM and quantitatively ranked the selectivity and potency of hundreds of compounds. In these screens we have identified previously unrecognized SRM selective inhibitors that are several orders of magnitude more potent compared to commonly used oil industry biocides such as THPS, bronopol or benzalkonium chloride, that, unlike these biocides, are selective against SRM. We have also identified important non-additive (synergistic/antagonistic) interactions between chemicals and have applied our high-throughput approach to improve formulations of oil recovery chemical mixtures to maximize efficacy and minimize cost. Finally, we have identified more cost-effective souring treatments based on nitrate that have been tested in column studies and mesocosms and are now moving into field trials.

*130 “Corrosion of low carbon steel by thermophilic sulfate reducing microorganisms (tSRM) enriched from oilfield produced water samples”.

Authors
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3. Gerrit Voordouw - University of Calgary

Thermophilic oilfields harbour mesophilic and thermophilic microorganisms that could impact oil production and distribution. One of the major group of microbes often studied in this area are the sulfate reducing microorganisms, because of their involvement in reservoir souring and biocorrosion. This study compared the corrosion on carbon steel caused by three thermophilic sulfate reducing microorganisms (tSRM) consortia enriched at 60°C from both onshore and offshore oilfields in the presence/absence of organic substrates. Examination of scanning electron micrographs of coupons retrieved from incubations with lactate showed extensive populations of
bacterial cells mixed with corrosion products. Coupons in medium with no organic electron donor, had few bacterial cells found only in FeS rich regions on the coupons. Higher corrosion rates corresponded to increased numbers of localized pits formed in incubations without lactate. Some tSRM were observed to attach to the metal surface with filaments under nutrient starved condition, which possibly contribute to the uptake of electrons for sulfate reduction by directly attacking the surface of carbon steel coupons. The thermophilic sulfate reducer Desulfotomaculum was found in both the planktonic and sessile microbial community in this study and could be the major corrosion causing organism in high temperature environments. However other community members like Syntrophus, Pseuodomonas and Methanoculleus were also detected, all known to participate in microbiologically influenced corrosion.

*169 “Determination of sulfur oxyanions in produced water using indirect detection capillary electrophoresis”

Authors
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4. Reza Akhoondi - Memorial University of Newfoundland

Sulfides and sulfur oxyanions found naturally and in industrial processes (e.g., oil production) have environmental and economical impacts. Chemical and biological reduction of sulfur-oxygen species can form toxic and corrosive hydrogen sulfide. Oxidative processes on sulfide and sulfuroxyanions causes reduction in pH and mobilization of toxic metals. The presence of these sulfur compounds can ultimately lead to higher operational costs, whether due to cost of treatment or repair of damaged equipment. Measurement of the major charged sulfur species, such as sulfate (SO\text{4}^{2-}), thiosulfate (S\text{2}O\text{6}^{2-}), tetrathionate (S\text{4}O\text{6}^{2-}), sulfite (SO\text{3}^{2-}), and sulfide (S^{2-}) is needed in developing a clear understand of the behaviour of these compounds in complex systems. However, for analysis of these charged species in produced water and aqueous reservoir fluids, high salinity is a major challenge for the analytical methods. We developed methods using chromatography (IC) and capillary electrophoresis (CE). Since most of our target analytes donot possess chromophores, indirect-UV method is selected for CE. All the key components for each method, including buffers, electroosmotic flow modifiers, and chromophores for CE with indirect detection and mobile phase composition for IC, were optimized. Fast analysis with high separation efficiency in low salinity matrices were achieved with the CE method. On the other hand, IC showed some advantages for analysis of samples with high salinity, though with considerably longer analysis times compare to CE. Results will be presented in detail.

*76 “Evaluation of Biocide Performance in Soured Oilfield Environments”

Authors
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One of the most problematic and costly microbial issues of the oil and gas industry is biotic reservoir souring. Reservoir souring can be mitigated by developing and applying effective and robust biocidal solutions which control the growth of problematic bacteria, including sulfate reducing bacteria. However, previously existing fundamental studies provide limited understanding of the performance of biocides in soured reservoirs where biocide encounter dissolved sulfide ions and interact with formation rock. This study investigates the stability and efficacy of commonly used oil and gas biocides in a soured environment to determine their suitability for use in souring remediation. Biocidal efficacy studies were performed against sulfate-reducing bacteria to determine minimum cidal concentration of each of these biocides in soured systems using 96-well plate most probable number assay. Due to the lack of a relevant test methodology, it has been very challenging to mimic the real-world dynamic flow conditions to determine the performance of biocides adsorbed on the surface of reservoir rocks in the near-wellbore. This study establishes a new test methodology to measure the efficacy of commonly used biocides in top-side facilities and near-wellbore environments where they are in contact with rock. Fundamental results of biocide adsorption on rock in static and dynamic flow conditions generate a selection tool for biocides that show efficacy when used in topside assets and the near-wellbore. In summary, these results offer guidance for selection of biocides that will remain stable and efficacious in soured reservoirs. The results showcase that glutaraldehyde is very stable in soured systems, does not adsorb to rock, and remains available for microbial control.

*40 “dsrAB gene expression - new approaches for biocorrosion studies”

Authors
1. Luiz Pinto - National Institute of Technology
2. Diogo Azevedo - National Institute of Technology
3. Walter Cravo - National Institute of Technology
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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. Molecular tools may help to mitigate these possible damages, however, studies are needed to understand molecular mechanisms involving microbial activity, especially the transcription of important enzymes such as sulfite reductase (dsrAB), present on sulfate reducing bacteria (SRB). Transcription of the mRNA (transcriptome) can represent the molecular scenario at the time of sample collection, indicating microbial responses to the conditions present at that moment, thus generating functional data of the microorganisms analyzed. Customized collect methods associated with expertise acquired over the years made possible the conduction of a study seeking the understanding of the relationship between number of microorganisms, gene expression and enzymatic conversion time. A dose response study was conducted, using a single strain of SRB (Desulfovibrio desulfuricans), so that the expression of this gene could be better understood, obtaining data of bacterial quantification, H2S
production and rate of gene expression. Based on the results obtained, the same number of bacteria can be stimulated to over express this gene, producing more $H_2S$ and consequently, causing large damages. Due to that, only bacterial quantification may not be the only tool to minimize biocorrosion, but knowledge of the substrate available at the site, may lead what decisions to take.

"Sulphur isotope fractionation behaviours during souring of an offshore oilfield"

Authors
1. Max Frenzel - Oil Plus Ltd
2. Hannah Mitchell - Oil Plus Ltd
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Reservoir souring is caused by the activities of sulfate-reducing microorganisms (SRM), primarily during water flooding associated with secondary oil recovery to maintain reservoir pressure or increase sweep efficiency. The hydrogen sulphide produced by SRM is a toxic, corrosive species that impacts on operational safety, infrastructure integrity, operational efficiency and increases capital and operational costs. We conducted a souring study of an offshore oilfield with significant confirmed SRM activity. A set of coreflood experiments was conducted to determine the scavenging capacity of the reservoir. Laboratory jar tests were used to determine the sulphide-generation capacity of microbes isolated from wellhead samples. Typically, sulphur isotope analysis of produced sulphide, and source sulphate, is used to identify biogenic souring via the enrichment of $^{32}S$ in produced sulphide. During bottle tests we measured a sulphide sulphur isotope fractionation of $-14\%$. From 2018 field data, the seawater sulphate had a $\delta^{34}S$ isotope ratio of $+30.5\%$, and the produced fluids a sulphide $\delta^{34}S$ isotope ratio of $+19.5\%$ to $+20.5\%$ (vs CDT). This contrasts with 2014 data, where the sulphide $\delta^{34}S$ isotope ratio was $0.0\%$ to $+10.0\%$ in production wells. These field data would suggest the source of souring was not primarily biological. The core material contains very little sulphur bearing mineralogy that might explain the shift to $^{34}S$-enriched $H_2S$. We hypothesise that the cessation of seawater injection, with the continuous addition of sulphate, and the establishment of the recirculation of produced water containing significant quantities of previously injected seawater sulphate, coupled with mineralogical fractionation of $H_2S$ during the scavenging reactions can explain the shift in isotope values recorded for both reaction source and product. This study highlights the use of a multidisciplinary approach in identifying the source of souring, and that isotope data needs to be applied and understood within the context of the field under study.

"Detection of Sulfate Reducing Bacteria in Oilfield Produced Water and Biocide Treatment of Desulfovibrio”.

Authors
1. Iris Porat - Kemira Chemicals
2. Virginia Turk - Kemira Chemicals

Growth of sulfate-reducing bacteria (SRB) has negative effects for the oil industry, including souring of oil and corrosion of pipelines and storage tanks. Biocides are in
use to inhibit the growth of SRB. Traditionally, the oil industry relies on cultivation techniques, like MPN liquid dilution series, to quantify SRB. The MPN method was accepted as the standard technique by NACE in 2004. Due to the long incubation time (28 days) and the low microbial detection (less than 10% of the viable microbes), molecular microbiology methods (MMMs) for the detection of SRB were included in the NACE Standard from 2012. qPCR is one the MMMs method for detection and quantification of SRB by proving one of the genes in dissimilatory sulfate reduction pathway. This research presents the detection of SRB in produced water samples by MPN and qPCR methods. Also, a number of biocides were tested against a strain of SRB. Produce water samples, collected over 5 months, were used for detection of SRB by MPN or by qPCR. Biocides effects were tested against the SRB model organism Desulfovibrio vulgaris. SRB were detected in all produced water samples, by qPCR, in the range of 2x104–1x106 gene copy number/ml. SRB were detected also by MPN, but in the range of 9x100–3x103 bacteria/ml. In most of the cases, the same trends over time were observed within the two methods. THPS (200-400 ppm), glutaraldehyde-quaternary ammonium compounds (150-500 ppm), dazomet (350-480 ppm), glutaraldehyde (500 ppm) inhibited D. vulgaris growth for up 33 days. qPCR provides higher detection resolution, and in some cases, predicts the bacteria trends before the results were observed by the MPN method. As a result, the use of qPCR would detect an SRB spike faster and more timely to alert the facility to revise the biocide treatment.

*103 “Towards Creating a Model Microbial Community to Study Microbiologically Influenced Corrosion”.

Authors
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2. Raymond Turner - University of Calgary

Aims: Laboratory based research on microbiologically influenced corrosion (MIC) has traditionally focused on pure cultures. Here, we aim to create a model mixed community composed of six unique genera aimed to represent key metabolic niches found in natural MIC-associated events. This work will help standardize laboratory research in a way to improve MIC research to more closely simulate in field conditions.

Methods: Six genera were grown in pure culture and combined in pairwise fashion. The community was composed of the biofilm former Pseudomonas putida, the iron-reducer Geoalkalibacter subterraneus, the sulfate-reducer Desulfovibrio vulgaris, the denitrifier Thauera aromatica, the acid-producer Acetobacterium woodii, and the fermentative Bacillus subtilis. Two approaches were used to create the model community, grown in anaerobic conditions. The first was using an artificial produced water media supplemented with hydrocarbons and a carbon steel coupon to promote MIC-associated growth. The successful pairwise combinations were further combined to create an additive community. The second was combining the six pure cultures in various combinations on polystyrene pegs inserted in a 96-well plate. In both cases, the presence of each member will be validated using terminal-restriction fragment length polymorphism (T-RFLP).

Observations: This work shows that laboratory work can successfully work towards a standardized approach of creating a model mixed MIC community. This work will serve
as a foundation for future MIC work and provide a more complete understanding of how microbial interactions in a biofilm influence corrosion beyond single culture studies.

*159 “Limiting nitrite reduction by Tepidiphilus sp. strain CC001 can control souring in high temperature reservoirs”

Authors
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Souring, the increase of sulfide concentration in production fluid by sulfate reducing bacteria (SRB), is deleterious to production facilities and human resources. To control souring, various biocides and/or metabolic inhibitors like nitrate or nitrite are applied in the field. The objective of this work was to evaluate nitrite accumulation by Tepidiphilus sp. strain CC001 to control reservoir souring. The nitrite accumulation leads to souring control by inhibiting bisulfite reductase enzyme of SRB. This phenomena was evident at high temperature. Enrichment of SRB were used for souring establishment in serum bottles at high temperature (at 550°C) and low temperature (370°C). Tepidiphilus sp. was injected in these serum bottles when half sulfate was reduced and further sulfate and nitrate reduction was observed. It was detected that sulfate reduction was stopped after Tepidiphilus sp. injection and nitrite accumulation was observed in serum bottles at high temperature. Results of present study showed that Tepidiphilus sp. strain CC001 reduces nitrate in N₂ at ambient temperature, while at high temperature (550°C) nitrate was reduced in nitrite and no further reduction was observed. The mechanism for this incomplete reduction of nitrate was confirmed by studying the nitrite reductase gene expression at high temperature. This study shows that incomplete nitrate reduction leads to inhibition of SRB growth and souring control at high temperature.

*101 “Biocorrosion behavior of API 5L X65 exposed to seawater under different flow conditions”.

Authors
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2. Cristiana Cravo-Laureau - Université de Pau
3. Marcia Lutterbach - National Institute of Technology
4. Eliana Flávia Camporese Sérulo - Federal University of Rio de Janeiro

The effect of fluid flow on the microbial corrosion behavior of API 5L X65 pipeline steel is investigated in product oil pipelines. It is confirmed that there is a small amount of trapped water accumulated at low elevation sections along the pipelines. The trapped water and dissolved oxygen in pipelines promote the growth of both aerobic and anaerobic bacteria, causing microbiologically influenced corrosion (MIC). MIC has been detected not only in static fluid systems, but also in flow systems. In product oil pipelines, MIC is conventionally attributed to three species of bacteria namely sulfate-reducing bacteria (SRB), iron oxidizing bacteria (IOB) and aerobic heterotrophic bacteria (AHB) which are influenced by the fluid flow condition. The aim of this study was investigating the MIC behavior of an API 5L X65 microalloyed steel exposed to
seawater. Comparatively, the steel biocorrosion was tested under laminar and turbulent flows during 15 days. Exogenous microorganisms were not added to seawater. By applying a laminar flow, higher abundance of sessile bacteria was detected than in the turbulent flow, especially SRB, which reached 107 MPN/cm² in 7 days of testing. In turbulent condition, SRB reached just 104 MPN/cm² at the same time. In addition, IOB was not detected on the steel surface under laminar flow, but it was in 105 MPN/cm² on steel under turbulent flow. Dissolved oxygen reduction in laminar condition may have created an inhospitable environment to aerobic IOB and favored anaerobic SRB. However, on the 15-day, sessile SRB abundance was similar to both flow conditions, even in oxygen-rich fluid. Metagenomic analysis suggests that there was variation in the bacteria community structure formed under laminar and turbulent flows, particularly in the early days of biofilm formation.

*200 “Zeolite-supported silver nanoparticles as biocidal additive for elastomeric coating formulations”.

Authors
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3. Vilma Rosato- Lemit, La Plata
4. Mario Moreno- Centro Atómico Bariloche
5. Marcia Soares Lutterbach- Centro Atómico Bariloche
6. Elena Basaldella-CINDECA, CCT- La Plata-CONICET

The presence of microorganisms on exposed surfaces leads to the spread of microbiological contamination. At present, the use of antimicrobial coatings is a methodology employed to provide an effective control of microbiological growth. Microbial adhesion begins when the microorganisms adhere to a surface embedded in a gelatinous matrix called biofilm, which promotes a significant modification of the interface and generally increases the substrate deterioration. The development of coating systems with antimicrobial properties based on film-forming materials suitable for particular protection of diverse substrates is nowadays an industrial requirement. For example, the outcome of reliable methods for the prevention and control of microbiologically influenced corrosion is one of the main achievements still pending for research and applied engineering. This work introduces an entirely new approach through the use of zeolite/nanoparticle composites as biocidal additive for coating formulations. The zeolite network would stabilize the delivery of active silver nanoparticles at a nanometric level facilitating the optimization of their dosage. Silver nanoparticles dispersed onto micrometer-sized crystals of A-type zeolite were prepared by reduction methods applied to a Ag-exchanged zeolite. TEM, XRD and UV-Vis analyses confirmed the presence of rounded crystalline silver nanoparticles, homogeneously dispersed on the outer surface of the zeolite crystals: Ag⁰ and Ag₂O nanoparticles coexisted with noncrystalline clusters and silver cations hosted inside the porous structure. The microbiological activity of elastomeric coatings formulated with the synthesized zeolite/silver nanoparticles, measured in terms of inhibition halo development, clearly indicated that the inhibition of Trichoderma sp. growth was similar to that obtained by adding a commercial biocide based on isothiazolinones. The chemical nature, size and size distribution of the nanoparticles were the main...
factors affecting the biocidal action of the prepared coatings. On this basis, the coatings formulated with the silver composite could be considered suitable to prevent microorganism growth and its consequential production of metabolic substances responsible for substrate deterioration. Also, the supported silver nanoparticles could be considered for the substitution of many of the organic biocides, which according to environmental regulations should be replaced by low-toxicity and environmentally friendly alternatives.

*205 “Zero-resistance ammetry as a low power approach to monitor microbially influenced corrosion”.

Authors
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Prasanna Chinthala - The University of Akron, Akron.
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John Senko - The University of Akron, Akron.

We are developing an electrochemical approach that uses zero-resistance ammetry (ZRA) measurements along a pipeline detect microbially influenced corrosion (MIC). To develop ZRA as an MIC monitoring tool, we have initiated split chamber ZRA (SC-ZRA) incubations that entail deployment of two steel working electrodes (WE1 and WE2) in chambers separated by a salt bridge. Subsequently, one chamber can be inoculated with an individual or consortia of microorganisms, and ZRA and potential measurements can be made in conjunction with evaluations of microbiological activities. Current direction and magnitude can be indicative of the mechanisms and extents of MIC. The SC-ZRA setup mimics the heterogenous biofilm coverage of metal surfaces that leads to MIC, and might be used to establish the electrochemical signatures of MIC. We have obtained samples from several natural gas transmission pipelines and are conducting preliminary physicochemical and microbiological characterizations of the slurries. In oxic SC-ZRA incubations, a negative current was observed between WE1 in non-sterile pigging slurry and WE2 in biologically deactivated slurry. This indicated that electron transfer occurred with WE2 serving as the anode and WE1 serving as the cathode, which was consistent with greater mass loss of WE2 than WE1. Additionally, the mass loss from WE2 was consistent with the magnitude of current between the two electrodes. Additional SC-ZRA incubations are ongoing in an effort to identify electrochemical signatures of microbiological processes.

Session 03: Hydrocarbon Biodegradation

*94 “Microbial hydrocarbon degradation potentials in shoreline and deep-sea sediments around Svalbard and possible ways for their stimulation”.

Authors
1. Sven Bartholomaeus - BGR
2. Nontje Straaten - BGR
3. Martin Krueger – BGR
The diversity and metabolic potentials of the microbial communities inhabiting sediments in the Northern Barents Sea are largely unknown. Recent reports on natural methane seeps as well as the increase in hydrocarbon exploration activities in the Arctic initiated our studies on the potential of indigenous microbial communities to degrade methane and higher hydrocarbons under in situ pressure and temperature conditions. Furthermore, the subseafloor geochemistry in these areas was studied, together with important microbial groups, like methanotrophs, methanogens, metal and sulfate reducers, which may drive seafloor ecosystems in the Northern Barents Sea. A recent focus is on the evaluation of different approaches to stimulate oil degradation by the indigenous microbial populations. The potential of the indigenous microorganisms to degrade methane and higher hydrocarbons as well as different oils under in situ temperatures and pressures was widespread in surface sediments. Degradation rates were higher under aerobic than under anaerobic conditions, and decreased with increasing sediment as well as water depths, i.e. increasing pressures. The addition of different nutrient solutions increased microbial biomass and oil degradation rates. The degradation of the added oils was verified by organic geochemical analyses. Similar activity distribution patterns were found for other metabolic processes, including sulfate, Fe and Mn reduction as well as carbon dioxide and methane production rates. Ongoing molecular biological analyses of original sediments and enrichment cultures indicate the presence of diverse and varying microbial communities.

*173 “Monitoring the Response of Microbial Communities to a Simulated Surface Oil Spill in a Coastal Ecosystem”.

Authors
1. Susan Cobanli - Fisheries and Oceans Canada
2. Camilla Ryther - Dalhousie University
3. Taylor Watts - Dalhousie University
4. Alison Gladwell - Dalhousie University
5. Jessica Mac Donald - Fisheries and Oceans Canada
6. Gary Wohlgeschaffen - Fisheries and Oceans Canada
7. Brian Robinson - Fisheries and Oceans Canada
8. Thomas King - Fisheries and Oceans Canada
9. Alice Ortmann - Fisheries and Oceans Canada

The fate of spilled oil in a coastal ecosystem depends on factors including the type of oil product and seasonal conditions. These factors can influence the bioavailability of oil to lower trophic level communities and how the oil and communities interact can change physicochemical characteristics of the oil. Mesocosm experiments were conducted by spilling unweathered oil products (3 diluted bitumens and diesel) on the surface of enclosures containing 215 L of coastal seawater. Experiments were replicated in the spring, summer and autumn of 2017 with biological and chemical samples collected over 14 days. Microplankton community changes were monitored using a dynamic imaging particle analyser (FlowCam®). Pico- and nanoplankton community abundance and diversity were measured using flow cytometry. Hydrocarbons were monitored using GC-FID to measure total hydrocarbons (TPH) and purge & trap to measure volatile hydrocarbons (BTEX). Seasonal differences in the amount and peak timing for TPH and BTEX were observed, with differences among oil types. Microbial communities varied at t=0 for each season and remained
different throughout the experiment. Abundances of procaryotes and microplankton did not differ from control treatments in the diluted bitumen treatments. The diesel treatment in summer saw a significant increase in microplankton, mainly short chain phytoplankton (> 70%) and short pennate diatoms (>15%) with a peak at day 6. Prokaryote populations in the diesel mesocosms increased significantly in all 3 seasons until 4 days post spill, then decreased, with the decrease most significant in the summer. Stimulation of distinct microplankton and microbial communities may have implications for the food web and on the eventual fate of spilled oil. Understanding the interactions of microbe and microplankton populations with different spilled oil products can provide information on how higher trophic levels of a community may respond and information for evaluation of oil spill countermeasures.

*63 “Transcriptomic Analysis Reveals Conserved Bacterial and Fungal Adaptive Mechanisms Essential for Survival and Proliferation in Hydrocarbon-Containing Environments”.

Authors
1. Oscar Ruiz - Air Force Research Laboratory
2. Thusitha Gunasekera - University of Dayton Research Institute
3. Osman Radwan - University of Dayton

Here we present an overview of a large-scale transcriptomics study performed on Gram-negative bacteria, Gram-positive bacteria, yeast and filamentous fungi under fuel conditions to elucidate genes and pathways important in microbial adaptation to hydrocarbon fuels. Many genes involved in the uptake and extrusion of hydrocarbons including membrane protein channels and efflux pumps, iron acquisition, hydrocarbon degradation via monooxygenases and hydroxylases, quorum sensing, and biosurfactant production to access hydrocarbons, were differentially expressed and regulated in a similar fashion in the different microbial groups. These results indicate that fuel microbial adaptation may take place via conserved global mechanisms that provide the capacity to the cell of surviving the toxic fuel. Evidence supports that resistance to fuel and hydrocarbon utilization are decoupled processes in terms of the signal pathways that regulate them. The global mechanisms identified through this study may become important targets in the development of microbial detection system and biocontrol technologies to prevent and mitigate fuel system biodeterioration.

*114 “Anaerobic degradation of polycyclic aromatic hydrocarbons (PAHs) by the sulfate-reducing bacterial enrichment culture TRIP”

Authors
1. Ivana Kraiselburd - University of Duisburg-Essen
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4. Markus Kaiser - University of Duisburg-Essen
5. Rainer Meckenstock - University of Duisburg-Essen

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed pollutants producing hazardous effects on human health. In PAH-contaminated sites, oxygen is rapidly
depleted. Thus, microorganisms able to use these compounds as a carbon source in the absence of molecular oxygen are crucial for their consumption. This work aims at elucidating the mechanisms for the anaerobic degradation of PAHs by a sulfate-reducing enrichment culture (TRIP) obtained from a natural asphalt lake. The TRIP culture was grown anaerobically in freshwater mineral medium with different PAHs as carbon and electron sources and sulfate as electron acceptor. Bacterial growth was evaluated by cell counting (DAPI-staining) and sulfate consumption (Ion Chromatography). Bacterial composition was analyzed by 16S rRNA gene amplicon sequencing. The metaproteome of the TRIP culture was obtained in the different growth conditions by liquid chromatography-tandem mass spectroscopy. The TRIP culture was able to anaerobically degrade the PAHs phenanthrene, acenaphthylene, 2-phenanthroic acid and 2-naphtoic acid. Shifts of the bacterial community were observed upon growth with each substrate, indicating that different members of the enrichment culture are involved in the degradation of these compounds. Proteomic analysis in comparison to previously reconstructed genomes from the TRIP culture, revealed the dominant enzymes involved in the degradation process, as well as the organisms encoding such proteins. While degradation of phenanthrene involves a carboxylation step, followed by CoA ligation and a stepwise reduction, by enzymes encoded by a candidate Desulfatiglans bacterium; degradation of acenaphtylene appears to be performed by another bacterium of the same genus, and to involve a different mechanism.

*152 “Unravelling the metabolism of Atribacteria”.

Authors
1. Sabrina Beckmann - University of Delaware
2. Glenn Christman - University of Delaware
3. Megan Ruhmel - Exxonmobil Research and Engineering
4. Dave Valentine - University of California Santa Barbara
5. Zarath Summers - Exxonmobil Research and Engineering
6. Jennifer Biddle - University of Delaware

Natural oil and gas seeps introduce hydrocarbons into the marine environment creating hot spots saturated in hydrocarbons. Bacteria belonging to the newly proposed candidate phylum Atribacteria, formerly referred to as OP9 and JS1 lineages, appeared to be highly abundant in these anoxic methane-rich sediments. Atribacteria have been observed through 16S rRNA sequencing surveys in a number of anoxic reservoirs as mud volcanos, tidal flats, geothermal environments and, in particular, from deep sea sediments saturated with hydrocarbons, e.g. oil and gas seeps3,4. Recent studies of non-marine Atribacteria suggested a heterotrophic lifestyle of these bacteria providing substrates as acetate, ethanol and CO2 to methanogenic archaea1. Considering the co-occurrence and high abundance of Atribacteria in methane-rich reservoirs in the deep sea, we suggest that Atribacteria species might have the potential to be involved in the cycling of methane3. In this study we aim to investigate the diversity and metabolic potential of Atribacteria representatives combining metagenomics with physiological approaches to elucidate the genetic blueprint. We examined the sequence abundance of this newly discovered phylum from two shallow oil seeps located offshore in Coal Oil Point. Sediment samples were obtained by push coring up to 20 cmbsf and DNA was extracted. Subsequent Illumina sequencing of the 16S rRNA gene revealed a large relative
abundance of sequence reads classified to Atribacteria with up to 17% of the overall microbial community at both locations. Preliminary metagenomics analyses showed the presence of atribacterial metagenome bins with a completeness greater than 80%. Interestingly, no methanogenic archaea nor known AMNE consortia were observed in these samples, contesting also the role of Atribacteria fueling methanogenic archaea in these methane rich reservoirs.

*90 “Assessing calcium naphthenate soap risk in an offshore installation”.

Authors
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2. Max Frenzel - Oil Plus Ltd

‘ARN’ acids are large (C80+) molecules that are thought to arise from the membrane lipids of thermophilic archaeal taxa, in particular, glycerol monoalkyl glycerol tetraethers. When ARN acids interact with divalent cations, particularly calcium, calcium naphthenate (CaN) soap scale can result. If not properly identified and managed CaN will reduce the efficiency of a number of critical oilfield operational activities, including: oil dehydration; desalting; produced and waste water treatment and disposal; oil storage and export. In extreme cases a complete shutdown of production can occur. An offshore installation was experiencing deposition of an unknown organic deposit within the heater treaters and crude oil transfer pumps. We analysed a sample of the deposit, as well as crude oil samples from the production risers, and first and second stage separators. Inductively coupled plasma mass spectrometry (ICP-MS), Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDS), Fourier-Transform Infrared Spectroscopy (FT-IR), and high temperature-gas chromatography (HT-GC) analyses were utilized for analysis. The deposit contained residual crude oil and wax (23%), asphaltenes (4%), and a solvent-insoluble (65%) fraction with calcium naphthenate (CaN) deposits and inorganic scales. Of the solvent-insoluble fraction, there were crude ARN acids; (44%), scale (20%) and the insoluble (1%) remainders. The presence of ARN acid was confirmed by HT-GC analysis for total C80-82:0-8 tetraacids. The eight oil samples analysed ranged in ARN acid levels from 0.5 to 10.1 ppm. In addition, di and tri-acid C80+ molecules were identified in the crude, thought to arise from biodegradation of glycerol monoalkyl glycerol tetraethers precursors. These have been identified as ‘pre-ARN’ molecules that may contribute to CaN formation. The levels of ARN and ‘pre-ARN’ identified in the crude and their positive identification within the organic deposit indicate the facility is at high risk of CaN formation.

*137 “Enhancement of methanogenic crude oil degradation using organic nutrients toward biotechnology applications in oil reservoirs”.

Authors
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2. Konomi Suda - aist
3. Hideyuki Tamaki - aist
4. Haruo Maeda - aist
5. Yoichi Kamagata - aist
6. Susumu Sakata - aist
8. Masayuki Ikarashi - INPEX Corporation
Methanogenic crude oil degradation is currently attracting much attention in biotechnology, i.e., microbial enhanced energy recovery (MEER) technology, converting the humanly inaccessible crude oil into recoverable biogas. In previous study, we reported on a unique methanogenic microbial community degrading toluene in crude oil recovered from a high-temperature oil reservoir. However, commercially practicable MEER technology using this microbial community is still challenging because toluene is a small component in crude oil hydrocarbons, and methane produced from only toluene was insufficient for the field application. Therefore, we focused on biostimulation for the microbial community aiming to degrade n-alkanes, major components in crude oil hydrocarbons. We conducted incubation experiments of the oil reservoir production water amended with inorganic (nitrogen and phosphorus sources, trace metals and vitamins) or organic (four types of commercially available yeast extracts) nutrients under the in situ oil reservoir condition, i.e., 55°C and 5 MPa. Methane production was drastically accelerated up to three to five times in incubations amended with two types of yeast extracts (1 to 1.6 mmol/ml-oil) compared with those amended with inorganic nutrients (0.33 mmol/ml-oil). This result was supported by hydrocarbon composition analysis showing that cultures amended with their yeast extracts degraded high-molecular weight n-alkane (C20 to C32) as well as toluene. Microbial community compositions in cultures amended with inorganic nutrients were dominated by uncultured Ca. Atribacteria (class JS1) which has been known to encode a bss gene, the initial activating enzyme for toluene, through our metagenomic analysis. Meanwhile, uncultured Ca. Acetothermia (class OPB14) and class JS1 dominated in cultures amended with yeast extracts, suggesting that these uncultured bacteria may play an important role in n-alkane degradation. This study demonstrates that the biostimulation strategy using our organic nutrients is effective for enhancement of methane production from crude oil, promoting the field application of commercially feasible MEER technology.

*170 “Tracking the behaviour and impacts of weathered fuel oil with artificial beaches”

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4. Taylor Watts - Dalhousie University
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8. Charles Greer - National Research Council
9. Thomas King - Fisheries and Oceans Canada

Beached oil often differs from freshly released oil in composition due to extensive weathering on water, and affecting its ultimate impact. To characterize the importance of season and sediment type on fate and effects, three micro-beach mesocosm experiments were carried out. An intermediate fuel oil (IFO40) was weathered in
seawater for three days on an orbital shaker and added to replicate outdoor artificial beaches to measure leaching hydrocarbons due to tidal flux and to monitor changes in the microbial communities. Water was drained and replaced daily (July) or every 2-3 days (February) for 5 to 7 days and analyzed to determine total petroleum hydrocarbon (TPH) concentrations, impacts on microbial activity (Microtox) and changes in the abundance and diversity of microbes. Season played an important role in weathering and movement of oil through the beaches. In February, weathering resulted in a thick mousse that slowly penetrated the silty beach, with a gradual increase in TPH over 7 days to 1 ppm while TPH increased rapidly in July to 3 ppm. TPH data indicates that sediment from one experiment had elevated hydrocarbon prior to the collection, although the source is unknown. Inhibition of microbial activity followed TPH, increasing slowly to 20% in February, but quickly increasing to an average of 60% in July. Prokaryote abundances did not increase in February but increased 10-fold in July. Although abundances did not increase in February, community composition changed over time, with no difference seen between treatments. Different community patterns were observed in July, when warmer temperatures and pre-contaminated sediment drove community changes. These preliminary experiments show how beached oil can affect local waters due to tidal flushing.

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

*154 “Clostridia in hot oil reservoirs of Texas”.

Authors
1. Glenn Christman - University of Delaware
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The importance of Clostridia to microbial community processes in oil reservoirs has mostly been ignored, but here we present data on an abundant, spore forming Clostridia organism that was found in producing wells from an oil field in Texas, offshore Gulf of Mexico. These reservoirs are hot, with temperatures greater than 100oC, and with salinity values exceeding 28. Nearly complete metagenome assembled genomes (MAGs) were retrieved from metagenomic assemblies of Illumina reads from each of the four sampled reservoirs. Completeness, based on the presence of marker genes, ranged from 99.2% to 100.0%. Based on phylogenetic analyses of both 16S rRNA genes and concatenated ribosomal proteins, this Clostridia is closely related to, but distinct from, Caminicella sporogenes and Clostridium caminithermale (aka Paramaledivibacter caminothermalis). These cultured organisms are at least moderately thermophilic and halophilic and were initially isolated from deep-sea hydrothermal vent systems. Given the thermophilic nature of these relatives, it is unlikely that our MAGs contain a mesophilic pipeline or infrastructure contaminant. Metabolic pathways found in the reservoir MAGs were generally similar to those found in C. sporogenes and C. caminithermale, which are spore forming anaerobic fermenters of carbohydrates and proteinaceous substances. The MAGs did include some differences not found in their cultured relatives, including a complete Wood-Ljundahl carbon fixation pathway. Although spore formation may have initially
helped the reservoir Clostridia resist the high temperatures of the oil system, 35-40% of the community appear to be in the process of replicating. The reservoir MAGs may then represent a distinct adaption of the moderately halophilic and thermophilic Caminicella/Paramaledivibacter group to the high temperatures found in oil reservoir system. But there is no evidence that any of these organisms are themselves directly metabolizing the hydrocarbons found in these reservoirs. Regardless, the worldwide occurrence of clostridial organisms in oil reservoirs should not be ignored.

*177 Distribution of Microbial Communities in Biodiesel and ULSD Storage Tanks

Authors
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2. Heather Nunn - University of Oklahoma
3. Caitlin Bojanowski - Air Force Research Laboratory
4. Carrie Drake - Air Force Research Laboratory
5. Wendy Goodson - Air Force Research Laboratory
6. Bradley Stevenson - University of Oklahoma

Biodiesel is a renewable fuel consisting of straight chain fatty acid methyl esters (FAME) derived from plant and animal oils and fats. Biodiesel is used as an additive (up to 5%) in ultra-low sulfur diesel (ULSD) to restore lubricity, or in higher proportions (e.g. 20% in B20 biodiesel) as a replacement, or extender of ULSD. As part of their ongoing Operational Energy Strategy, the Department of Defense has adopted B20 biodiesel for use in non-combat ground vehicles. In compliance with this plan, many military bases are now using B20 biodiesel and have infrastructure dedicated to its storage and dispensing and as a result are experiencing microbial contamination problems. FAME and petroleum diesel have similar physical and chemical properties; however, biodiesel contains more oxygen, is more hygroscopic, and is more oxidatively unstable which increases its susceptibility to microbial contamination and degradation. We conducted a survey of storage tanks containing B20 or ULSD across several military bases to detect the extent and composition of microbial contamination. Fuel from the bottom of each tank was collected and the microbial communities present were monitored using high throughput sequencing of small subunit ribosomal RNA (SSU rRNA) gene libraries. The level of metabolic activity in each fuel was determined using ATP measurements, and the fuel composition was characterized with GC-MS. Noticeable differences in fuel composition were observed and correlated with changes in the microbial communities. Factors such as FAME composition and geographic distribution appeared to play a role. Notably, many of the ULSD tanks containing 5% FAME or less had significant contamination. This information will guide decisions for fuel infrastructure management in order to reduce the incidents of fouling, degradation and corrosion.

*57 “A Comprehensive Microbial Assessment of Hydraulic Fracturing Stimulations Using Molecular Microbiological Methods: Three Western-Canadian Case Studies”.

Authors
1. Marc Demeter - OSP Microcheck
Experience has shown hydraulic fracturing operations can introduce and/or stimulate microbial populations in the wellbore that in turn may lead to undesired corrosion, souring or other production issues. Biocides are applied to prevent the establishment of problematic microbes downhole; a strategy hinging on a detailed understanding of the quantity, composition and source of the microbial contaminants faced. Once the standard for microbial assessments in the oil and gas industry, culture media bottles are now just one of many available tests including ATP, qPCR and 16S Sequencing. Tests vary by the resolution, and the information they yield. Some will target very specific microbial subgroups of concern, while others evaluate all microbes within the sample. In the case studies presented, water/produced fluids were collected from pertinent frac sample points and were assessed using the four tests listed above.

Sources of microbial contamination, effectiveness of the microbial control program, and insights into the value microbial tests provide to frac operations were made: Culture media-based testing consistently resulted in incoherent and confusing data that failed to correlate with other methods. ATP technology lent itself well to on-site, evidence-based decision making, allowing for evaluation of the control program on-the-fly. DNA-based testing (qPCR and 16S Sequencing) provided the most comprehensive insight into the microbial communities exposed to the well, and those that established post-completions. Overall, holistic microbial testing offers the user key information required to design and implement successful microbial control programs for frac. The acknowledgment of the role microbes play in well completions, and the testing technology to evaluate oilfield microbes is rapidly advancing. Here we present some of the first case studies highlighting the use of molecular, DNA-based technology for assessing hydraulic fracturing operations, demonstrating the fallacy of culture media based testing which is the current industry standard.

*51 “Development and Initial Validation of an In-Field qPCR Testing Platform for the Rapid On-Site Detection and Characterization of Microorganisms in Fuel”.

Authors
2. Wei Huang - LuminUltra Technologies, Inc.
3. Oscar Ruiz - Air Force Research Laboratory

Fuel-system biodeterioration is a significant issue that affects both commercial and military systems containing conventional and alternative fuels. The increasing use of more environmentally friendly fuels, such as biodiesel and ethanol, has further exacerbated this issue in recent years. Biodiesel blends have a higher propensity for biocontamination due to the increased content of fatty acid methyl esters, which increase fuel moisture content and act as an exceptional microbial food source. Early detection of damage-causing microorganisms and subsequent treatment can help to minimize costly and potentially detrimental biodeterioration-related problems such as reduced fuel stability and quality, tank and coating degradation, deactivation of fuel-water separators, and injector and filter fouling.

Recent work has demonstrated that quantitative polymerase chain reaction (qPCR) can be employed in fuel microbiology to quickly detect and quantitate biodegradation-related microorganisms. Furthermore, qPCR has been shown to overcome many of
the important drawbacks of existing culture-dependent methods including the proclivity to underestimate viable microbial population densities and long wait times for results. Despite these advances, employing qPCR in fuel microbiology still requires transporting a sample to a well-equipped laboratory for analysis, which has prevented its universal use. To overcome these limitations and to provide a more comprehensive solution, we will report on the development and initial validation of a new mobile, field-capable qPCR analysis platform allowing on-site fuel microbiology results to be generated in less than three hours from sample collection to data analysis. This new platform includes a novel protocol for rapidly extracting microbial DNA from fuel and water samples and a new portable device for qPCR analysis. In-field results were successfully validated against laboratory generated results. Field-based qPCR has the potential to significantly reduce testing time, costs, and provide industry with a powerful new on-site fuel microbiology analysis technology.

*120 “Assessment of the source and fate of intact polar lipids in hydrothermally altered sediments”.

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Hydrocarbon generation has widely been studied in conventional petroleum forming basins. The kinetics of these processes can be difficult to fully constrain due to the excessive depths and extended lengths of time required for converting sedimentary organic matter into petroleum forming hydrocarbons. This study evaluates whether these limitations are overcome within the pyrolytic conditions naturally present at Cathedral Hill, a sedimented hydrothermal vent systems in Guyamas Basin, Gulf of California. Here, in-situ petroleum formation may occur due to high temperature conditions projected to have reached 155ºC within the upper 21 cm of sediments. In a push core transect we have identified and quantified bacterial and archaeal intact polar lipids (IPLs) and core lipids (CLs), which are cell membrane constituents. This study is tracking the distribution of IPLs and CLs to: (1) assess the microbial community that inhabits these sediments at chemotaxonomic level, (2) determine the thermochemical stability of these lipids, and (3) determine degradation pathways that may result from their pyrolytic conversion into hydrocarbons. Currently, we have assessed archaeal IPLs including 1G-GDGTs, likely sourced from hyperthermophilic archaea, and 2G-GDGTs, possibly derived from methanotrophs. Preliminary data shows an upper limit of ~145°C for the persistence of 1G-GDGTs, and ~50°C for 2G-GDGTs. The abundance of core GDGTs also decreases with sediment depth, but little to no biphytanes, which are break-up products of GDGTs, have been found. This indicates that at elevated vent temperatures more time is needed to crack these lipids into hydrocarbons. Evaluation of additional IPLs and CLs will allow us to better constrain the reaction pathways to form hydrocarbons from lipids.

*81 “Long-term preservation of water based drilling muds”.
Drilling muds are essential components for drilling almost all oil, gas and injector wells. Uncontrolled in the drilling mud, degradation of mud thinners will negatively impact control of fluid rheology and loss as clays used to build viscosity will flocculate with pH drops and thinner consumption. Use of biocides to control bacterial in drilling traditionally favors quick kill biocides, added continuously or with frequent batch additions to maintain the QA/QC specification of the mud. However, use of preservative biocides, such as Kemira AMA®-324 (dazomet) can provide an alternative long-term approach to traditional quick kill biocides. In this investigation, components of a commercial, water based mud were evaluated as well as efficacy of a long-term preservative, AMA-324 biocide, over 29 days in this made-down mud. Drilling mud aliquots were treated with 250 to 1500 ppm of preservative biocide along with an untreated control sample and were incubated for 29 days at a constant temperature of 32°C while gently rotating. Periodic media enumerations of AMA-324 aliquots showed effective control of aerobic bacteria vs the control. After 15 days, a 1% challenge of spoilage organisms were introduced to the system. Mud filtrate was collected from API fluid loss apparatus to run aerobic bacteria plating tests to get the bacteria counts. Data confirms AMA-324 to be a slower acting biocide taking more time than traditional biocides to begin acting. After 8 days incubation the bacteria have been reduced considerably versus the control. At 1500 PPM dosage, control is maintained past 29 days even with the 1% bacterial challenge of 106 aerobic bacteria at 15 days. Susceptibility of water based muds to bacterial action limits their useable lifespan. Use of a long-term preservative such as AMA-324 means that water based mud can be reused more often and effectively stored as oil based muds are.

*147 “Comparative study of a novel thin-film culture device, serial dilution bottles, and 16S metagenomics for enumeration of sulfate reducing bacteria in field samples”.

Identification and enumeration of microbes associated with microbiologically influenced corrosion (MIC) in oil field operations is essential, as it informs an effective mitigation strategy. The past two decades have seen a revolution in the speed, accessibility, and price of molecular microbiology methods (MMMs), specifically quantitative PCR and 16S metagenomics. While MMMs are becoming more ubiquitous in the O&G industry, the primary means of day-to-day enumeration of microbes traditionally associated with corrosion, such as sulfate reducing bacteria (SRB), remains culture based. Low cost and relative ease of use are the primary drivers for the predominant culture-based method, serial dilution bottles, despite widely accepted issues with false negatives and underreported results. Recently, a new thin-film culture device for the detection and enumeration of SRBs in surface, flow-back, cooling, and produced waters has become available. Here we attempt to enumerate SRBs in field water samples using this new technology, as well as serial dilution bottles, and compare the results to those obtained using quantitative PCR and 16S metagenomics. Using MMMs as the comparator, our results indicate that the thin-film
culture device technology accurately enumerated SRBs from complex communities of SRB and non-SRB microorganisms. Additionally, this technology showed fewer false negatives than serial dilution bottles and returned results days to weeks faster than serial dilution or 16S metagenomics. Ultimately, the use of periodic MMM testing with more frequent use of the thin-film culture device will aid the O&G industry in constructing a more complete picture of microbial issues as they occur.

*79 “How homogeneous are microbial communities distributed in subsurface sediment layers?”

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Microbial consortia from subsurface habitats play a key role in all biogeochemical cycles. In coal subsurface deposits, hydrocarbon-degrading bacteria as well as methanogenic archaea are converting coal organic compounds into natural gas known as coalbed methane. Over the last decades, an increasing demand and utilization of natural gas has been observed all around the world but biogenic coal conversion to methane is limited by the low nutrient availability and low water content in the subsurface coal deposits. In addition, little is known about the spatial distribution of indigenous microbial communities and how it is related to depth and geochemical parameters. Hence, we wanted to understand the microbial ecology of coal seams. Here we determined whether microbial consortia are heterogeneously or homogeneously spatially distributed in a subsurface lignite coal seam. Coal cuttings were collected along vertical (3 m) and horizontal distances (3,8 km). We successfully isolated DNA for sequencing of bacterial and archaeal 16S ribosomal RNA genes. Microbial biogeographic patterns and the effects of geochemical parameters are analyzed with multivariate statistics.

*112 “Thermophilic endospores associated with petroleum and geofluid seepage in Gulf of Mexico marine sediments”

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Dormant endospores of thermophilic bacteria (thermospores) can be routinely detected in permanently cold marine sediments. These hardy and low-abundance endospores, belonging to the phylum Firmicutes, go undetected in typical nucleic acid based community surveys, but proliferate when sediments are experimentally heated. The occurrence of thermospores in the seabed may be explained by upward migration from deep petroleum reservoirs along with naturally seeping hydrocarbons and other geofluids. Thermospores were compared in near-surface sediments from three locations associated with natural hydrocarbon seepage (i.e. “seep”), as well as three locations without hydrocarbon seepage (i.e. “non-seep”), all from the Gulf of Mexico.
From each location, triplicate sediment slurries were amended with organic substrates and 20 mM sulfate, pasteurized at 80°C for 1 hour, and incubated at 35°C, 50°C or 65°C for 14 days. At regular intervals microbial activity was assessed by monitoring sulfate depletion, consumption of organic acids and total cell counts using fluorescence microscopy, and microbial community composition was determined by 16S rRNA gene amplicon sequencing. At higher temperatures, sulfate reduction and fermentation occurred to a greater extent in seep sediments relative to non-seep sediments. At 65°C, microbial activity was prominent only in the seep locations. 16S rRNA gene amplicon libraries revealed elevated relative abundance of sequence reads affiliated with Firmicutes lineages in all incubations where microbial activity was observed. At 35°C, Clostridia and Bacilli were detected at all locations, with Clostridia representing the larger fraction of the community. On the contrary, certain thermophilic Clostridia and Bacilli were exclusive to seep locations at 65°C. Metagenome sequencing to better understand these organisms may reveal the genetic potential for living in sub-seafloor petroleum reservoirs, which would be consistent with a dispersal history of deep-to-shallow transport out of oil reservoirs via hydrocarbon seeps.

*128 “Advanced Diagnostics of the Microbial Contamination and Field Trial of Glutaraldehyde/THNM in a Water Treatment Plant in Brazil”.

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A Petrobras water treatment plant processes 100,000 m3/day of fluid transported by four pipelines (3 onshore/1 offshore). Approximately 90% of the treated water is disposed throughout a submarine outfall, while the remaining 10% receives final physicochemical treatments in a water injection station (WIS) to be used for secondary recovery in an offshore field (26 km distant). Samples were collected from each stream/equipment along the water flow and analyzed by serial dilution enumeration (SRB/APB/TAnB), ATP and molecular biology techniques (qPCR/NGS). A glutaraldehyde/THNM (tris(hydroxymethyl)nitromethane) combination was selected from a laboratory assay comparing 12 different biocide systems to be tested in a 3-month field trial. For the trial, the co-dosed combination replaced the incumbent treatment (THPS, 300 ppm/2 h batch-dosed biweekly & ammonium quaternary, 50 ppm, continuously-dosed). Samples collected weekly on the WIS inlet/outlet (72 h after dosage) were analyzed by serial dilution and ATP. NGS and qPCR were performed for selected samples. Key findings were: Before trial: Production fluids contained 10^2-10^5 cfu/mL for SRB/APB counts and 10^3-10^6 total cells/mL. The community in one pipeline was composed of 73% SRP, identified as Deltaproteobacteria, which differed significantly from other streams (<9% SRP) where Gammaproteobacteria were dominant, followed by Epsilonproteobacteria. Some 70% of the population at the WIS was composed of genera Thiofaba/Sulfuricurvum which contains many members described as Sulfur Oxidizing Bacteria (SOB). Archaea represented 1-3% of population. After trial: Counts were reduced at the WIS outlet from 10^4 to 10^1 cfu/mL while ATP decreased from 650 to 50 pg/mL. NGS showed that Gammaproteobacteria expanded its dominance on the production side (>75%) while Betaproteobacteria...
(Thauera) increased at the WIS outlet (28%) and in the injection water (46%). Results demonstrated that glutaraldehyde/THNM is very effective in providing long-lasting microbial control across a diverse set of microbes in topside, including water that is transported within long pipelines.

*201 “In situ gas production: geomechanical implications and oil displacement”.

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Denitrification is one of the biogeochemical processes that contribute to dissolved, and potentially free phase, gas production in subsurface (oil reservoirs). It is a microbially facilitated process performed by facultative anaerobic bacteria capable of reducing nitrate (NO₃⁻) to nitrite (NO₂⁻) to di-nitrogen (N₂) via other gas species (NO, N₂O). Free phase gas production (bubble formation) in the subsurface occurs when the total dissolved gas pressure (P₇D₉G) generated by increasing dissolved gas concentrations exceeds the bubbling pressure (P₈UB). The P₇D₉G, which is the sum of partial gas pressures (∑P₉) of dissolved gases, can be measured in situ by a simple adaptation to pressure transducers. Nitrate-reducing bacteria (NRB) isolated from a produced water sample from a nitrate injected crude oil reservoir were used to study N₂ production in microcosms. When minimal medium containing 100 mM nitrate and 100 mM acetate was incubated with NRB, 49.8 ± 0.2 mM of N₂ (i.e. >99% of the initial NO₃-N added) were produced. The partitioning of the N₂ between the aqueous and free gas phases in the microcosms in equilibrium with local atmospheric pressure (~0.88 atm) resulted in 82 ± 2.8 mL of N₂ in the free gas phase (i.e. microcosm headspace). Subsequent incubations in six sand-packed columns (including two control columns) were conducted at a temperature of 25°C for 21 days, with continuous P₇D₉G monitoring. The columns were injected with one pore volume (PV=30 mL) of minimal medium, followed by injection of NRB in a subsequent single PV of minimal medium containing 100 mM nitrate and 100 mM acetate. After 21 days of incubation, the P₇D₉G in columns increased from 0.88 atm to 2.2 atm. This P₇D₉G increase resulted in free phase gas exsolution (which was visually observed), which displaced between 2 and 5 mL of fluids from the columns with NRB. The optimization of increased P₇D₉G to the point of free phase gas production on displacement of oils with API gravities of 15° to 42° and its impact on the integrity of reservoir matrix is being investigated.

*202 “Genomic insights into microbial processes in Permian Basin produced waters”.

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With the growing importance of hydrocarbon extraction technologies, the role of microorganisms associated with oil and gas wells and infrastructure has gained significant industrial and engineering interest. Microbial activity in production waters from unconventional oil and gas operations can lead to the degradation of hydrocarbons and cause damage to the well, equipment and surface infrastructure via corrosion, sulfide release, and fouling. Using genome based, culture independent methods, several studies have explored microbial life around hydraulic fracturing operations in some of the most important oil and gas regions across the United States, highlighting the risk of deleterious microbial processes. However, no such data currently exists for the Permian Basin, one of the most important hydrocarbon producing areas in North America. Here, we use shotgun metagenomic sequencing to investigate the microbial taxa and microbial processes that may contribute to corrosion, fouling, and souring events in Permian Basin oilfield waters from West Texas. Using metagenomic binning we recovered ten metagenome assembles genomes (MAGs), representing halophilic, thermophilic, sulfate reducing, fermentative, and methanogenic Bacteria and Archaea, such as *Orenia*, *Desulfohalobium*, *Geoglobus*, *Methanohalophilus* and *Methanothermococcus*. Reconstruction of metabolic pathways allowed us to demonstrate that both classical sulfate reduction and thiosulfate reduction may contribute to gas souring in this region. Similarly, the recovery of multiple methanogenic Archaea suggests a high potential for biological methane production. Annotation of *Geoglobus* and *Marinobacter* draft genomes revealed the microbial capabilities for iron reduction, autotrophic carbon oxidation, and anaerobic degradation of aromatic compounds. Results from this study show produced waters from oil and gas extraction to represent unique microbial ecosystems, hosting rare microbial taxa and processes. In addition, these findings also expand the current understanding of the microbial ecology in hydrocarbon environments and may aid in the development of more specific and efficient biocide treatments, with the goal to improve operational efficacy and safety.

*204 “Impacts of geological carbon sequestration on subsurface microbial communities”*

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Geological carbon sequestration (GCS) is the process of capturing CO2 from industrial sources, compressing it into a supercritical fluid (scCO2) and injecting it into geologic repositories such as enhanced oil recovery (EOR) or deep saline aquifers sites for long term storage. Microorganisms are effective geochemical catalysts for processes that can affect efficacy of carbon storage, such as biofouling, biomineralization, and biocorrosion. Comprehensive characterization of the biogeochemistry of these complex systems is essential to enhance carbon storage procedures and industrial usage. Our current study investigated the microbial diversity at field GCS sites. We examined overall shifts in dominate microbial taxa in response to the CO2 in these environments. Our results show dominant taxa within relevant GCS systems and highlight the important biogeochemical characterizations of these systems to target when mitigating deleterious reactions.
Hydrolyzed and non-hydrolyzed polyacrylamide (HPAM and PAM, respectively) are commonly used in upstream oil and gas operations such as for enhanced oil recovery and hydraulic fracturing processes. HPAM and PAM can act as thickening agents, helping to increase viscosity and reduce friction in the reservoir. The constant use of PAM and HPAM can result in residual polymers getting trapped in the formation (e.g., forming filter cakes), ultimately affecting oil production. Chemical oxidizers (breakers) are often used to degrade the residual polymers, but their ongoing use can lead to safety concerns, corrosion of pipelines, and inefficient polymer degradation.

Understanding how microorganisms within oil reservoirs and other polymer-associated environments can utilize HPAM and PAM will help in the development of biotechnologies that can improve oil recovery and remediate the polymers. Microbial communities obtained from oilfield production waters (PW) and wastewater sludge were tested for their ability to utilize HPAM and PAM as sources of C and N at 50°C. PAM and HPAM were partially deaminated by microbial communities enriched from PW and sludge when supplemented as N sources. When HPAM was added as a N source, higher CO2 production was observed in sludge-derived microbial communities compared to PW-derived microbial enrichments. In addition, significant viscosity reduction was observed when HPAM was amended as the N source within the sludge enrichment, while no viscosity reduction was observed when PAM or HPAM was amended as the only C source. Microbial isolates obtained from the PW enrichments showed 83% identity to Pseudoxanthomonas species and 95% similarity to Bacillus haynesii. Isolates obtained from wastewater culture had a 97% similarity to Chelatococcus composti and 94% identity to Bacillus species. This study shows the ability of microbial communities enriched from oilfield PW and wastewater sludge to readily utilize PAM and HPAM as N sources at 50°C, with a metabolic preference for HPAM. Thus, these polymers may serve as nutrient sources for reservoir-associated microbial communities.

*207 “Coupled laboratory and field investigations resolve microbial interactions that underpin persistence in hydraulically fractured shales”.

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Hydraulic fracturing of shale is the industrial process behind the surging natural gas output in the United States. This technology inadvertently creates an engineered microbial ecosystem thousands of meters below Earth’s surface. Here, we show that persisting microbial communities in fractured shales converge to a similar membership and structure, despite differences in operators, shale formations, and input communities/chemistries. Based on the detection of the metabolite glycine betaine across all Appalachian Basin produced fluids sampled to date, we used laboratory reactors to manipulate persisting shale microbial communities that are currently not feasible in field scenarios. Metaproteomic and metabolite findings from the laboratory were then corroborated using regression-based modeling performed on field metagenomic and metabolite data from more than 40 produced fluid samples from five hydraulically fractured shale wells. Collectively, our findings show that *Halanaerobium*, *Geotoga*, and *Methanohalophilus* strain abundances predict a significant fraction of carbon and nitrogen metabolites at the field scale. Our combined laboratory and field results revealed that microorganisms persisting in hydraulically fractured shales must maintain osmotic balance in hypersaline fluids, gain energy in the absence of exogenous electron acceptors, and acquire carbon and nitrogen to synthesize cell building blocks. We provide evidence that that co-fermentation of amino acids and their derivatives, like glycine betaine, meets these organismal needs and thus Stickland fermentations function as a keystone metabolism conserved across hydraulically fractured shale communities. Additionally, laboratory experiments showed that prophage-induced bacterial lysis releases intracellular metabolites that can sustain these fermentations, supporting the persistence of microorganisms in this ecosystem. These research findings likely extend across subsurface ecosystems, both marine and terrestrial, where amino acid fermentation and viral predation are increasingly recognized as important drivers of community structure.

**Session 05: Emerging Innovation and Applications in petroleum microbiology**

*125 “QUOR’em DB: A database to Query, Update, Organize and Research metagenomics data”.

Authors

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With an ever-increasing number of tools available for analyzing microbial community sequence data, the task of organizing bioinformatic pipeline data can be daunting. The result is often an ad-hoc storage solution for intermediate files, but the domain knowledge holders do not always have the training to locate and retrieve information from these files. We have developed the QUOR’em DB, a database package that facilitates the organization, querying, extraction, and visualization of large amounts of microbial community data. Our Django-based utility leverages the data provenance tracking features of QIIME2 artifacts into a simple-upload process that minimizes the risk of input errors. As a deployable web utility, QUOR’em DB streamlines the data-exploration process by allowing geographically distant team members to separately process and explore results, without the need to manually compile and send the results by e-mail or third-party file storage services. The database supports multiple studies and sample replicates, allowing for meta-analytical queries that facilitate computational and wet-lab protocol validation. For example, to identify how primer selection influences alpha diversity, QUOR’em can query all relevant results from samples that have been sequenced with multiple primer sets and aggregate and contrast the results. To ensure that uploaded data are valid for a given project, QUOR’em can interact with an ontology, such as the Microbiologically Influenced Corrosion Ontology (MICON), to enable semantic interoperability and add another layer of quality control for data input. Another advantage of QUOR’em is its open source licensing and extensibility to other projects. QUOR’em DB is being used to store and analyze microbial data being produced by the geno-MIC project (https://bio.ucalgary.ca/microbial-corrosion/research), enabling fast and reliable analyses from a variety of sources and technical expertise.

*126 “Microbial bioindicators for de-risking offshore deep water oil and gas exploration”.

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Deep water oil and gas exploration off the Atlantic Coast of Canada has increased in recent years. This exploration has largely employed conventional geochemical analyses of subsurface marine sediments to identify the presence of a working petroleum system. In the interest of further de-risking exploratory drilling, molecular microbiological methods are being investigated as a complementary tool for hydrocarbon seep detection by uncovering anomalous diversity and distribution patterns in the seafloor. Piston coring was performed in >2500 m water depth off the
coast of Nova Scotia resulting in samples from as deep as 10 metres below seafloor (mbsf). Over 500 archaeal and bacterial 16S rRNA gene amplicon libraries were sequenced to compare surface (0 and 20 cmbsf) and subsurface (>20 cmbsf) communities in 42 geographic locations along the Scotian Slope. In subsurface communities, similar bacterial OTUs (97% identity) were dominant at all 42 locations, featuring the phyla Aminicenantes (up to 47% relative sequence abundance) and Atribacteria (up to 74%). In surface communities, Atribacteria were mostly absent except in locations where migrated thermogenic hydrocarbons were detected in the same piston core by GC-MS. In these “hydrocarbon positive” surface sediments Atribacteria were observed in high relative abundance (9-40%). Archaeal OTUs in these same locations included members of the ANME1b and ANME2a-b clades. ANME2a-b OTUs were detected in high relative abundance (up to 20%) in locations with biogenic and/or thermogenic gas whereas ANME1b OTUs were prevalent (up to 37%) only in locations with thermogenic gas. These patterns suggest that microbial screening has the potential to play a key role in offshore exploration, and that specific taxa e.g. within Atribacteria and ANME1 lineages may be useful bioindicators for hydrocarbon seep detection in the deep sea.

*50 “Examining the role of thermophilic, endospore-forming bacteria in de-risking offshore petroleum exploration”.

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De-risking offshore petroleum exploration requires a multitude of strategies including conventional geophysical and geochemical techniques to identify the presence of a petroleum system. To maximize exploration efforts, novel molecular technologies focusing on the marine microbiome have been proposed as complementary tools. Hydrocarbon seepage from deep, hot subsurface reservoirs has been invoked to explain the presence of thermophilic bacterial endospores, i.e. “thermospores”, in cold seabed sediments. A biogeography informed approach to identifying petroleum reservoir derived thermospores in marine sediments around seeps could offer a marker for hydrocarbon presence as well as a strategy for de-risking offshore exploration. Marine sediment samples were obtained during piston coring expeditions aboard the research vessel CCGS Hudson in deep water (>2500 m) prospective areas offshore Nova Scotia, Canada. Geochemical analyses on subsections of cores confirmed the presence (oil-positive) or absence (oil-negative) of migrated hydrocarbons. To test for viable thermospores, six sediments were amended with 20 mM sulfate and 5 mM organic substrates. The slurries were subsequently pasteurised at 80°C to eliminate viable vegetative cells, and incubated at 40, 50, and 60°C for 56 days. Sulfate and organic acid profiles indicated activity of sulfate-reducing and fermentative populations in both oil-positive and oil-negative cores. However, 16S rRNA gene amplicon sequencing results before and after incubation revealed
statistically different microbial communities, with thermophilic spore-forming Desulfitomaculum, Tepidimicrobium, and Candidatus Desulfurudis sp. in significantly higher relative sequence abundance in the oil-positive core. This thermospore signal may be explained by a dispersal of spores out of a warm, deep biosphere setting via hydrocarbon seepage. Ongoing efforts to correlate petroleum reservoir microbiome data, obtained via surveying prior published sequences, with communities identified in these incubations will reveal the utility of microbial biosensors for de-risking offshore oil and gas exploration.

*78 “Tracking active petroleum systems with intact polar lipids in shallow marine sediments of the Scotian Margin”.

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This study aims to develop novel indirect hydrocarbon indicators (IHIs) through the detection of intact polar lipids (IPLs) derived from hydrocarbon oxidizing bacteria and archaea. IPLs are considered diagnostic markers for living microbial cells and are ubiquitous as trace compounds in marine sediments. To test whether IPL fingerprinting techniques can be used as an IHI, surface and piston core sediments were collected during recent expeditions (CCGS Hudson 2015 & 2016; RV Coriolis 2017) from the light oil and condensate prone Scotian Margin, off Nova Scotia. IPLs were extracted using a modified Bligh & Dyer protocol and analyzed with an UHPLC-qToF-MS and UHPLC-QQQ-MS. Total lipid extracts (TLEs) are significantly more variable and enriched from cored sites containing seep hydrocarbons (ranging from ~300 to 800 µg/g sed.) relative to that of a hydrocarbon negative site (~200-300 µg/g sed.). The same trend is reflected in the concentrations of IPLs where, for instance, archaeal IPLs reach 1000 ng/g sed. in hydrocarbon positive sites and only 160 ng/g sed. in ambient sediment samples. Thus far, we have been able to identify 8 different bacterial IPL compound classes and 10 different archaeal compound classes with significant numbers of yet unknown bacterial IPLs. Glycosidic saturated and hydroxylated archaeal ether lipids (e.g. 1G-GDGTs, 2G-OH-GDGTs), likely produced by marine benthic archaea, and unsaturated dietherglycerols with phosphate-based headgroups (e.g. PE-DEGs, PME-DEGs), likely produced by sulfate-reducing bacteria, have been found in both hydrocarbon positive and negative sites. Further, differentiating hydrocarbon positive sites from ambient surface sediments is the presence of cardiolipins and an array of unknown bacterial IPLs. Collectively, these lipid fingerprints may provide key information for the search of offshore petroleum in order to further reduce front-end exploration risks to oil and gas companies.

*55 Evaluation and recommendation of sample preservation techniques for 16S rRNA sequencing in oilfield systems
The implementation of metagenomics in oil and gas industry has drastically increased in the last several years. It is being used for a host of applications, including: microbiologically influenced corrosion (MIC) origin detection, biocide efficacy studies, and microbial content evaluations of injection water and water used for hydraulic fracturing. One major deficiency with the application of metagenomics, is the lack of standardized protocols. Due to the remote nature of oilfields, sample preservation and maintaining integrity of microbial population during transport is of particular concern. If not preserved properly, the microbial community composition can change between sample collection and DNA extraction. Several studies have been conducted recently on DNA preservation of environmental samples. However, no consensus has been reached on a best practice. Results are often dependent on the preservation techniques tested, the types of samples, and the target organisms examined. Furthermore, most studies were focused on samples from the natural environment, with very few studies examining oilfield associated samples. The objective of this study is to evaluate several common preservation techniques for both liquids and solids that are suitable for remote oilfield locations. Techniques tested will include: 1) filtration followed by air drying, 2) storage of sample at low temperatures (refrigeration or on-ice), 3) freezing, and 4) chemical preservation. The air dried and chemically preserved samples will be exposed to different storage temperatures (20-50°C) and transportation times. Comparative data analysis will be conducted on the preserved samples against unpreserved sample used for DNA sample preparation immediately after collection. All DNA samples prepared will be sequenced using Illumina 16S rRNA sequencing. This comprehensive study will fill the gap pertaining to oilfield sample preservation and will contribute towards the development of standard protocols for metagenomics analysis.

*58 “Characterization of a thermophilic microbial community that determines the performance of an electromethanogenic biocathode”.

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Electromethanogenesis is a bio-electrochemical system to synthesize methane from CO2. Catalytic microbes on a cathode surface utilize electrons from the electrode for
CO2 reduction. As such biocathode enables conversion of electrical energy into methane with high energy efficiency, applications in renewable electricity conversion (Bio Power to Gas) and CO2 utilization have been proposed. We discovered a thermophilic microbial community, which originated from formation water from a petroleum reservoir and was capable of catalyzing electromethanogenesis at 55°C with cathode poised at –350 mV vs. a standard hydrogen electrode. Both methanogenesis and electron consumption at the biocathode depended on the presence of CO2 and were strongly inhibited by the methanogen inhibitor 2-bromoethanesulfonate, suggesting that electrons from the cathode were mainly consumed directly for the production of methane (rather than mediator compounds). 16S rRNA gene clone libraries initially suggested that a methanogen related to Methanothermobacter and several bacterial species were enriched on the biocathode. In this study, we further analyzed the community using metagenomics and metatranscriptomics to address in situ functions of the primary constituents. Moreover, cell density and morphology of the surface community were examined in relation to internal resistance of the biocathode, leading to an initial insight into the relationship between the surface community and the biocathode performance. Thus, besides potential advantages of thermophiles for industrial applications, this study provides useful knowledge to understand microbial mechanism catalyzing electromethanogenesis as well as for developing a strategy to improve the biocathode performance.

*66 “Pilot Trial of Indigenous Microbial Enhanced Oil Recovery in Conglomerate Reservoir”

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Microbial enhanced oil recovery technology has made great progress in recent years thanks to the rapid development of technology of microbial and molecular biology. To verify the validity of microbial enhanced oil recovery (MEOR) technology in conglomerate reservoir, A pilot test of microbial flooding by activating endogenous was carried out in the conglomerate reservoir of Block 7 Xinjiang oilfield in China from 2014-2018. Prior to the field test, the indigenous bacterial 16Sr RNA clone library was constructed to analyze the community diversity in formation water, the number of the hydrocarbon oxidation bacteria (HOB) in formation water also was tested. The activation system of microorganism was established through experimental study in laboratory, and the injection process of microorganism field test was optimized by physical simulation and numerical simulation. A total of 16.2 x 104 m3 activator solution and 130 x 104 m3 air were injected into an oil reservoir which includes four injection wells and nine production wells during actual field test. Production performance of oil wells and parameters of produced fluid from oil wells were monitored throughout the test period. The oil production increased by 1.2 times in the
testing area, cumulative oil increase 39,300 ton, oil recovery increased by 5.46%, water cut of oil well decreased by 8.8% average, the concentration of Hydrocarbon-Oxidizing Bacteria (HOB) in produced fluid from oil wells increased by 100~1000 times and maintained at a high level as the end of September in 2018. Results of the pilot trial shown that air-assisted endogenous microbial flooding is an effectively technology to enhanced oil recovery in Conglomerate Reservoir, which will play important role in the oilfield development in future.

*167 “Levan produced by Zymomonas mobilis grown on carbon supplemented produced water effluent from the petrol industry”.

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The aim of this research is to optimize the production of exopolysaccharide levan using produced water from oil exploration as the medium base for growing Zymomonas mobilis. Such a polysaccharide may be used by the petrol industry for improving fluids viscosity, which is important during oil recovery processes and/or as a protection against wear and tear of specific tools. Levan production by Zymomonas mobilis CCT 4494 was tested with and without agitation and using distinct carbon supplementation (sucrose). The highest growth coefficients (0.23 and 0.11 h⁻¹) were observed in produced water supplemented with 150 g/L of sucrose with and without agitation, respectively. Such a high growth rate was observed at 200 rpm incubated at 30°C. Levan production was of 10 and 6.1 g/L with and without agitation, respectively. The results suggest that agitation can double the growth coefficient but such effect does not directly reflect in levan accumulation. This microbial strain is known for exhibiting a complex metabolism varying the production of byproducts such as acetate, ethanol and levan according to the medium conditions. At the moment, some attempts of growing such strain within a bioreactor with controlled periods of agitation have shown the potential for increasing levan production above the mark of 12 g/L. This result is promising taking into consideration the nature of the medium base used in the process. Viscosity assays and levan molecular characterization show that the produced polysaccharide is similar to the commercially available counterpart. This work was supported by PETROGAL BRASIL and ANP - Brazilian National Agency for Petroleum, Natural Gas and Biofuels.

*164 “The influence of nitrogen supplementation in produced water amended with crude glycerin for the production of rhamnolipid by Pseudomonas aeruginosa”.

Authors
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The diverse metabolic capability of Pseudomonas aeruginosa allows such a strain to produce biosurfactant (rhamnolipid) using recalcitrant or low weight hydrocarbons as source of carbon and energy. The produced water generated during the exploration of oil from rock formation is an environmental problem. However, it has been demonstrated that such liquid waste can be used as a base medium for the culturing P. aeruginosa. Particularly if such effluent is amended with additional organics such as crude glycerin waste. The aim of this work is to test different nitrogen supplementation sources and concentrations that can increase the production of rhamnolipid using produced water amended with crude glycerin. It was tested 11 different combinations using yeast extract (0.25; 0.5; 0.75; 1 and 1.25 g/L) and NaNO3 (1, 2, 3, 4 and 5 g/L). The concentration of rhamnolipid assessed by the sulfuric orcinol assay and the emulsification index after 24 hours (IE24) were also evaluated. The best results for biosurfactant production with yeast extract were found in the concentration of 0.75 g/L (6.99 ± 0.84 g/L) and the NaNO3 concentration of 4 g/L (5.83 ± 1.75 g/L of ramnolipid). The best emulsification result was obtained using media containing 0.75 g/L of yeast extract (63.93 ± 12.42%) and 4 g/L NaNO3 (63.81 ± 3.3%). Therefore, either yeast extract or NaNO3 can be used as nitrogen supplementation in the production of rhamnolipid by P. aeruginosa in culture medium prepared with produced water and crude glycerin. This work was supported by PETROGAL BRASIL and ANP - Brazilian National Agency for Petroleum, Natural Gas and Biofuels.

*163 “The effect of distinct sources of produced water and activated coal pretreatment on Enterobacter amnigenus exopolysaccharide production”.

Authors
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The aim of this research is to optimize the production of Enterobacter amnigenus polysaccharide using produced water effluent from the oil industry as the main medium base. Such a polysaccharide may be used by the petrol industry for improving fluids viscosity, which is important during oil recovery processes and/or as a protection against wear and tear of specific tools. This research tested two distinct sources of produced water effluents (SA and CA, respectively). Activated coal was also used for pretreating the effluents and the results compared with the respective controls. A
factorial experiment was also carried out for testing distinct combination of carbon supplementation (sucrose and glycerol). Produced water SA and CA showed significant variance on oil and grease (11 and 80 mg/L, respectively) and salinity (62 and 76 g/L, respectively). The highest production of exopolysaccharide (7.5 g/L) was obtained using the activated carbon pretreatment of CA effluent; which was further supplemented with 2 and 2.5% of sucrose and glycerol, respectively. On the other hand, the highest production with SA effluent was observed using produced water without pretreatment (6.5 g/L) and at the same carbon supplementation previously reported. Therefore, exopolysaccharide production by Enterobacter amnigenus can be significantly influenced by oil and grease concentrations present in the produced water effluent. At high oil and grease concentrations, activated coal can significantly improve exopolysaccharide production when used as a pretreatment step during medium preparation. The presence of 11 mg/L of oil and grease in SA effluent increased in 32% the production of polysaccharide. Thus, this strain shows potential for using such hydrocarbons as carbon source at the reported concentration. This work was supported by PETROGAL BRASIL and ANP - Brazilian National Agency for Petroleum, Natural Gas and Biofuels.

*107 “Field Application of Bio-Augmentation processes for Microbial Enhance Oil Recovery Targeting Depleted Oil Field”.

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The joint research project was started in 2008 to study new Microbial Enhanced Oil Recovery (MEOR) method, targeting depleted oil filed in Japan. We focused on microbial reactions which degrade and convert oil into methane in the reservoir. In order to evaluate the potential of this method, microbial cultivation experiments have been conducted under the reservoir condition (55°C, 5 MPa). The bio-augmentation processes were reproduced in the cultivation bottles by mixing crude oil and formation water containing the functional microbe communities. As indications of oil degradation, several measurement items were observed and analyzed including the isotope ratio of reaction gas, microbial metabolism and properties of oil itself. The experimental results showed that the oil conversion reactions can be promoted by optimizing cultivation conditions, and there is an opportunity for the field application. Based on
the results of experiments, a field pilot operation is being planned. One oil field located in the north part of Japan is selected as a pilot field. In the field, the presence of microbe communities which convert oil to gas is not observed. By injecting water containing the microbes brought from other field, it is expected that the microbial reactions starts in the reservoir automatically without any artificial approaches. This method is intended to recover not only oil and gas converted from oil. Therefore, we refer to this method as Microbial Enhanced “Energy” Recovery (MEER), defining as a kind of new MEOR methods. For estimating the gas conversion and production rate in a well scale, a simple tank model is constructed to evaluate oil conversion reaction near wellbore. Calculation results show a positive potential of the new MEER method.

“Development of a biotechnological tool to remove filter cakes during the hydraulic fracturing process”.

Authors
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Hydraulic fracturing (fracking) is a widely used technique to extract hydrocarbons from unconventional reservoirs. In this technique, gelling agents are added to fracturing fluid to help proppant reach the fractures, keeping them open. These highly viscous gelling agents (polymers) tend to accumulate at the fractures’ faces and block them, forming what is known as a filter cake, preventing gas (or oil) flow thus impeding recovery. Chemical oxidizers are commonly used to remove filter cakes, but their toxicity remains a serious concern. They are also used in combination with strong acids, potentially leading to corrosion. These problems highlight a need for a more sustainable and safer technology to degrade filter cakes. In this study, we investigated the use of a biotechnological tool as an alternative to chemical filter-cake degradation. Specifically, we sought to identify enzymes capable of degrading carboxymethyl cellulose (CMC), a cellulose-based polymer used as a gelling agent. To date, no known work has been done on enzymes degrading CMC as a gelling agent under fracking conditions. We detected enzymatic CMC biodegradation in the supernatant of a mixed bacterial culture enriched from cattle manure. Viscosity, a parameter directly affecting filter cake formation, was quantified as an indication of activity. Within 5 days, cultures reduced viscosity by 90.5% compared to controls. Endo-β-1,4-glucanases reached their highest activity at 0.3 U/mL and β-1,4-glucosidases reached their peak at 0.6 U/mL. Characterization of these CMC-degrading enzymes indicate that they are extracellular, stable between 50°C and 80°C, at pH values 5 - 8, and can tolerate salinity up to 18% (w/v). Significant viscosity decrease was also measured when enzymes (in cell-free extracts) were tested on oilfield produced water samples, showing that this approach holds promise for use in the field. Our results present an encouraging non-toxic, environmentally-friendly alternative for effective fracking treatment protocols.

“Experimental Study on Treatment of Oily Sludge by Washing with Bio-Surfactant”.

Authors
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Oily sludge has become a major impediment to petroleum development industries because of its hazards to environment and human health. In order to reduce the oil content of sludge to an acceptable level of environmental protection standard and recover oil from heterogeneous sludge complex, ahamnolipid, a bio-surfactan, produced by Pseudomonas aeruginosa was used to washing tank bottom oily sludge .

A serious experiments of washing sludge were conducted in laboratory so as to obtain a good washing technical. Oil content of sludge samples was tested before and after washing. The optimum washing parameters such as bio-surfactants concentration, treatment temperature, ratio of liquid to sludge, stirring intensity, washing time, were determined by testing results. Good results were obtained from experiments that the oil content of sludge was reduced from 19.16% to 1.48% by washing with bio-surfactant, which can meet bury standards of environmental environment necessary, and recovery oil from sludge reach to 82.24%. It was shown that washing oil with bio-surfactant is an effective method of treatment of oily sludge, it has an a application prospect in oily sludge treatment technology because of high treatment effects and environment friendly.

*178 “Improved methods for trace analysis of polycyclic aromatic sulfur heterocycles in seawater using novel molecularly imprinted polymer sampling devices”.

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Thiophenes are polycyclic aromatic sulfur heterocycles (PASHs) present in petroleum products; they are considered problematic components due to their mutagenicity, carcinogenicity, and acute toxicity and lead to sulfur dioxide during combustion. PASHs are markers for regulated oil discharges, accidental oil spills and shipping related inputs. Current analytical methods for PASHs typically combine solid-phase extraction (SPE) with gas chromatography (GC) or high-performance liquid chromatography (HPLC). Matrix complexity, low concentrations, and the lack of selectivity of SPE hamper the efficiency of existing analytical methods. To overcome these problems, a selective sorbent material, such as molecularly imprinted polymer (MIP) can be employed. MIPs can selectively adsorb the targeted analyte(s) from complex matrices such as seawater and wastewater. Compared to other traditional methods, the MIPs offer faster sample preparation that is also more environmentally-friendly and cost-effective. In this study, a thin film MIP is engineered and fabricated on glass substrates to selectively extract thiophenes form complex matrices. This
extraction technique is combined with headspace-GC equipped with sulfurchemiluminescence (SCD) and FID to simplify the analytical procedure for the extraction and analysis of thiophene compounds in water samples. To obtain lower detection limit GC-MS was also employed (data will be presented). This technology is applicable for sampling in remote or inaccessible areas in harsh conditions using remotely operated vehicles.

*161 “A Combined Approach to Improve Oil Recovery by using Nickel Nanoparticle and Xanthan Gum as Injection Fluid”.

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Polymers are used for many decades for increasing the efficiency of water-flooding by improving sweep efficiency of injected fluid. Although polymer flooding is the most widely used method for chemical enhanced oil recovery (CEOR), there are critical difficulties faced in using polymers, such as, a risk of chemical degradation and low permeability. Thus, there is a need to develop new materials which can be stable at reservoir conditions. In the past decade, nanoparticles have gained wide application in different areas and is now applied in the field of oil recovery. Being smaller than the size of pore throats in the reservoir rocks, these nano-size particles can move through the rocks and mobilize residual oil. Therefore, in the current study, a novel nanoparticle assisted polymer flooding approach was carried out to tap the potential of nanoparticles alongside drawing benefits from polymer flooding. To determine whether nanoparticles influences the displacement efficiency of injected fluid we used a blend of xanthan gum and nickel nanoparticles. Initially we had evaluated the changes occurring in the intrinsic viscosity of xanthan when the nickel nanoparticles are added to the dispersing medium. We had found, xanthan in nanoparticles containing medium had a higher intrinsic viscosity of 55.25 dl/gm compared to 49.13 dl/gm for the sole gum solution. This suggested higher stability of the polymer nanoparticle blend in high salinity medium. Later the efficiency of nanoparticle assisted polymer flooding was evaluated in sand packed bioreactors with ~0.6 PV of residual oil in place (ROIP). Flooding results demonstrated a highest recovery of 5.98% of additional ROIP with xanthan-nickel nanoparticle mixture compared to 4.48 and 4.58%ROIP during separate flooding of xanthan and nanoparticles respectively. The injection fluid were able to flow through the porous medium without any hindrance and was effective in increasing recovery of residual oil.

*162 “Production of Exopolysaccharide by two distinct bacterial strains Using Oil Produced Water Effluent”.

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Oil is yet the most economically convenient answer to the world growing demand for energy. As energy resources directly influence our economy, it is essential to sustain an oil production. In Brazil, most of the oil obtained from the sea, however, due to the complexity of the offshore operations, renewed attention has turned to onshore fields. Innovative biotechnological strategies are emerging in order to improve the percentage of oil recovered in onshore fields. Approaches that are of low cost and more environmentally friendly. This work aims to associate the utilization of produce water effluent with the production of bioactive such as exopolysaccharides (EPS) and rhamnolipids. Culturing medium were prepared with distilled water medium (controls) and produced water (trials). Species of Enterobacter and Pseudomonas were used to produce exopolysaccharide and biosurfactant, respectively. Trials were carried out in pure culture, but also in mixed cultures. Incubation was carried out using 240 rpm, 35°C for 72 hours. The results showed a production (g.L-1) of 26.7; 25.44; 17.5; 21.2, for (I) EPS-pure culture distilled water; (II) EPS pure culture produced water; (III) EPS mixed culture distilled water and (IV) EPS mixed culture produce water. According to the results of the isolated productions and coproduction, we observed that the water produced proved to be a strategic component to produce bioactive products used in the advanced oil recovery, besides reducing its disposition in the environment. This work was supported by PETROGAL BRASIL and ANP - Brazilian National Agency for Petroleum, Natural Gas and Biofuels.

*87 “New microbial polysaccharides customization and their use in oil field”.

Authors
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Microbial polysaccharides can also profile the channel of oil reservoir and suspend drill mud in oil industry. The glycosyl arrangement of microbial polysaccharides, the important industrial chemicals, determines their conformation and physicochemical property. We customized eight polysaccharides containing uniformly distributed repeating units based on xanthan in vivo. Our work provided a promising theoretical foundation for studying the structure-activity relationship of xanthan and helping xanthan-containing block copolymers synthesis. The performance of a series of polysaccharide mutants was evaluated. We found that viscoelasticity of some mutants was significantly improved, and temperature resistance increased obviously. These polysaccharides are expected to be used in oil field drilling mud and water shutoff profile control.

*203 “Electrochemical screening techniques towards the microbial role in corrosion of biofuel storage tanks”.

Authors
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Biofuels are utilized in several industries and military branches. During storage, these biofuels begin to adsorb water forming a fuel-water interface. Microorganisms thrive at the fuel-water interface, resulting in fuel degradation and enhanced corrosion rates. Due to the variety of different microorganisms found in biofuel storage tanks, it is difficult to quickly ascertain the role of a microorganism on corrosion. In this work, we developed two electrochemical techniques to screen microorganisms for their ability to cause uniform and/or pitting corrosion in B20 storage tanks. Specifically, we focused on isolates *Byssochlamys* sp. SW2, *Yarrowia lipolytica*, and *Wickerhammomyces anomalus* SE3 growing in B20 for one month. Initially, a split chamber zero resistance ammetry (S-ZRA) technique was used to predict uniform corrosion in microbial incubations. The S-ZRA technique monitors the movement of electrons, in real time, between two shorted electrodes. Fluctuations in the magnitude and direction of current can be indicative of microbial activity. Our S-ZRA technique was able to accurately predict uniform corrosion with approximately 70% accuracy. Microscopy techniques indicated that deviation of our S-ZRA experiments from predicted corrosion rates was caused by localized, or pitting, corrosion. Therefore, it was necessary to develop an electrochemical impedance spectroscopy (EIS) technique capable of predicting pitting corrosion. Preliminary data shows that the EIS technique is able to predict localized corrosion with 92% accuracy. Together, these techniques can be used to quickly determine the type and extent of corrosion caused by a given organism or consortia. Understanding the microbial role in corrosion of biofuel storage tanks could allow field operators to develop more effective mitigation techniques and more quickly identify microbially influence corrosion (MIC).