# ISMOS4

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Dear Delegate,

**Welcome to ISMOS$^4$, Brazil !**

This is the 4$^{th}$ meeting of the International Symposium on Applied Molecular Microbiology in Oil Systems (ISMOS). The meeting is being hosted by PETROBRAS CENPES Convention Center in Rio de Janeiro.

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 problems (e.g. souring, biocorrosion) and encourage beneficial activities (e.g. MEOR, biofuels, hydrocarbon biodegradation for bioremediation). We are also pleased to introduce two new sessions as part of ISMOS$^4$ on 'Deepwater Horizon Blowout' and 'Microbes in Extreme Operating Conditions'.

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. A workshop is also included in the programme aimed at PhD students and hosted by industry speakers to foster discussions on careers in the oil industry.

We will also have an opportunity to visit some laboratories that work within the field of the Symposium including a visit to a site that has been developed by the industry with pipelines, pigs etc.

For the social events there will be a typical Brazilian barbecue and conference dinner for delegates.

We are very grateful to the Technical & Scientific Committee (TSC) and the Local Organising Committee (LOC) INT and ABRACO for their organisation and support for this conference. We also thank the sponsors and supporters (BP, Dow Microbial Control, Genome Alberta, Nalco Champion, Zeiss, Rhodia Solvay group, Petrobras and CTS Soldas - SENAI) for their support to ISMOS$^4$.

We hope you have an interesting and enjoyable meeting!

Yours,

Marcia Lutterbach
National Institute of Technology - INT

## Deepwater Horizon

### Hydrocarbon chemistry modulated the microbial response to Deepwater Horizon

**D. Valentine**

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The environmental discharge of oil and gas from the Macondo well introduced ~ 670 million kilograms of hydrocarbons into the Northern Gulf of Mexico. The trapping of oil and gas into the deep ocean that occurred during this event provided scientists a first opportunity to track a massive hydrocarbon discharge into this environment. In this talk I will chronicle some of the key lessons learned about the microbial response to hydrocarbons is the deep ocean setting. Principle amongst these lessons is that the microbial response tracked the physical and chemical properties of the hydrocarbons in the deep plumes, with one key factor being the solubility of the hydrocarbon compounds. Results will be presented from measurements taken during the event and from subsequent modeling studies with the aim to explain the primary drivers of microbial ecology during this event.

### Multi-omics assessment of the impact of the Deepwater Horizon oil spill on microbial communities and functions

*Janet Jansson*

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The Deepwater Horizon (DWH) oil spill in the fall of 2010 resulted in an input of approximately 4.9 million barrels of oil to the Gulf of Mexico, much of which remains unaccounted for. A hydrocarbon plume formed during the spill and caused a significant enrichment of specific members of the indigenous microbiota, including members with hydrocarbon degradation potential. A draft genome of a single cell of Oceanospirillales, showed that it had genes for chemotaxis and motility as well as a pathway for cyclohexane degradation. Many of these genes were among the most abundant expressed genes in the plume. During the spill history there was a succession from Oceanospirillales to Colwellia. This microbial succession was replicated in laboratory microcosms during which time large flocs formed and the oil was degraded. Similar flocs formed during the DWH spill, thus leading to the “dirty bathtub hypothesis” suggesting that the flocs could deposit to the deep sea floor carrying remaining hydrocarbons together with biomass. Therefore, we investigated the impact of oil deposition on surface sediments at 64 locations in the Gulf of Mexico. Amplicon 16S rRNA V4 sequencing of all 64 samples was complemented with a terabase of shotgun metagenomic data from 14 of these samples. In samples with the higher concentrations there was a higher proportion of several characterized as well as previously uncharacterized microbial populations. The presence and activity of key degradation pathways by sediment microbes was confirmed by determining the mineralization of $^{14}$C-labeled model substrates in the order: dodecane, tolune, phenanthrene. The combined data suggested differences in catabolic potential in the deep-sea plume compared to the sediment surface communities. In addition, we used metagenomics and metatranscriptomics to reveal that the oil also caused shifts in microbial community composition and function on coastal communities, but distinct from that that occurred in the water column and sediment surface.

### Evolution of Hydrocarbon-Degrading Microbial Communities in the Aftermath of the Deepwater Horizon Oil Spill in the Gulf of Mexico

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The Deepwater Horizon oil spill produced large subsurface plumes of dispersed oil and gas in the Gulf of Mexico that stimulated growth of psychrophilic, hydrocarbon degrading bacteria. We tracked microbial succession before, during and after the 83-day spill to determine the microbial response and biodegradation potential throughout the incident. Dominant bacteria in plumes shifted substantially over time and were dependent on relative quantities of insoluble and soluble oil fractions. Unmitigated flow from the wellhead early in the spill resulted in the highest concentrations of oil and higher proportions of n-alkanes down current from the wellhead. These conditions resulted in dominance by alkane-degrading Oceanospirillales, *Pseudomonas* and *Shewanella.* Once partial containment of oil and gas began, relative concentrations of *n*-alkanes in plumes decreased relative to BTEX and other soluble petroleum fractions, and an assembl age of aromatic and natural gas degraders became dominant consisting of *Colwellia*, *Pseudoalteromonas* and *Cycloclasticus*. Bacterial isolates obtained from water column samples confirmed that several of the dominant taxa identified by molecular analysis were capable of growth on crude oil. After the well was completely contained, petroleum hydrocarbons and associated plume bacteria rapidly disappeared but anomalous oxygen depressions persisted at plume depths for at least six weeks and were enriched in Flavobacteria and Rhodobacteraceae, common marine heterotrophs associated with degradation of high-molecular-weight organic matter. These results indicate that microbial communities in post-spill oxygen anomalies were primarily structured by degradation of the decaying microbial bloom rather than methane consumption as previously suggested.

## MIC, biocorrosion, souring, monitoring & treatment

### Reservoir Souring Challenges and Solutions from the Operators Perspective

**P. Evans**

*Chevron, UK*

Microbial reservoir souring results in an increase in the $H_2S$ concentration in produced gas, typically associated with secondary recovery by water injection, with the $H_2S$ first appearing some time after the breakthrough of injection water to the production well. The historical approach to controlling reservoir souring has been to shut-in high $H_2S$ wells and chemically scavenge the $H_2S$ from the produced fluids. However, this has cost implications from lost production and CAPEX and OPEX of $H_2S$ scavenging. Concentrations of gas phase $H_2S$ as high as several thousands of ppm have been measured from individual wells in reservoirs which are activity souring, which also presents an issue for operator safety. A potentially better solution is to prevent reservoir souring development by injection water treatment. A number of injection water treatments have been proposed to control reservoir souring including batch dosing of biocides, continuous dosing of nitrate salts and nanofiltration for sulphate removal. These have shown varying degrees of success, with perhaps sulphate removal having the highest confidence for limiting produced gas $H_2S$ concentration over field life, but at a high equipment cost. Accurate reservoir souring simulations are key to evaluating the optimum control strategy for field development, considering all aspects of the project i.e. reservoir characteristics, injection water chemistry, microbiology, metallurgy, topside facilities, and environmental impact. Ongoing research studies focus on reducing uncertainty in the understanding of reservoir souring mechanisms, the prediction of reservoir souring and efficacy of injection water treatments in controlling souring development.

### Using Stable Isotopes to Monitor Reservoir Microbial Souring Processes

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Microbial sulfate reduction is well known to impart large, characteristic shifts in the sulfur and oxygen isotope compositions of the residual sulfate and produce sulfide with very low $\delta^{34}S$ values. As microbial sulfate reduction is considered to be a major factor affecting reservoir souring, monitoring the isotopic composition of the dissolved sulfate and sulfide presents a promising tool for tracking microbial sulfur cycling in oil reservoirs. In this contribution, we present a combination of experimental and simple modeling approaches to explore the use and sensitivity of isotopic signatures of sulfate and sulfide to monitor reservoir souring. Preliminary column experiments with environmental communities showed enrichment in $\delta^{34}S$ of dissolved sulfate of up to 30‰ compared to baseline values, whereas produced sulfide is depleted in $^{34}S$. Under strongly sulfidogenic conditions, sulfate-oxygen isotope analyses show an increase of up to 15‰ compared with the $\delta^{18}O$ of influent sulfate and up to 21‰ compared with the $\delta^{18}O$ of water, indicative of microbially-mediated oxygen fractionation with water. These results also demonstrated the potential for isotopic analyses to discriminate between competing biological (microbial sulfate reduction), hydrological (mixing of formation and injection waters) and geochemical (e.g. precipitation of sulfide minerals) processes affecting sulfur cycling in reservoirs. To extend the applicability of these initial results we performed sensitivity analyses to explore the effects of a range of isotopic fractionation values, extent of microbial sulfate reduction, hydrological mixing and mineral precipitation on isotopic signatures. Ongoing efforts are being made to incorporate these effects into reservoir reactive transport models.

## Simulation of Reservoir Souring Considering Multiple Carbon Sources and Environmental Aspects

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The development of microbial reservoir souring, associated with water flooding, can have a significant economic impact on field operations. High levels of produced $H_2S$ can result in affected wells being shut-in due to HES, metallurgy and gas export quality limitations. The mixing of seawater and formation water provides a favorable environment for the development of sulfate reducing bacteria and/or Archaea. These microbes convert the seawater sulfate into hydrogen sulfide, and consuming soluble carbon compounds in the formation water as the electron donor in the metabolism. Water analysis reveals that a high content of volatile fatty acids (VFA) can be found in the formation water, and these can be the dominant utilizable carbon source consumed for biomass growth and respiration in the early life of the reservoir. With ongoing seawater injection the availability of VFA in water swept regions decreases, but microbiological activ ity is sustained by other carbon sources, such as partitioning of light hydrocarbons to the water phase and biodegradation. Environmental aspects such as temperature and salinity will also affect the microorganism's metabolism. These effects are incorporated into the computational model currently in use for reservoir souring development. Results of a field case showing the development of biomass inside the reservoir and consequent $H_2S$ production are presented. The effects of VFA or other sources of carbon are incorporated during the reservoir souring calculation together with the temperature and salinity impacts on metabolism. The model can be used for history-matching and subsequent prediction of $H_2S$ levels in reservoirs with souring potential.

## Pipeline Integrity Monitoring Programmes for Monitoring MIC using Molecular Microbiological Methods (MMM)

**A. Price**, R. Johnson, M. Frenzel,

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Microbiologically influenced corrosion (MIC) is estimated to cost the oil and gas industry billions of dollars each year due to capital costs in replacing and repairing pipelines and equipment, and from revenue losses due to lost production. Therefore, it is crucial that well planned pipeline monitoring programmes are in place to assess the corrosion status of a pipeline and ensure effective mitigation strategies are in place to minimise the risk of a pipeline failure due to MIC. This presentation will discuss the key aspects that form the basis of an effective pipeline integrity monitoring programme for MIC and biofouling, with the need to take both biotic and abiotic samples. The application of molecular microbiological monitoring (MMM) techniques and the advantages that they convey over traditional microbiological methods will be discussed. Some of the MMM techniques that will be discussed include reverse transcription quantitative PCR (RT-qPCR) for the assessment of microbial activity e.g. sulphate-reducing microorganisms, and the application of Live / Dead qPCR for rapid enumeration of live microorganisms using qPCR. Finally the presentation will conclude with several case studies where good monitoring regimes have identified potential problems with pipelines allowing proactive decisions to be made to rectify the problem before a severe pipeline failure could occur, resulting in very significant cost savings for the operator.

**Potentials of Chlorate Reduction for Souring Mitigation**

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Mitigation of reservoir souring is one of the key challenges that production chemists face in upstream oil business. Moreover, development of sulfate reducing communities, causing reservoir souring, has also been linked to microbially influenced corrosion. Methods for mitigation of reservoir souring are continuously investigated and cost the oil industry millions of dollars annually. The most commonly used methods for mitigation of reservoir souring include; nitrate injection and sulfate removal. Biociding is widely used, but most effectively for general microbial control in surface facilities, although certain biocides have been claimed (and to some extend proven) to be specifically effective against SRP (e.g. THPS). Sulfate removal is a very effective method, but is rarely used exclusively for mitigation of reservoir souring due to the costs, footprint (in case of offshore), incompatibility with produced water re-injection (PWRI) and cases in which the formation water contains significant amounts of sulfate. Nitrate injection has been shown to be successfully combating development of SRP communities and thereby limiting reservoir souring in several cases. The proposed mechanisms of mitigation by nitrate include scavenging of 'SRP-substrates" by nitrate reducing bacteria (NRB), inhibition of SRP by nitrite (intermediate of nitrate reduction), and high redox stress to SRP by nitrate/nitrite. Some cases that provided evidence of temperature limitations of application of nitrate are presented. These findings resulted in interest to search for alternatives for nitrate that are applicable at higher temperature. The potentials of application of chlorate, its combined use with other mitigation strategies and the impact on sulfate reducing communities is discussed.

**Role of Thiosulfate-Reducing Bacteria and Methanogenic Archaea in the Biocorroion of Oil Pipelines**

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Sulfate-reducing bacteria (SRB) are considered the major culprits responsible for oilfield biocorrosion and are frequently investigated in this regard. However, non-SRB sulfidogenic procaryotes and methanogens are often numerically dominant in oilfields. To investigate the role of microorganisms other than SRB in biocorrosion processes, we used scrapings from the inside of a pipeline to establish thermophilic enrichments under both thiosulfate-reducing (TSR) and methanogenic conditions. Molecular methods revealed that both enrichments harbored the same dominant bacterium that belonged to the genus Anaerobaculum. The methanogenic enrichment produced methane with abiotically produced hydrogen when a carbon steel coupon was used as the source of electrons, but this process did not increase corrosion relative to sterile controls. The dominant archaea in this enrichment was affiliated with the genus Methanothermobacter. When the en richments were amended with yeast extract as a complex source of organic matter, coupon weight loss by the TSR enrichment (11.13 ± 1.40 mg) was about 5 times greater than the abiotic control (1.97 ± 0.15 mg), while the comparable measures for the methanogenic culture were 3.45 ± 0.49 mg and 1.05 ± 0.07 mg, respectively. Total iron analysis in the cultures supported the weight loss conclusions. When cultivated in the presence of polished steel coupons, profilometry analysis revealed that the TSR enrichment caused 59 pits, while only 6 pits were evident in the corresponding methanogenic incubations. The results suggest that microorganisms catalyze more corrosion through thiosulfate reduction than when they exist as syntrophic methanogenic associations.

**Bacteria control in Unconventional Gas developments**

**Michael Scheck**,

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Bacteria control is of outmost importance to avoid corrosion and reservoir souring. Bacterial contamination of fracturing fluids can happen very easily as the source water can contain a lot of bacteria and nutrients. Some of the chemicals used in the fracturing process can act as nutrients too and thus lead to a rapid increase in bacteria population. This paper discusses: - How waste water from the city of Dawson Creek is treated in order to make it a suitable water source - How flowback water is monitored (and how the various methods used compared to each other) - What was done when wells were for the first time fracked with guar gum (which essentially is a sugar-based polymer; the use of it enhances the risk of bacteria growth).

**Control of the Biogenic Sulfate Reduction by Molybdate**

**E.B. de Jesus**, L.R.P. de Andrade Lima, L.A. Bernardez, P.F. Almeida

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Oil fields acidification is a major problem produced during the recovery stage conventional use of water injection. This phenomenon occurs also due to the action of sulfate reducing bacteria during their metabolic process that produces hydrogen sulfide from the reduction of sulfate in the water injection. Various methods have been employed to control the activity of these microorganisms, although an alternative method based on the use of inorganic compounds, such as molybdate and nitrate has been investigated. In this work, the efficiency of inhibition of the activity of sulfate reducing bacteria through the use of the molybdate is investigated. The criteria used to determine bacterial activity were browning of the medium and the pH and redox potential. Batch tests in liquid medium containing 1000 to 2000 mg/L sulfate and excess carbon source were performed. The inoculum was prepared from a microbial consortium dominated by Desul fovibrio vulgaris enriched from produced water from oil wells in the Reconcavo Basin, Brazil. The results show that using 0.08 mM molybdate is sufficient to inhibit the activity of sulfate reducing bacteria for up to 168 hours.

**Development and Application of the Biocompetitive Exclusion Technology in the Control of Biogenic Sulfide in the Oil Industry**

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The aim of this research was to identify and evaluate the main variables involved with the microbial biocompetitive exclusion technology (BET) approach for controlling the activity of sulfate reducing bacteria (SRB) at offshore oil fields. Biogenic sulfide generation may be controlled by the activity of physiologically competing strains, which are collective identified as petrobiotic bacteria. Produced water and injected water samples were obtained from four distinct PETROBRAS fields located at Bahia, Brazil. The following SRB were identified (16S rRNA): *Desulfovibrio vulgaris, Desulfovibrio alaskensis, Desulfovibrio capillatus, Desulfovibrio* sp., *Desulfobacter* sp., *Desulfosarcina* and *Desulfotomaculum* spp. The following petrobiotic bacteria were identified: *Halomonas salina, H. aquamarina, H. meridiana, Halomonas* sp., *Marinobacter aquaeolei, M. hydrocarbonoclasticus* and *Marinobacter* sp. BET approach was stimulated by the addition of sulfate analogs, such as: nitrate, sodium molybdate, tungstate, metavanadate and, SRB inhibiting agents, such as nitrite. The effects of three distinct matrices were also evaluated in combination with the former substances. The goal was to improve BET process efficiency. Statistical analyses confirmed that, in average, sulfide production was reduced in about 200 folds (~0.82±0.11 mg $L^{-1}$) when compared to the averaged values obtained with the controls without BET treatment (~200±23.5 mg $L^{-1}$). Although *Marinobacter* and *Halomonas* species were the most predominant petrobiotic organisms detected by molecular and culture methods, it seems to be that helicoidal-type forms of bacteria were the organisms involved in BET as showed by fluorescent microscopic analysis.

**Reducing Souring from Oil Wells: an Eco-physiological Study of using Bacterial Isolates with Antagonistic Activity owards Sulphate Reducing Bacteria**

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"Souring" is a common problem for the oil industry and it is associated with the production of corrosive sulphite ($H_2S$) by sulphate-reducing bacteria (SRB). Some companies estimate that the economic impact caused by the activity of such SRB may reach values of US$ 30 million a year. Therefore, the aim of this research was to selectively isolate bacterial strains with the capacity of producing antagonist substances to the in situ activity of BRS. Isolation was carried out using oil-production water samples from Reconcavo Baiano basin - Brazil, thus aiming to select strains, which are already present at the respective oil wells. Isolation was carried out from samples obtained from the most probable number culture counts (MPN) using CSB and Postgate media, respectively. It was selected about 120 nitrate-reducing distinct strains and 2 species were tested positively for producing a SRB antagonistic substance. In average, MPN counting showed that nitrate-reducing bacteria were 4 folds more abundant than SRB ($1.0 \times 10^2$ cfu. $ml^{-1}$). This suggested oil well pre-exposition to nitrate. At the moment, co-culturing inhibition tests using SRB and nitrate-reducing bacteria with SRB antagonist effect are showing to be directly affected by initial inocula concentration and high $NO_3$:$SO_4$ ratios.

**Metagenomic Bacterial Population Characterizations of FPSO Slop Tanks Reveal a Dominance of Sulfate Reducers**

**J. Wrangham**

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Hydrogen sulfide generation is a major operational and safety concern in floating production storage and offloading (FPSO) vessels. The $H_2S$ generation is largely attributed to the activity of sulfate reducing bacteria (SRB). The population consortia of FPSO Slop Tanks from offshore Africa, Malaysia, and Newfoundland were characterized in order to identify, if present, any similarity of species between the units, as well as the dominance of SRBs. Metagenomic analyses revealed that the three FPSO units displayed a narrow diversity of species. In addition, all slop tanks were dominated by SRB, with surprising similarity of species between the vessels given the geographical distance of the units.

**Sulfidogenic Prokaryotes Diversity and Composition in Production Fluids from High Temperature Corroding Oil Reservoirs**

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Oil reservoirs and production facilities are generally contaminated with $H_2S$ resulting from the activity of sulfidogenic prokaryotes (SRP). Sulfidogenesis plays a major role in reservoir souring and microbial influenced corrosion in oil production systems. In the present study, sulfidogenic microbial diversity and composition in production fluids retrieved from three blocks of high temperature (79~95°C) oil reservoirs with high sulfate concentrations were investigated by phylogenetic analyses of gene fragments of the dissimilatory sulfite reductase (*dsr*). Analysis of dsr gene fragments revealed the presence of several clusters of sulfidogenic prokaryotes that cover the orders Desulfovibrionales (*Desulfovibrio, Desulfomicrobium thermophilum*), Desulfobacterales (*Desulfobacterium, Desulfosarcina, Desulfococcus, Desulfotignum*, *Desulfobotulus, Desulfobulbus*), Syntrophobacterales (*Desulfacinum, Thermodesulforhabdus*, *Desulforhabdus*), Clostridiales (*Desulfotomaculum*) and Archaeoglobales (*Archaeoglobus*); among which sequences affiliated to members of *Desulfomicrobium, Desulfotomaculum* and *Desulfovibrio* appeared to be the most encountered genera within the three blocks. Collectively, phylogenetic and non-metric multidimensional scaling analyses indicated that similar but structurally different sulfidogenic prokaryotes communities within the waters retrieved from the three blocks. This study show the diversity and composition of sulfidogenic prokaryotes that may play a role in the souring mediated corrosion of the oilfield and also provides a fundamental basis for further investigation to control oil reservoir souring.

**Assessment of Microbial Diversity and Corrosion Risk in Tanks Storing Heavily Fouled Biodiesel**

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The introduction of next generation renewable fuels to the current infrastructure potentially poses new challenges with regard to biofouling and biocorrosion. In an effort to increase energy independence, the US Department of Defense has mandated the conversion of all operations to the use of next generation alternative fuels. The risk to fuel infrastructure was studied in a series of three in-ground storage tanks containing B20 (20 percent biodiesel and 80 percent conventional diesel) experiencing various degrees of biofouling. Bulk fluid and biofilm were sampled from each tank. Enrichment cultures were used to conduct corrosion rate measurements, putative metabolites of hydrocarbons were profiled using gas chromatography-mass spectrometry and the microbial community was characterized through metagenomic analyses. *Cryptococcus saitoi, Pseudomonas oleovorans, Bacillus cereus*, and *Burkholderia tropica* were isolated aerobically and an anaerobic enrichment from tank two was chosen for additional corrosion testing. The metabolites $C_4$-$C_6$, $C_{16}$, $C_{18}$, 9-octadecenoic and 9,12-

octadecanoic acids were present in the aqueous phase of tank 1 while all were absent in the parent fuel profile indicating potential microbial degradation of the fuel. Additionally, $C_4$-$C_{10}$ diacids, pentanol, and propanediol were observed. The microbial communities in each tank were similar in composition. This work represents a comprehensive analysis of the microbial ecology associated with the biodeterioration and biofouling of $B_{20}$ biodiesel and the associated corrosion risk for exposed infrastructure. This knowledge will be used to monitor these activities in service, predict suitable targets for mitigation and assess the efficacy of any mitigation activities deployed.

**Biofilm Activity Monitoring In Flow System: Carbon Steel Application**

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Whereas aerobic corrosion of iron is a chemical process, anaerobic corrosion of iron is frequently linked to microorganisms such as prokaryotes representing a various physiological groups. Among them, sulfate-reducing bacteria (SRB) are known to promote strong corrosion of carbon steel which is widely used in oil and gas transport and processing systems. One explanation for the occurrence of the Microbial Influenced Corrosion (MIC) process is the formation of biofilms structure on the surface of steel. The intrinsic properties of the biofilm makes the bacteria embedded within remarkably difficult to reach by antimicrobials; biofilms are often more resistant to doses of antimicrobials higher than the minimum lethal dose for microbes outside of biofilms. In this study we made an attempt to investigate the behavior of the SRB species *Desulfovibrio desulfuricans* ATCC 27774 and *Desulfovibrio alaskensis* AL1 (an oil field marine isolate), on carbon steel surface. Many parameters of SRB biofilm formation such as adhesion, growth, maturation and their corrosive impact have been investigated under variable condition, anaerobic flow system and specific chemical treatment. Complementary analytical tools such as microfluidic analysis and electrochemical measurement were used to monitor and better understand microbiological processes in relation with the unfolding on the surface of carbon steel. In addition, all steel samples were microscopically examined at the termination of the exposure period. The overall test results seems to indicate the necessary take into account of integrated evaluation methods for monitoring bacterial fouling and biofilm dynamics formed at pipe surface, in view of an anti-biocorrosion application.

**Electronic Equipment Failure due to Corrosion**

**Y. Tariq**

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There are many reason which are involved in electronic equipment failure i.e. temperature, humidity, dust, smoke etc. Corrosive gases are also one of the factors that may involve in failure of equipment. Sensitivity of electronic equipment increased when "lead-free" regulation enforced on manufacturers. In data center, equipment like hard disk, servers, printed circuit boards etc. have been exposed to gaseous contamination due to increase in sensitivity. There is a worldwide standard to protect electronic industrial electronic from corrosive gases. It is well known as "ANSI/ISA S71.04 - 1985 - Environmental Conditions for Control Systems: Airborne Contaminants". ASHRAE Technical Committee (TC) 9.9 members also recommended ISA standard in their whitepaper on Gaseous and Particulate Contamination Guideline for data centers. TC 9.9 members represented some of the major IT equipment manufacturers e.g. IBM, HP, Cisco etc. As per standard practices, first step is to monitor air quality in data center. If contamination level shows more than G1, it means that gas-phase air filtration is required other than dust/smoke air filtration. It is important that outside fresh air entering in data center should have pressurization/re-circulated process in order to absorb corrosive gases & to maintain level

within specified limit. It is also important that air quality monitoring should be conducted once in a year. Temperature & humidity should also be monitored as per standard practices & to maintain level within specified limit.

**Influence of Presence of Waste at Crude Glycerol from Biodiesel in Sulphide Production**

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Glycerol from biodiesel is an attractive electron donor for biological reduction processes, and could as such be used for reduction processes that are relevant for environmental biotechnology. However, crude glycerol waste has been shown to be toxic to microorganisms even at low concentrations. In the current work, the aim was to evaluate the influence of crude glycerol (CG) as substrate in microbial reduction processes, like sulfate and sulfur reduction. The samples of CG originated from the biodiesel production plant Biofuel Plant Petrobras SA at Candeias, Bahia, Brazil. The control was technical grade glycerol. Tests were performed under anaerobic conditions, using Postgate medium inoculated with 10-3, 10-4 and 10-5 bacterial cells.mL-1 of a sulfate-reducing (SRB) consortium. SRB were isolated from produced water of an oil well of Bahia, Brazil. The experiment was monitored by measurement of sulfide using a colorimetric metho d. Results show that the SRB grew and produced sulfide at CG contents below 3%. Above concentrations of 3% CG the biogenic production of sulfide was reduced/stopped. We suppose that waste compounds from biodiesel production, such as particular carbon compounds (acrolein and formaldehyde) and the high concentration of catalysts (NaOH or KOH) in CG act directly or via an associated way on the metabolism of SRB. The CG showed to be an interesting and cheap source carbon for microbial sulfate and sulfur reduction at low concentrations, but more extended studies have to be conducted to understand the inhibitory effect of CG on souring at higher concentrations.

**Development of a Novel Biocide for Enhanced Biofilm Control**

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Microbiologically influenced corrosion (MIC) is a significant challenge in the oilfield that results in significant cost for the operator in downtime, pipe and equipment replacement, and safety hazards associated with failures. One option for minimizing the risk of MIC is to efficiently treat the system with a biocide to kill microbes. However, effectively controlling a microbial population present as a biofilm is much more challenging than controlling microbes present in planktonic form. Most of the currently available biocides simply kill the outer layer of the biofilm, resulting in minimal MIC risk reduction. Furthermore, biofilm removal is just as important as the microbial kill in order to ensure that under-deposit corrosion does not occur. To this end, a novel biocide has been developed that provides microbial kill within a biofilm as well as biofilm removal. This novel biocide was initially evaluated against a biofil m in a stagnant environment and shown to be very effective. It was then tested in a dynamic flow loop against a best in class commercially available biocide. The new biocide was shown to more effectively kill and remove biofilm at a dosage significantly less than the incumbent product. This data suggests that this novel biocide has the potential to be used to treat systems where MIC is a concern.

**Multi-technique Approach to Assess Oilfield Microbial Populations' Response to Biocide Treatments**

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Microbial populations in oilfields are quite complex and often comprise of species that can impact the production and the integrity of the assets through the generation of metabolic by-products. The main problems caused by microbial activity are bio-fouling, well/reservoir souring and microbiologically influenced corrosion. Recent advances in monitoring methodologies have substantially improved the ability to detect microorganisms in oilfields and efficiently assess the risks they pose to a particular system. These include the use of immediate response ATP assays and molecular techniques, such as quantitative PCR (qPCR) and DNA sequencing. Despite the improvements in monitoring methods, little is known about how the dynamic in microbial metabolism varies in response to changes in the system, including during biocide treatments. We have taken a multi-technique approach to assess how microbial populations respond to biocide treat ments in order to determine the efficiency of treatment at different dosages. For this, we treated production water samples with several commonly used biocides at 3 different dosages (sub-lethal dosage - 10ppm; frequent dosage -250ppm and over-dosage - 1000ppm). Samples were taken over time within 5 hours of treatment for further analysis. Microbial enumeration was determined by qPCR for total bacteria and SRBs, metabolic changes were determined by ATP/AMP assays and species were identified by DNA sequencing using Ion Torrent platform. Particular trends in microbial survival/dormancy in response to biocide treatment will be further discussed.

**Use of Molecular Microbiological Methods to Develop Effective Microbial Control Strategies for Hydraulic Fracturing**

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Biocides are added to hydraulic fracturing injection water to control the growth and development of problematic microorganisms. Recent studies have shown a wide diversity of microorganisms in samples from hydraulic fracturing systems in various shale formations. A better understanding of microbial diversity and population shift during the fracturing and production processes, as well as biocide effectiveness under relevant environmental conditions will help in developing more effective microbial control strategies for longer term microbial control. In this study, molecular microbiological methods (e.g. qPCR, DGGE) were used in combination with traditional microbial detection techniques to monitor microbial diversity and population shift in a shale gas field during hydraulic fracturing and production. In addition to traditional SRB and APB evaluation, total bacteria, total archaea and sulfate-reducing prokaryote were also monitored using molecular methods. Endospore-forming sulfide-producing bacteria have been detected in many flowback/produced water sources, which are often recycled as injection water. Their growth and states (endospores vs. vegetative state) can affect the souring process and biocide effectiveness in both lab evaluation and field application. Use of qPCR together with microscopic staining technique can quickly detect this bacterial group and assess their souring-causing risk and treatment strategy in water reinjection. By using both molecular and traditional microbial monitoring techniques, as well as test methods that better simulate relevant environmental conditions, a new biocide combination and long-term microbial control strategy were developed for hydraulic fracturing at this shale gas field.

**CIM Monitoring using 96-Well Microplates in Production Systems for Oil and Gas**

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Microbiologically Influenced Corrosion (MIC) is formed by a consortium of various microorganisms, especially those microorganisms sulfate reducers (MRS) given its importance

in the formation of biofilm promoter of the problems associated with biogenic souring. The sulfate reducers Microorganisms (MRS) are a diverse group of bacteria and archaea and the heterotrophic anaerobic growth, reduce sulfates and produce hydrogen sulfide ($H_2S$) which can be detected by the presence of colored precipitates in the liquid medium. The search for methods of identifying and counting MRS has been increasing in recent years, particularly with regard to reducing the incubation time of the bacteria and less costly development of methods for monitoring and controlling these microorganisms. In this approach, the main objective of this research was to evaluate a new methodology for detection and enumeration of microorganisms sulfate reducers (MRS) in samples of the production of oil and gas. For thi s, we carried out the monitoring of microbial growth over 06 months (May 2011 to October 2011) pipeline (aqueduct, pipeline, pipeline) and storage tanks of water produced. The results of statistical analyzes regarding the detection of microorganisms sulfate reducers (MRS) showed a significant advantage in reducing the processing time of the sample using the method of microplates (7 days) compared with the traditional method (28 days), allowing obtaining more rapid diagnosis for biocorrosion.

**Diagnostic, Predication and Control of Microbiologically Influenced Corrosion in the Oil & Gas Industry**

**T.L. Skovhus**

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Monitoring of Microbiologically Influenced Corrosion (MIC) in the oil industry has in the past been conducted mainly on sulfate-reducing bacteria (SRB) using cultivation-based techniques. However, with the introduction of novel DNA-based methods for enumeration of microbes, more accurate and fast methods are now available which offer better determination of MIC on a routine basis. This presentation presents the latest knowledge on how state-of-the-art DNA based-methods can be applied by E&P companies in diagnosing, predicting and controlling MIC. The presentation discusses field cases from the Danish Sector of the North Sea of how microbial numbers are evaluated and interpreted in the best way with respect to risk assessment and system integrity measures of aging offshore assets and pipelines. Also the presentation will highlight a number of newly published latest industry standards on MIC detection with DNA-based methods.

**Search for Chemical signatures for Microbially-Induced Corrosion in Laboratory and Field Samples**

**Vincent Bonifay**, Whitney Smith, Jan Sunner

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Microbially-induced corrosion (MIC) is commonly found in both man-made and natural systems. There is an urgent need for novel approaches to better predict, diagnose and mitigate MIC. We are using state-of-the-art mass spectrometry for broad-based chemical characterization of samples obtained from both laboratory and oil-field environments, with thousands of compounds detected in each sample. Here, we report results of statistical analysis of the chemical data obtained from MIC environments and chemical patterns observed in their correlations with severity of corrosion. Water samples from oilfield pipelines and laboratory cultures were submitted to limited extraction and concentration procedure and analyzed using an Agilent 1290 HPLC coupled to an Agilent 6538 Q-ToF mass spectrometer. A robust workflow for the analysis of metabolomics data from these samples was developed: The data were processed using dedicated software (IDEOM, XCMS and metaXCMS); lists of putative metabolites were generated and compared to online databases, such as KEGG, ConsensuspathDB, and ECMDB. Results from different oilfield environments will be reported.

The ability of field samples to induce corrosion was determined in the laboratory by exposing 1018 steel coupons to oil-field water samples. The results were correlated with the metabolomics/chemical patterns. This approach revealed interesting correlations between metabolome and severity of corrosion observed in the laboratory experiments. The analysis was able to differentiate the samples by their metabolomics signature induced by bacteria understood to cause MIC. This research demonstrates that a mass spectrometry-based metabolomics approach has great potential for oilfield management and, in particular, for MIC monitoring and mitigation.

### Factors Associated With Microbial Contamination in Diesel Fuel

**A. Ramos**

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To characterize the factors that directly influence the proliferation of microorganisms in diesel fuel in petrol station was investigated knowledge about microbial contamination. To do so a questionnaire was applied in ten Brazilian states. In each state were selected randomly picked ten heads of service stations to respond to a questionnaire with open and closed questions, dealing with operational parameters and level of technical knowledge about microbial contamination and its treatment. It was shown that there is little work routines which aims to eliminate the factors that lead to microbial contamination and its treatment, or when they show up are often ineffective. The water draining from the tank does not have a standard routine cleaning and maintenance in the systems are often lacking, as well as exchange of paper filter press. This stems from ignorance on the subject that the vast majority of respondents demonstrated. The prevention of this framework with the use of biocides was also ignored by the majority of respondents. The study demonstrates that the issue is still a subject not yet widespread, and there is still a lack of preparation involved in indicating the need for further treatment of these factors.

## Molecular Microbial Methods (MMM)

### Comparative metagenomics of deeply buried oil reservoirs suggests very slow rates of evolution in the deep biosphere

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It is well established that microorganisms colonize a variety of extreme environments, including habitats like oil reservoirs deep inside the earth crust. To investigate differences in microbial communities in oil reservoir on the Norwegian Continental Shelf, we recently performed a comparative high coverage DNA sequencing study of metagenomes derived from two different oil reservoirs, both located about 2.5 km sub-seafloor. A bioinformatic analysis of DNA sequence data derived from one of the reservoirs (Well I) indicated that the community is dominated by bacterial species with a smaller fraction of Archaea. However, results of a similar analysis from another reservoir (Well II) located in the same geographical area, but according to available geological knowledge lacking direct physical contact with Well I surprisingly revealed

that the Well II community is largely dominated by Archaea with a subordinate fraction of Bacteria. Nevertheless, comparison of the two datasets sh owed that large fractions of the sequences are extremely similar, both with respect to identity (typically above 98%) and gene organization. We therefore conclude that both wells contain essentially the same organisms, but in different relative abundances. Assuming that the communities have been separated for very long times, the results also indicate that cell growth rates in the reservoirs are extremely slow, and hence seemingly have very slow efficient mutation rates.

### *Halomonas sulfidaeris*-dominated Microbial Communities Inhabit 1.8 km-deep Subsurface Cambrian Sandstone Reservoirs

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An indigenous low-diversity microbial community, dominated by the *Gamma-Proteobacterium Halomonas sulfidaeris*, inhabits warm saline formation pore water in the Cambrian Mt. Simon Sandstone of the Illinois Basin, North American Midcontinent (1.8 km-5872 ft. burial depth, 50o C, pH 8, 181 bars pressure). These highly porous and permeable quartz arenite sandstones are directly analogous to reservoirs around the world targeted for large-scale hydrocarbon extraction, as well as subsurface gas and carbon storage. A new downhole low-contamination subsurface sampling probe was used to collect in situ formation water samples for microbial environmental metagenomic analyses. Metabolic pathway reconstruction, constrained by the geology, geochemistry and present-day environmental conditions of the Mt. Simon Sandstone, implies that the native microbes utilize iron and nitrogen metabolisms and extensively recycle indigenous nutrients and subs trates. The presence of aromatic compound metabolic pathways suggests this microbial community can readily adapt to and survive subsurface hydrocarbon migration.

### Evaluation of Flow Cytometry as a Tool for Biocide Efficacy Testing and Monitoring as Compared to Classical Culturing Methods and ATP Photometry

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Current biocide performance testing relies on classical microbiological culturing techniques and other non-culture based methods such as ATP photometry and fluorescence microscopy. Although each of these methods has its merits, there are significant drawbacks as well. For example, results via culturing require a two-four week incubation, which delays optimization of the biocide application. It also assumes that the microbes are actually able to grow in common oilfield culture media. ATP photometry has reduced the time necessary for system optimization (1 day), but there can be challenges with quantification of dying (but still membrane intact) microbes. It is our hypothesis that multi-parameter flow cytometry can outperform both culture and ATP-based methods in terms of speed to results (1 day), absolute quantification of dead microbes, and insight into biocidal mechanisms of action. Flow cytometry has historically been used to evaluate antimicrobials in biomedical microbiology, but this is the first time it has been used in a petroleum microbiology application for biocide evaluation and selection. The data presented here will compare and contrast the advantages and disadvantages of using flow cytometry, culture-based methods, and ATP quantification using the model organism *E.coli* as well as microbial consortia from oilfield water samples.The results indicate that there are significant advantages in using flow cytometry over either of the other methods as there is significant differentiation of biocidal performance and insight into the actual biocidal killing mechanism.

**Characterization of Oil-Field Samples with Emphasis on Biocorrosion Monitoring and Diagnostics**

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Recent progress in mass spectrometry, in particular the ability to detect many compounds simultaneously, is revolutionizing our ability to characterize complex chemical systems, as more than $10^4$ organic compounds can be detected in a single sample. This "metabolomics" ability holds tremendous promise for the characterization and diagnosis of microbially-induced corrosion (MIC) systems, and, thus, for early mitigation and remediation of MIC in field environments. For about two years, we have used HPLC coupled to a QToF mass spectrometer to analyze a wide variety MIC-related samples. The data has been analyzed with metabolomics software packages and databases. Biofilms from corroding environments are sampled by laser (UV, VIS, or mid-IR) ablation followed by HPLC/MS analysis. These data-rich chemical profiles contain a wealth of information about fuel degradation processes; microbial activities; the presence of additives, such as anti-corrosion and antimicrobial agents; as well as other important aspects of sample history and status. A number of applications will be presented, such as the detection of microbial activity through the presence of fuel degradation products; the detection of metabolic degradation pathways; sulfur speciation; and chemical characterization as well as imaging of MIC biofilms. A particular focus of this work concerns the detailed relation between the chemical profiles and the severity of microbially-induced corrosion. This is discussed in a follow-up presentation. It is anticipated that as our ability to interpret the detailed chemical profiles of MIC-related samples, MS-based techniques will prove invaluable in oilfield management.

**MIMESH, Metagenomics Standards for the Cataloguing and Analysis of Hydrocarbon Environments**

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The biological processes that are taking place in hydrocarbon environments are still largely unknown and very hard to investigate. Recent developments in DNA sequencing technologies and Bioinformatics revolutionized the field of Metagenomics (aka Environmental Genomics). Metagenomics circumvents the need for microbial cultivation in the lab and provides a clearer picture of the microbial assemblages and biochemical processes that are taking place. However, like with all biological data, biological interpretation of metagenomic data can be very difficult and potentially erroneous. This statement holds true especially when the data are not properly linked to the associated environmental conditions and experimental procedures commonly known as metadata. The capturing, storing, management and analysis of such metadata is an arduous task, but this information can be essential and probably as important as the associated sequenced metagenomic data. Currently any knowledge related to metadata from Hydrocarbon environments is scattered across the public literature and databases. As a result, researchers cannot harness the full potential of this combined knowledge through data integration. We propose a set of standards, MIMESH (*Minimal Information about Metagenomic Samples from Hydrocarbon environments*) for the capturing, cataloguing and handling of metagenomic data and metadata from hydrocarbon environments. These standards adhere to the widely accepted MIGS/MIMS specifications, enabling data integration comparison and interpretation.

**Metagenomics of a Methanogenic Toluene Degrading Enrichment Culture**

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Hydrocarbon biodegradation in the subsurface can occur under methanogenic conditions. Crude oil degradation in reservoirs lowers the API gravity of oil, reducing its value and making it more difficult to extract. Conversely, the conversion of residual oil to methane could be a means to extract energy from marginal reservoirs. Furthermore, methanogenic hydrocarbon degradation has proven to be useful in the bioremediation of hydrocarbon-impacted shallow subsurface environments. A better understanding of methanogenic hydrocarbon degrading communities will improve our ability to regulate subsurface hydrocarbon degradation and harness methanogenic communities for MEOR or *in-situ* bioremediation technologies. We carried out metagenomic sequencing of an established methanogenic toluene degrading enrichment culture that has recently been found to degrade a range of hydrocarbon substrates including alkanes, benzene, xylenes and ethylbenzene. The microbial community is highly diverse and dominated by the bacterial phyla Firmicutes and Proteobacteria, and both hydrogenotrophic and acetoclastic methanogens. The metagenome contains genes involved in the anaerobic biodegradation of hydrocarbons such as benzylsuccinate synthase and alkylsuccinate synthase, putative benzene and naphthalene carboxylases and genes involved in benzoate metabolism. In addition, the metagenome is enriched in genes involved in methanogenesis and energy conservation relative to metagenomes from other environments. Key pathways involved in carbon metabolism include beta-oxidation, and the reductive TCA and glyoxylate cycles. Due to its enriched nature and ability of this culture to degrade a range of hydrocarbons, we propose that the metagenome of this culture serves as a blueprint to better understand methanogenic hydrocarbon degrading communities.

**In Search Of An Oil Sands Tailings Pond Core Microbiome**

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Tailings ponds (TPs) store the solid and liquid wastes generated from bitumen extraction of mined oil sands. TPs support varied microbes, including those involved in elemental cycling, methane production, and hydrocarbon degradation. We sought to determine if there is a tailings core microbiome (a subset of microbes shared across most TP samples), when comparing ponds with different management strategies. Partial 16S rRNA gene pyrosequencing data from 130 samples from six TPs within the Athabasca region (Alberta, Canada) were analyzed using an in-house bioinformatics pipeline (Phoenix II) and MOTHUR. Core biomes were determined per pond at the operational taxonomic unit (OTU) level. Additionally, all TP samples were analyzed together to determine if there was an overall TP core biome. Results indicated that each TP had a core microbiome of 4-10 OTUs, which were shared across at least 75% of the samples. Surprisingly, even though different ponds had distinct community profiles, there were also 5 OTUs shared across the majority of the ponds. Although the core only consisted of 0.1 to 0.5% of the total OTUs, these 4-10 OTUs constituted 39-54% of the total sequence reads. The core microbes have been affiliated with the iron, nitrogen and sulfur cycles, syntrophy, methanogenesis and fermentation, with the latter two groups being the most predominant. Overall, our results indicate that TP microbial communities consist of both core and variable biomes.

**Metagenomics Analysis of Production Water from a Deep and Hot North Sea Oil Reservoir**

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DNA extracted from hot water produced from the North Sea Troll-B oil reservoir, with an *in situ* temperature of about 70°C was amplified using whole genome amplification and sequenced. From 465,000 reads and a total of 228 Mb nucleotide sequences, 6.9 Mb of assembled sequence data distributed over 1920 contigs were obtained, with an N50 contig size of 12,590. The estimated metagenome size was estimated to ~76 Mb. 77.0% of the genes encoded known functions while 23.6% encoded unknown or unpredicted proteins. 95.5% of the predicted genes belonged to the Bacterial domain, while 3.6% to Archaea. Phylogenetic analysis of the predicted genes indicated that Firmicutes and Proteobacteria constituted the major bacterial phyla (42 and 36%, respectively), with Thermoanaerobacteriales, Campylobacteriales and Clostridiales as the most prominent orders. Thermotogales appeared to constitute 6.7% of the community. Based on phylogenetic relatio nships and functional genes, major energy-conserving metabolic reactions are discussed.

**The Influence on the Indigenous Microorganism after Injecting Sewage Treatment Additive into the Oil Reservoirs**

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Various kinds of additives for sewage treatment have been injected into oil reservoirs during the water flooding. The effects of these additives on the indigenous microbes are still unknown. In this study, most probable number (MPN) method was used to analyze the biological activity of hydrocarbon-oxidizing bacteria (HOB) in the enrichment cultures. In addition, 16S rDNA clone library technique was performed to analyze the compositions of HOB bacterial communities. Biomass of HOB in the enrichment cultures in injection wells and production wells fell by more than 4 orders of magnitude due to injection of high concentrations of bactericide, and biomass of HOB in production well decreased 1-2 orders of magnitude after injection of different concentrations of demulsifier, flocculants and scale inhibitor. Obtained results of 16S rDNA clone library showed that the microbial community structure of HOB changed greatly. After the additives were added, *Pseudomonas stutzeri*, covered a dominant percentage in the production well sample, was not detected in the enrichment cultures, which indicated that *Pseudomonas stutzeri* was sensitive to additives. In addtion, *Pseudomonas aeruginosa*, was not detected in production well sample, became the dominant group in the enrichment cultures after demulsifier, bactericide and scale inhibitor were added. For injection well sample, after bactericide was added, the only detected bacterial group was *Exiguobacterium* sp. Interestingly, *Thauera* sp. was dominant in the injection well samples before and after demulsifie, flocculant and scale inhibitor were added, which indicated that this bacterial was tolerant to these additives.

**Diversity of Genetic Markers from Sulfate Reducing Prokaryotes to Monitor and Manage Oil Reservoir Souring**

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Oilfield reservoir souring caused by sulfate reducing prokaryotes is responsible for annual loss of millions in assets. Genetic diagnostics that can be used to rapidly assess, predict, monitor, and manage oil reservoirs and production water sources prior to or during treatment of a souring event is critical. However, there is always concern on the coverage and specificity of different primer sets and functional genes. As such, the coverage and diversity of various functional markers was examined along with community structure for 22 samples from reservoirs with and without soured production. In detail, eight amplicon pools were assembled from conserved genetic signatures using both novel markers and an extensive search of the literature for primers targeting the dsrA, dsrB, apsA, and 16S rRNA gene. The workflow for sequence library preparation included PCR amplification, harvesting and purifying amplicons via gel purification, and next generation sequencing on the 454 platform. Sequence diversity related to sample and markers was quantified using tools available on the Functional Gene Pipeline Repository. Overall, these results will be useful in identifying genetic signatures that can be used to test production waters in oilfield operations.

**Euryarchaeota Dominate the Brazilian Petroleum on Campos Basin**

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The interest of the Brazilian Petroleum Company (PETROBRAS) in the study of the microbiota of petroleum reservoirs is increasing nowadays mainly due to the need to establish a microbial oil recovery (MEOR) in different National Petroleum Reservoirs. In addition, the study of waterflooded reservoirs may help the understanding and the further control of biogenic souring. This study focused in an analysis using Roche´s 454 sequencing high-throughput sequencing of metagenomic DNA recovered from samples of crude oil extracted from an offshore platform localized in Campos Basin, southeast of Brazil. A metagenomic analysis using WebCarma pipeline revealed a predominance of Euryarchaeota. At the genus level, most of the environmental gene tags (EGTs) were related to Archaeoglobus, a sulfate reducing prokaryote group commonly found in high-temperature oil fields. Therefore, this genus can be a potential contributor to oil field souring. A deeper analysis of this metagenome is currently being performed together with culture dependent techniques. In the near future, a broader scenario of this niche in Brazil will be disclosed.

**The Role of *rhlA* Gene Homologue on the Population Profile and Surfactant Properties of Rhamnolipids Produced by *Burkholderia kururiensis***

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Biosurfactants are amphipathic compounds of great interest in the oil industry, which include rhamnolipids, typically produced by *Pseudomonas* and *Burkholderia*. RhlA is a biosynthetic enzyme, which produces β-hydroxy fatty acids, major rhamnolipid precursors. The present work is focused on the functional analysis of r*hlA* homologue from *Burkholderia kururiensis* and its relation to rhamnolipid production and structure profile. Two strains were generated from wild-type *B. kururiensis* M130 strain: LMM24, containing a 230-nucleotide deletion within a rhlA homologue, and LMM25, the complemented rhlA-mutant strain, carrying plasmid pUCP18-rhlABK. The strains were cultivated for 24 hours in LB medium, and the cell-free supernatants were used for the purification and physical-chemical analyses of rhamnolipids. Organic extracts

were used for the analyses of rhamnolipid populations in TLC and LTQ-ESI-Hybrid Orbitrap Mass Spectrometry (MS). Our results showed a significant difference between the wild-type strain M130 and LMM25, as the latter presented 270% higher rhamnolipid yielding. The LMM24 strain yielded 61% less rhamnolipids when compared with the wild-type strain. Surface tension tests with rhamnolipid extracts resulted in similar values for the strains, although a substantially higher emulsification value (E24) was observed for strain LMM25. Structural analyses resulted in a significantly different profile, both in TLC analysis and, most importantly, the MS analysis. These results confirmed the major role of the RhlA enzyme on the production of rhamnolipids in *B. kururiensis*, and represents a powerful target for metabolic engineering aiming at improved rhamnolipid-producing strains.

**Diversity and Distribution of Bacteria Community in Different Type Oil Reservoirs in China**

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Diversity and distribution of bacteria communities in three type oil reservoirs, including polymer flooding oil reservoir represented by block DQ, high water cut oil reservoir represented by block Q and water flooding heavy oil reservoir represented by block L, M and B, were investigated based on constructing 16S rRNA gene clone library methods. Principle component analysis and canonical correspondence analysis were used in bacteria communities clustering and correlation analysis with environmental factors respectively. The results indicated that diversity of bacteria community in polymer flooding oil reservoir was lower than the other two type oil reservoirs. Phylogenetic analysis showed that the bacteria community was dominated by clones affiliated to uncultured bacteria and *Epsilon-Proteobacteria,* in polymer flooding oil reservoirs. While it had some similarity in high water cut reservoirs and heavy oil reservoirs, both of which wer e dominated by clones affiliated to proteobacteria, especially α-and γ-proteobacteria. It suggested that chemical process had significant influences to bacteria community rather than water content and stratal condition. Correlation analysis between bacteria communities and environmental factors showed temperature had significant influences to bacteria communities ($P$=0.0280). Crude oil component also could influent bacteria community and resin fraction had significant influence to bacteria community ($P$= 0.059). The rest of environmental factors also had contributed to microbial communities, though not significantly. These results revealed that bacteria community in oil reservoir was deeply influenced by oil displacement process and environment condition, especially the former.

**Assessment of New Detection Method of Potential Biocorrosion (MIC) in Oil and Gas Pipelines and Tanks**

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Microbiologically influenced corrosion (MIC) is process led by various microorganisms, especially of sulphate reducing microorganisms (MRS), which are a diverse group of Bacteria and Archaea characterized by sulphate reducing and sulfide producing compounds, that can be detected in the media. The objective of this study was to evaluate a new method for detection and enumeration of sulfate reducers (MRS) in samples of the oil and gas by comparing the traditional flask MPN procedure with new microplate MPN procedure using real samples of Oil and Gas Industry from Petrobras at Linhares ES, Brazil. We carried out the monitoring of microbial growth by the presence of sulfate-reducing microrganisms for 06 months (May 2011 to October 2011) in pipelines and tanks terminals of Petrobras production, Linhares, ES. The presence of MRS was performed by comparing the traditional method with the new proposed

method using 96 well microplates (NMP) and the physico-chemical quality of the water produced. Statistical analysis (correlation tersts) were used to verify the agreement between the two detection methods of MIC. The enumeration of sulfate-reducing microorganisms (MRS) by the traditional method ranged from 0 to 4.2 x 104 MRS NMP / mL and the new proposed microplate method ranged from 0 to 1.6 x 104 MRS (NMP / mL). Statistical analyzes relating to MRS detection indicated that both methods were equivalent. The main advantage of microplate method is the reduction in processing time (7 days) as compared with the traditional method (28 days). Thus, the use of this new proposed method can significantly improve the biocorrosion monitoring in oil and Gas industry.

**Characterization of Microbial Community of an Onshore Oil Reservoir Candidate for $CO_2$ Injection**

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Advanced methods of oil recovery (EOR) are required when conventional technology reaches its limit. In mature fields $CO_2$ injection has been used to increase the recovery factor. However, the impacts of $CO_2$ injection on the microbial community of the oil reservoir must be evaluated and therefore it is necessary to characterize this community. The present study aimed to characterize the microbial community using culture-dependent and culture-independent methods through the analysis of fluids of three production wells and the injection water from an onshore oil reservoir with high salinity and temperature, located in the northeast of Brazil. Samples were grown in selective media for sulfate-reducing bacteria (Postgate E modified medium), iron-reducing bacteria (Basal medium with peptone) and nitrate-reducing bacteria (Nitrate broth). DNA from the samples and from the cultures were extracted and used for microbial characterization by sequencing 16S rDNA. The bacterial genera detected as prevalent in the samples were *Marinobacter, Marinobacterium, Thioclava, Arcobacter* and *Pelobacter*. In the cultures, the predominant genera were *Desulfotomaculum, Thermovirga, Burkholderia, Garciella, Desulfonauticus, Thermohalobacter, Hydrogenophilus, Anaerobaculum* and *Bacillus*. The genus *Thioclava* was also quite represented in one of the cultures. The difference in the diversity observed among the identified microorganisms in fluids and the ones recovered by the cultures corroborates with previous researches regarding the necessity of using two approaches to microbial characterization of this type of environment.

**Metagenomic Analysis and Biodegradative Potential of Microbial Communities in Deep Oil Sands**

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Petroleum and petroleum-based products are cornerstones of modern civilization. As the supplies of conventional oil decline, more focus is being directed towards unconventional sources. Extraction and refinement of unconventional oil, such as the bitumen found in the Alberta oil sands, requires a greater energy input than what is required for processing of conventional oil. Bitumen is a viscous mixture of high molecular weight hydrocarbons, which are left following degradation of low molecular weight hydrocarbons by subsurface bacteria. Understanding the microbial communities found in the oil sands and their role in bitumen degradation can help in the development of biotechnology-based extraction methods that may be less energy demanding, and therefore more cost-effective and environmentally responsible. For instance, viscosity reduction, the holy grail of unconventional oil recovery, may be achieved

by cleaving large hydrocarbon aggregates into smaller fragments through microbial degradation. Alternatively, stimulating microbial subpopulations could result in partial conversion of bitumen to methane. Previous microbial analysis using preserved oil sands core samples indicated that the resident microbial communities include known hydrocarbon degraders. Fresh core samples, traversing the cap rock and the bitumen pay zone, have been obtained and are currently being used to further determine the microbial community composition at varying depths and geologies through metagenomic analysis. Whether the resident hydrocarbon-degraders only attack low molecular weight hydrocarbon or can also use high molecular weight components is being investigated. This information could help suggest possible strategies to increase recovery and lower the environmental impacts of extraction.

**Effect of Film-Forming Corrosion Inhibitors on Biofilm Microbes and Pitting Corrosion in a Model Flow Cell System**

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Film-forming corrosion inhibitors are used to control corrosion but contain chemicals that can be nutrient sources for microbes. It may be particularly important to determine if stimulation of biocorrosion occurs with "green" corrosion inhibitors, as they are more biodegradable. Model flow cells containing mild steel corrosion coupons were used to evaluate the effect of two film-forming corrosion inhibitors on pitting corrosion. A continuous flow of a nutrient solution and a sulfide-producing microbial inoculum was pumped through 6 flow cells, 2 which received corrosion inhibitor "A", 2 corrosion inhibitor "B", and 2 with no inhibitor (controls). After operating for one month coupons were removed and analyzed for pitting and general corrosion, viable microbial biomass (phospholipid fatty acids), DNA was extracted from biofilm microbes and 16S rRNA gene sequence libraries constructed. Coupons from treated cells had ten to twentyfold higher biomass values and higher maximum pitting rates than control cells. Biofilms from the treated cells had higher proportions of Clostridia, Beta- and Gammaproteobacteria and a lower proportion of Deltaproteobacteria. The dominant species of Deltaproteobacteria varied, with *Desulfomicrobium* in higher abundance on coupons from the treated cells. The maximum pitting rate was positively associated with the estimated abundance of *Desulfomicrobium* plus clostridia but less so with the abundance of total Deltaproteobacteria. Monitoring single SRB-specific genes or species by qPCR may be less useful than multiple targets for detecting biocorrosion. Biodegradation of film-forming corrosion inhibitors could stimulate biocorrosion by decreasing the protective layer and by providing additional nutrient sources to biocorrosive microbes.

**Process Issues Caused by 'Slime': The Use of Analytical Chemical and Molecular Microbial Methods (MMM) to Identify and Treat Flow Issues, a Case Study**

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In order to treat onshore produced water, reduce oil in water and chemical oxygen demand (COD), a plant design incorporating bioreactors was constructed to allow for produced water disposal to the environment. However, since the attempted commissioning of the plant, process issues have been encountered due to the formation of an unidentified 'slime' upstream of the bioreactor, resulting in an inability to transfer the produced water for treatment in the bioreactors (due to fear of fouling the patented matrix). Here we used analytical chemical techniques such as Fourier transform infrared spectroscopy (FTIR) in conjunction with 454 DNA pyrosequencing to identify the slime. An in-depth site audit was carried out to isolate the cause of the problem

and recommend steps to eradicate the issue. Initial analysis by FTIR indicated that the slime was biological in origin. Microscopic analysis suggested it was a complex mix of Prokaryotic and Eukaryotic microorganisms, forming a microbial mat, intercalated with oil droplets. Extraction with 90% acetone and scanning spectroscopy identified the presence of chlorophyll a. DNA sequencing using 454 pyrosequencing of eubacterial, fungal and algal genes confirmed that the slime was microbial in nature and revealed the dominant microorganisms present. The site audit identified areas, which were contributing to the problem, and recommendations were given to rectify this, to reduce microbial slime formation. This case study shows how cutting-edge molecular microbial methods (MMM) such as 454 pyrosequencing can add value to conventional analytical approaches to identify and solve water treatment process problems within the oil industry.

**Application of Molecular Biology Methods in Identifying Biosurfactant Spore Forming Producers for Oil Recovery Enhancement**

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Petroleum oil is a major source of energy globally and it is a backbone economy for Oman. Recently this industry is facing many difficulties in driving oil from matured oil wells. Many Enhanced Oil Recovery (EOR) techniques are used including microbial enhance oil recovery (MEOR). Microbes produce different metabolites: biosurfactant, biopolymer, acids, solvents, and gases which are used to reduce the residual oil saturation. Biosurfactant is an amphiphilic molecule, which is used widely in MEOR to decrease surface tension (ST) and interfacial tension (IFT) between oil and water releasing trapped oil in the rock pores by capillary forces. Surfactin and lichenysin are the most reported lipopeptidal-biosurfactants. In this study, seventeen biosurfactant Bacillus producing strains previously isolated in Oman from oil-contaminated samples were used. After DNA extraction, the polymerase chain reaction (PCR) was used to detect surfactin/lichenysin (*srfA3/licA3*) gene responsible for bio surfactant production. Fourteen bacterial isolates showed the presence of *srfA3/licA3* gene. These isolates were compared for the optimum biosurfactant production and three were selected based on lower ST and IFT. These are B30, W16 and W19. Crude biosurfactant was isolated using acid-precipitaion and spray-dryer. To identify the chemical nature of the biosurfactants, TLC, HPTLC and FTIR were used. Initial characterization showed that it is lipopeptide. The results revealed that there is potential of using molecular biology methods for identifying biosurfactant producers from oil-contaminated sites.

**Improving of Indigenous Nutrients System and Its Effect to Enhanced Oil Recovery**

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In indigenous microbial flooding, the nutrient systems is effecting on the growth, metabolite and degration. At Zhuangyi Block, there were 4 types nutrients used during 6 years oilfield application The nutrient were mixture of chemicals including nitrogen & p and air, mixture of air and double function particles nutrient and complex nutrient with malas and particles. With nutrient systems improving the microbial chemistry of produced water from oil wells and oil production changed much, meanwhile injection system changed, and match technology completed. The parameters of produced water show that number of indigenous bacteria increased more than 4 orders, the microbial type and structure changed and show strong bacteria group controlling oil reservoir environment. The oil production of the block or wells improved and water cut decreased.

**Quantitative PCR Assays For Monitoring Oil Facility Microorganisms**

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Control over microbial fouling and the associated biocorrosion-related failures require monitoring methods that are both rapid and accurate. Molecular biology techniques have become more commonly used for the identification and estimation of target microorganisms present in corroded samples. High-throughput sequencing analyses are commonly used to assess the microbial diversity of environmental samples, although they are not well suited for monitoring purposes due to cost considerations. Quantitative PCR (qPCR) is designed to estimate gene copy numbers for sequences amplified by specific primers, but primers designed to monitor oil field microbes are scarce. Therefore, we designed and tested a set of qPCR primers targeting the 16S rRNA gene of thermophilic and hyper-thermophilic microorganisms ("core taxa") prevalent within and common to multiple sites throughout an oil production facility. Primers specific to the resident bacter ial and archaeal core taxa were designed and PCR amplification conditions optimized to provide high efficiency amplification (> 90%). We present the results of initial qPCR validation studies using three pipeline biofilm samples and find that the qPCR results are largely congruent with the relative abundance data derived from 454-pyrosequencing analysis. These primer sets should prove useful in monitoring dominant microbial taxa at oil facilities processing fluids from hot, anaerobic reservoirs.

**Isolation and *in situ* Detection of Thermophilic Microorganisms from Brazilian Oil Reservoirs**

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Petroleum reservoirs were long considered inhospitable environments to the existence of life. Nowadays it is clear that oil reservoirs constitute deep geological environments with diverse *in situ* physicochemical conditions where indigenous microbial communities grow and survive. Despite of the current knowledge of microbial diversity, community dynamics in oil reservoirs is far from being complete, a consequence of the difficulty in sampling and recovering the complex community present in such extreme environments. Previous studies have reported that the use of cultivation-dependent and independent methods have shown profound differences between dominant groups in oilfields. In this work, enriched culture media (Nutrient Broth, Tryptone Soya Broth, Grabowski, BH, Marine Broth and R2A) were used for recovering culturable microorganisms from three oil reservoirs. Two reservoirs were drilled in the onshore Recôncavo Basin (Brazil) and one in the offshore (post salt) (Brazil) under thermophilic conditions (≈50$^{O}$C). DAPI staining and Fluorescent *in situ* Hybridization (FISH) were applied to detect and identify the enriched microbial community. Eight phenotypes of thermophiles were isolated from the three oil reservoirs while diverse morphological forms were detected with DAPI and confirmed as Alphaproteobacteria, Gammaproteobacteria and Actinobacteria after FISH assays. These results corroborate previous findings that the use of culture media leads to biased and underestimated characterization of the real microbial community composition and that the application of DAPI staining and FISH may offer a more accurate tool to detect, characterize and quantify the microbial diversity in such environments.

**Characterization of cultivated bacterial diversity in sludge of a bioreactor under high salinity conditions from a Brazilian oil terminal.**

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A highly saline effluent, known as produced water, containing mainly sulfide and a complex mixture of hydrocarbons, is generated during oil extraction. The residual produced water that arrives in oil terminals needs to be properly treated for environmental disposal. However, these systems depend on a combination of parameters such as biomass, organic and inorganic mater relationship, pH, dissolved oxygen, salinity, and mostly operational control. In this context, the objective of the present research is to characterize the cultivated bacterial diversity recovered from the sludge of a bioreactor under high salinity conditions, from an oil terminal, aiming at future application in the treatment of hypersaline produced water. The bacterial isolation was performed in different culture media from a saline acclimatization batch process (4% to 20% NaCl). Bacterial identification was carried out by sequencing of 16S rRNA genes. Based on macromorphology, 72 isolates were selected and identified. Sequence analysis revealed the presence of the classes *GammaProteobacteria* 82%, *Alphaproteobacteria* 3%, Actinobacteria 8%, Bacilli 3% and Flavobacteria 4%, showing a dominance of the *GammaProteobacteria*. It is worth to mention that the percentage of distribution of the bacterial isolates among the classes was found to be similar when the isolation was carried out directly from the sludge sample, before the acclimatization process. The genera *Idiomarina*, *Halomonas*, *Brevibacterium, Salegentibacter*, *Staphylococcus*, *Halobacillus*, *Marinobacter*, *Pseudoalteromonas*, *Vibrio*, *Shewanella*, *Alcanivorax*, *Nitratireductor* and *Mesorhizobium* were identified among the isolated bacteria. Investigation of the presence of catabolic genes and hydrocarbon degradation capacity in hipersalinity conditions are in progress.

**Sulfate reducing prokaryotes PCR-DGGE bio-prospecting in sensitive environments in potiguar oil basin**

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The sulfate-reducing prokaryotes (SRP) are responsible for damage to the oil industry, as souring, bio-degradation of oil and bio-corrosion of metal surfaces in industrial installations. The Potiguar Oil Basin (POB) composes 119,300 $Km^2$, comprising more than 3,500 producer wells, over 5.000 Km of pipeline and storage capacity around 211,500 $m^3$, exposed to SRP action. This work, pioneer in environmental studies of native SRP of the POB, contemplates the use of molecular techniques applied to access the microbiome from Caatinga and mangrove biomes in response to oil contamination. Were evaluated PCR-DGGE metagenomic fingerprints with dsrB gene as molecular biomarker. The sediment were obtained from mangrove of Diogo Lopes-RN, Areia Branca-RN, Paracuru-CE, Fortim-CE and Caatinga soil of Macau-RN and stored in microcosms. Of a total of 31 samples tested, 18 without contamination and 13 with oil contamination, 134 and 98 organismal tax onomic units (OTUs) were detected, respectively. Were observed variation in the number of OTUs with the prevalence of SRP in contaminated soil microcosms from Caatinga biome (Macau-RN). For other sediments analyzed, there was no variation of number of OTUs between contaminated or not contaminated samples. Were observed spatial and temporal variations in SRP communities. According to the sampling periods and the presence / absence of oil in microcosms, was observed a relationship between seasonal OTUs of SRP. Data on the status quo of SRP native communities from the studied

microbiome is of interest to the oil and natural gas industry in eventual actions of safety, environmental and health in oil activities.

**Metagenomic monitoring and evaluation of mangrove microbiome response to contamination by polycyclic aromatic hydrocarbons**

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The Potiguar Oil Basin (POB) composes 119.300 Km2, with in situ volume of 5.82 billion barrels and 0.411 billion barrels in proven reserves. Although the Polycyclic Aromatic Hydrocarbons (PAH's) match the smallest fraction present in the oil, are noted for being stable, recalcitrant in the environment and toxic. Access to composition of microbiome by classical and omics strategies leads to studies about the "status" and its "capabilities". Considering the mangrove biome as a delicate and critical interface between earth and ocean, our study aim to partial characterization of microbiome from mangrove sediments that exhibit a potential for PAH's biodegradation. The metagenomic DNA were isolated according to the sampling periods relating 0 to 120 days using microcosms pure and contaminated with 3% petroleum. To examine the samples from metagenome were used the pcaF gene as biomarker. PCR-DGGE fingerprints obtained from Paracuru-CE, Fortim-CE and Areia Branca-RN samples revealed the occurrence of fluctuations of microbial communities of Prokaryotes that Degrade PAH's (PD-PAH's). PD-PAH's were detected In metagenomic analysis from Areia Branca-RN and Paracuru-CE with fluctuations in biomass and Organismal Taxonomic Units (OTU's) diversity. Results from Fortim-CE shown OTU's variations, however there was no detection of PD-PAH's, which makes this environment more vulnerable to oil contamination. Additional studies of others pristine mangrove locations of POB are in progress. Data about the status quo of PD-PAH's is of interest to the oil industry in eventual actions of safety, environmental and health in petroleum activities.

**Whole Genome Analysis of the Hydrocarbon-Degrading Bacterium, *Brevibacillus agri* Strain 5-2, Isolated from Deep Oil Reservoir**

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*Brevibacillus agri* has been widely used in the fields of microbial enhanced oil recovery and bioremediation of hydrocarbon contamination, but no genes or metabolism pathways involved in n-alkane degradation in Brevibacillus have been reported. In this study, we report the whole genome sequence of crude oil-degrading Brevibacillus agri strain 5-2, isolated from a deep oil reservoir in Changqing Oilfield, China. Multiple genes potentially involved in crude oil degradation were identified. The results showed that the chromosome of strain 5-2 was 5,543,507 bases in length, with a G+C content of 54.1%. The genome contains 5750 predicted protein-encoding open reading frames (ORFs) that account for 86.4% of the genome, with an average length of 833 bp. The genome encodes 116 tRNA genes, 6 rRNA loci and one copy of 16S-23S-5S operon. In particular, we analyzed genes possibly responsible for crude oil degradation. Genes encoding alkane hydroxylase, alcohol dehydrogenase, FAD-dependent oxidoreductase, rubredoxin, and cytochrome P450 were found in the genome. Moreover, methane monooxygenase and four genes encoding alkanesulfonate monooxygenase required

for the utilization of alkanesulfonates as sulfur sources were also found in the draft genome sequence. The strain 5-2 genome sequence and its accurate annotation are important assets to better understand the physiology and metabolic potential of *B. agri* and will open up new opportunities in the functional genomics of this species. A further and deep exploration of the genome sequence of *B. agri* is now under way, which will provide the basis to elucidate the putative gene determinants of *B. agri* for survival in deep oil reservoir.

### Tropical Soil Multi-Contamination: a Metagenomics Approach to Evaluate Nickel Influence in Petroleum Biodegradation

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Petrochemical industry is responsible for most of the soil multi-contamination with organic compounds and metals. The negatives effects of metals in the biodegradation of organic compounds turn the decontamination of these multi-contaminated sites challenging. Understanding the microbiological processes can help manipulate of remediation strategy. The aim of this study was to evaluate the effects of nickel on the biodegradation of crude oil in a tropical soil. To achieve this, we did a microcosm experiment under4 conditions: soil (control), soil contaminated with oil (5% w/w), with oil (5% w/w) and nickel (260 mg/Kg) and soil contaminated with nickel during 30 days. Metagenomics and 16S rRNA libraries were sequenced using Ion Torrent technology. The results showed that taxonomic changes were more affected by oil contamination and the order of Actinobacteria was predominant and increased in the presence of oil. The abundance of se quences related to organic compounds degradation increased in oil and Ni containing treatment, in contrast to previous studies that shown the negative effect of metals in degradation activity. Even nickel caused significant changes in the microbial community, it was possible to observe a functional redundancy between Beta-Proteobacteria and Actinobacteria (oil, oil-Ni, respectively) compared the degradation of aromatics compounds, this kind of redundancy can explain the fact that the levels of removal have not been different between treatments. This soil, regardless of the changes undergone by different pollutants, was able to remove the oil accessing microbial diversity, demonstrating the importance of the preservation of genetic resources in the environment.

## Hydrocarbon biodegradation

### Enrichment of Crude Oil degrading Microbial Consortia and the Production of 25-norhopane by Microbial Metabolism

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There is an emerging interest in the anaerobic degradation of crude oil. However, the knowledge about both the geochemical effects and microbiological activities that are associated with this process is limited. In this study, we used a mixed inoculum of anaerobic sludge and the production water from an oil well to construct four consortia, which were incubated under sulfate-reducing or methanogenic conditions at either mesophilic or thermophilic temperatures for 540 d. The significant degradation of the aromatic and saturated hydrocarbons was observed. Interestingly, the quantity and ratio of hopane to norhopane suggested that the microorganisms in the consortia produce norhopane. However, terminal electron-accepting conditions and temperature strongly affected the diversity and structure of the enriched microbial communities and the degradation of the oil. After a 540-day incubation, bacteria of unknown taxonomy were dominant in the thermophilic methanogenic consortia, whereas Clostridium dominated the mesophilic methanogenic consortia. Except for the dominant phylotypes shared with the methanogenic consortia, the sulfate-reducing consortia were predominantly composed of Deltaproteobacteria, unclassified Spirochaetaceae and the phylum Synergistetes. This study revealed an artificial microbial consortium for crude oil degradation under varying conditions and demonstrated that norhopane could be produced by microbial activity during incubation.

**A Methanogenic Consortium Capable of Large Molecular Weight Paraffin Biodegradation**

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Large molecular weight (MW) paraffins can form waxy deposits and reduce oil recovery operations. Microbial formulations have been used to treat paraffin deposition problems, but the organisms involved and their mechanistic basis is unclear. We used contaminated marine sediments to enrich for a methanogenic consortium capable of the stoichiometric biodegradation of long chain *n*-alkanes ($C_{28}H_{58}$ to $C_{40}H_{82}$). Amplicon cloning, 454-pyrosequencing and isolation attempts were used for characterization of the consortium. 16S rRNA bacterial and archaeal libraries along with the metagenomic sequencing indicated that that the dominant bacterial and archaeal lineages were 97% and 99% similar to an uncultured deltaproteobacterium Synthrophobacterales and several uncultured *Methanosaeta*, respectively. Sequences affiliated with the Methanomicrobiaceae were also detected. Culturing resulted in isolation of two archaea, an acetoclastic *Methanosaeta harundinacea* and a hydrogenotrophic methanogen (94% similar to *Methanogenium cariaci*). A deeply branched deltaproteobacterial strain SPR (91% similarity to *Desulfarculus baarsii*) was also isolated, but unlike it closest relative, the organism was incapable of sulfate reduction. Metagenomic analysis of the consortium for the alkylsuccinate synthase gene (assA) revealed seven assA genotypes. Five of the *assA* genotypes were subsequently shown to be expressed via RT-qPCR in the presence of $C_{28}H_{58}$. However, the *assA* sequences were not detected in strain SPR suggesting that it likely did not catalyze the primary activation of the paraffin substrate. The utilization of larger MW alkanes by this consortium suggests that a biotechnological approach to selective paraffin removal is at least feasible.

**Methanogenic Degradation of Crude Oil Alkanes under Different Temperature Conditions**

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Methanogenic consortia degrading crude oil and hexadecane originating from Shengli oilfield were enriched at 15°C, 35°C and 55°C, respectively. GC-MS analysis revealed that crude oil *n*-alkanes ($C_9$-$C_{37}$) were totally degraded at 35°C, short chain *n*-alkane was degraded under at 15°C, while high molecular weight alkanes was removed at 55°C. The referential degradation of long chain n-alkane was also observed under mesophilic and thermophilic conditions over time. An as-yet -uncultured member of *Syntrophaceae* was identified as a key player by DNA-stable isotope probing with U-$^{13}$C-hexadecane at 35°C. The other lineage of *Syntrophaceae* dominated in bacterial communities at low temperature condition using terminal restriction fragment length polymorphism (T-RFLP) fingerprinting and sequencing of their 16S rRNA gene fragments. While approximately four-fifths of bacterial clones exhibited less than 90% similarity to the sequences of known type strains dominated in the thermophilic consortia degrading crude oil and hexadecane, respectively. These results expand substantially our knowledge of the extent of microbial diversity associated with methanogenic degradation of alkanes under different temperature conditions.

**Are Diamonds Forever? Biodegradation Of Diamondoid Naphthenic Acids By Microbial Consortia**

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Oil-sand deposits constitute more than half of global oil reserves and during surface oil-sand refining, vast quantities of oil sands process affected waters (OSPW) are generated that contain naphthenic acids (NAs). NAs are highly toxic and thus the remediation of OSPW is of great environmental concern. Recently, tricyclic diamondoid NAs were found in OSPW but there is currently a lack of information on the microorganisms that can degrade them. The aim of this study was to investigate the efficacy of microbial consortia isolated from two different OSPW to degrade the diamondoid NAs, adamantane-1-carboxylic acid and 3-ethyl-adamantane carboxylic acid. Gas chromatography-mass spectrometry analysis showed that the microbial communities obtained from both OSPW samples degraded the tricyclic diamondoids, adamantane-1-carboxylic acid and 3-ethyl-adamantane carboxylic acid by approximately 71% and 50% respectively by day 33, resulting in a 50% reduction in toxicity as determined by the Microtox® assay. Significant changes (ANOSIM $p < 0.05$) were found in bacterial community composition (analysed by PCR-denaturing gradient gel electrophoresis), regardless of the carbon source. Dominant bacteria included *Pseudomonas, Microbacterium, Streptomyces* and *Acidovorax* spp. at day 11 and *Exiguobacterium, Bacillus, Thauera* and *Hydrogenophaga* spp. at day 33. These data suggest that complex communities are involved in the degradation of both adamantane-1-carboxylic acid and 3-ethyl-adamantane carboxylic acid and will enable more cost-effective bioremediation strategies to be developed for removing recalcitrant NAs from OSPW.

**Anaerobic degradation of polycyclic aromatic hydrocarbons in oil**

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Anaerobic degradation of polycyclic aromatic hydrocarbons is an important process in oil aging. We studied the biodegradation of naphthalene as a model compound for PAH with sulfate and iron-reducing microorganisms. The biochemical pathway was elucidated to large extent with

biochemical and genomic studies and several novel enzyme reactions were described on enzymatic level. This knowledge could be used to identify specific metabolites and prove anaerobic degradation in field studies as demonstrated at the example of a contaminated aquifer. Here, also limitations of biodegradation were identified such as the spacial separation of electron acceptors and ecological fluctuations. Finally, we could show that degradation does probably not only take place in the water compartment but also in water inclusions in the oil itself.

**Sulphate-Dependent Anaerobic Hydrocarbon Degradation In Estuarine Sediments**

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Anaerobic biodegradation of petroleum hydrocarbons under sulphate-reducing conditions was investigated in sediment microcosms amended with brackish medium containing 28 mM sulphate, three different levels of nutrient (N, P) concentrations and either (i) North Sea crude oil containing volatile low molecular weight (LMW) ("dead" oil), or (ii) the same oil from which LMW hydrocarbons were removed ("topped" oil). Sulphate reduction was most rapid with topped oil, compared to dead oil, indicating that LMW compounds may partially inhibit sulphate-reduction under these conditions. Controls with no crude oil amendment had much lower sulphate reduction rates, indicating that hydrocarbon-degrading sulphate-reducing populations were responsible for sulphate removal from oil-amended microcosms. Crude oil degradation was confirmed through GC-MS analysis of hydrocarbons and metabolites from fumarate addition to hydrocarbons. Aromatic and satu rate hydrocarbons were degraded at all nutrient levels but the patterns of compound removal were different at different nutrient concentrations. Interestingly even though the LMW hydrocarbons, e.g. toluene and LMW alkanes, were inhibitory to the degradation of dead oil, they were effectively degraded over the time course of the experiment. PCR screening revealed different variants of benzylsuccinate and alkylsuccinate synthetase genes (*bssA, assA*) were detected under different nutrient regimes and at different times, suggesting that different hydrocarbon-degrading sulphate-reducing bacteria may be enriched using this approach.

**The Paradox of "Aerobic" Bacteria in the Petroleum Reservoir Microbiome**

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Geochemical and microbiological evidence suggests that petroleum reservoirs are largely anoxic ecosystems where biodegradation has been ongoing for long geological timescales. Nevertheless there are many reports from both culture-independent and cultivation-based studies that suggest that some petroleum reservoirs may also harbour populations of "aerobic" bacteria. In a recent study of the oil water transition zone (OWTZ) of a Canadian heavy oil reservoir we have found the bacterial population to be dominated by organisms that would be conventionally considered to be versatile aerobic heterotrophs. We consider four explanations for this apparent paradox. 1. Exposure of samples to oxygen during sampling, transport or storage leading to over growth of the populations that are dominant *in situ*. 2. The reservoirs harbour a "cryptic" aerobic community, which use *in situ* generated oxygen, perhaps from radiolysis of water. 3. Oxygen is supplied externally with meteoric water. 4. The organisms we

think of as aerobes; are in fact anaerobes! While these are all possible, oil field gas compositions and carbon isotope ratios suggest that degradation of oil in these reservoirs occurred under methanogenic conditions and that any aerobic organisms, if indeed they are aerobic, are comparative latecomers in a succession of reservoir microbial populations. We examine the implications of this for genomic studies and biotechnology applications in petroleum reservoirs.

**Methanogenesis from Heavy Oil in Long-Term Microcosms from a Canadian Oil Sands Reservoir**

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Understanding microbial populations and activities in oil reservoirs is central to bioengineering interventions in areas such as souring control and enhancing residual oil recovery. The former can be achieved by stimulating nitrate-reducing bacteria, which oxidise sulphide or block its production by competing with sulphate-reducing bacteria for electron donors. The latter may be achieved by stimulating microbial methane production. We have investigated the microbiology of basal water and surface-mined bitumen from Athabasca oil sands (Alberta, Canada) in 2500-day microcosms composed of bitumen and basal water containing sulphide as a reductant. Replicate vials were sealed with anoxic headspace (85% $N_2$, 10% $CO_2$, 5% $H_2$) and contained either nitrate, sulphate or bicarbonate as the electron acceptor. Aerobic conditions (air in the headspace) were also tested. The only organic substrate in the microcosms was the bitumen. In nitrate-co ntaining microcosms nitrate reduction and sulphide oxidation were detected after 12 days of incubation. In all microcosms that did not receive nitrate, methane was eventually produced reaching maximum values of ca. 8,000 to 40,000 ppm. Methanogenesis in microcosms originally set up under sulphate- or oxygen-reducing conditions suggests that these electron acceptors were consumed first. Methane levels exceeded the yield attributable to the presence of 5% $H_2$ in the headspace of initially anoxic microcosms, and methanogenesis also occurred in the initially aerobic microcosms (0% $H_2$). These results demonstrate that microbial communities in Athabasca oil sands are capable of utilising organic carbon present in severely biodegraded heavy oil as substrates for further biodegradation and methanogenesis over long time scales.

**Selection Of Filamentous Fungi For Bioremediation Of Diesel Oil Contaminated Soils**

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Environmental contamination with petroleum and its products are extremely harmful to ecosystems, most of them often requiring an intervention in order to accelerate the decontamination process. Among the possible methods to be used for this purpose, bioremediation is considered as less expensive and less aggressive to the environment as it usually does not generate environmental liabilities. In this context we are searching for a technology using microorganisms capable of degrading contaminants such as diesel oil, which are so common in environmental accidents. We have isolated filamentous fungi from diesel-contaminated soils, a forest soil and from a compost pile of grape wastes. The fungi were tested for phenol oxidase production in potato dextrose agar with resorcin. Five of the 35 isolated fungi produce this kind of enzyme, which is important to initiate the organic matter degradation. The five selected fungi were inoculated in a forest soil artificially contaminated with diesel oil and the treatment was performed for 30 days in glass flasks at controlled moisture and temperature. At the end of the experiment, the amount of total petroleum hydrocarbon (TPH) was measured by

gas chromatography (GC-FID) and the results were compared with a control treatment (without any inoculant). One strain of fungus was able to reduce the amount of THP. This strain is currently being tested in a bioreactor to develop an efficient method to treat contaminated soils industrially.

**Metagenomics of Microbial Communities from a Transect of Non-contaminated to Highly Hydrocarbon-contaminated Soils in King George Island, Maritime Antarctica**

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Natural environments are negatively affected worldwide by petroleum spills. Antarctica represents one of the last remaining pristine zones on Earth, thus understanding the ecological consequence of petroleum spills in this environment is essential. Therefore, the aim of this study was to understand the impact of oil on microbial communities in Antarctic sites. Samples from a transect of non-contaminated to highly hydrocarbon-contaminated soils were collected around the Brazilian Antarctic Station, located at King George Island. Deep sequencing of community 16S rRNA genes and shotgun metagenomic sequencing were used to characterize the indigenous microbial communities' petroleum degradation potential. The 16S rRNA gene sequencing revealed a successive change in the microbial community according to the level of contaminant concentration. The richness and diversity of Bacteria and Archaea (based on Chao1 and Shannon-H index) decreased as the concentration of oil contamination increased. The most prominent effects of hydrocarbon contamination levels were observed in the relative abundance of Crenarchaeota, Acidobacteria and Gemmatimonadetes phyla, which increased in low and non-contaminated soils. By contrast, the genus *Cytophaga* was detected only in highly hydrocarbon-contaminated soils (11.9%), and the abundance of *Methyloversatilis* progressively increased from 5.43% to 27.2% as the concentration of hydrocarbons increased. The genera *Polaromonas* and *Williamsia* were dominant (up to 13.54% and 12.52%, respectively) in both medium and low hydrocarbon-contaminated soils. Analysis of metagenomic data showed that sequences related to house-keeping genes of Polaromonas were mostly found in metagenomic data obtained from medium and low hydrocarbon-contaminated soils, reaching 18.2% of the metagenomic reads.

**Diesel Oil Degradation Triggered By Plant Growth Promoting Bacteria Isolated From Poplar Trees Growing On A Diesel Contaminated Plume**

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Diesel fuel is a complex hydrocarbon mixture, characterized by high toxicity and carcinogenic potential. This study was performed to unravel the potential role of plant - bacteria partnerships in bioremediation processes, aiming to develop a robust system for the remediation of soils and groundwater contaminated with petroleum hydrocarbons. For that reason, hybrid poplars [*Populus deltoides* x (*trichocarpa* x *deltoides*) cv *Grimminge*] were planted as a means to contain and remediate a diesel plume at a contaminated industrial site in Belgium. Plant roots and soil were sampled in order to isolate bacteria able to grow in the presence of, and to biodegrade, diesel fuel. The isolated bacteria were genotypically characterized based on the 16S rDNA gene and the analysis revealed 20 different taxa, with *Pseudomonas* and *Acinetobacter* as the most abundant genera. All the isolated cultivable bacteria were tested for

their capacity to produce various plant growth promoting traits. The 2,6 DCPIP assay and GC-MS analysis were used for a more in depth examination of the diesel degradation rate and revealed that 25 of 380 isolates, were able to degrade diesel. The two strains with the highest degradation rates were selected for a draft genome sequencing using the Ion Torrent technique. Data from a greenhouse study along with bioinformatics analysis are used to unravel whether the corresponding strains can be exploited for increased remediation efficiency of petroleum hydrocarbon contaminated environments.

**Analysis on Alkane-oxidizing Methanogenic Community from a Production Water of an Oil Indication in Japan**

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Methanogenic alkane degradation has been firstly reported in 1999 by Zengler et al. Since then, many reports have documented the structures of methanogenic communities degrading alkanes or crude oil. However, mechanism of the reaction still remains to be clarified. In particular, the initiator microbe(s) of alkane degradation is regarded as a key player of the reaction and seems to be phylogenetically diverse, hence their diversity and functions should be further studied. In this study, we constructed and analyzed alkane-oxidizing methanogenic community from a production water of an oil indication. Among examined alkanes ($C_8$, $C_{12}$, and $C_{16}$), the community preferably degraded octane. About 85 % of electron from degraded octane was consumed for methanogenesis after 441 day of incubation at 30°C. Pyrosequencing analysis based on bacterial 16S rRNA gene showed that the most dominant taxa were changed from the phylum Proteobacteria in the production water into Firmicutes in the alkane-amended culture. At the family-level classification, uncultured Peptococcaceae bacteria were dominated and followed by uncultured Syntrophaceae in the culture. The function of the former was uncertain yet, but related sequences were also found in oil sands tailings and oil reservoirs. The relatives of the latter were often found and yet to be identified. Based on information of genera in Syntrophaceae, we subcultured the culture into media containing several fatty acids. Methanogenesis occurred in other than propionate-amended medium, however, no bands affiliated to the Syntrophaceae were detected in DGGE gel, indicating that Syntrophaceae-related microbes in our culture might not utilize fatty acids.

**Dynamic Analysis of Methanogenic Communities from Oil Reservoir Production Water After Nutrient Substance and Exogenous Microorganisms Addition by using Functional Genes and Quantitative Real-Time PCR**

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Biologically conversion of in situ $CO_2$ stored in oil reservoirs into methane as a recycled energy source is a potential way, which has attracted more and more attention. Specific primers for the amplification of functional genes concerning this bioconversion as molecular markers have been used to comprehensively detect the variation of methanogenic communities in the production water of an oil reservoir during and after nutrient substance and exogenous microorganims injection. Formyltetrahydrofolate synthetase (FTHFS) and [Fe-Fe]-hydrogenases were used to detect acetogenic bacteria and hydrogen producers, respectively. Methane producers were detected via amplification of the gene *mcrA* encoding for methyl coenzyme M reductase. Quantitative analysis of methane producers was conducted via q-PCR with group-specific primer sets. Results show a rich diversity of functional genes, which substantially implicates the potential of bioconversion of $CO_2$ into $CH_4$. As to the methanogens, the archaeal members

affiliated with the order Methanobacteriales appeared to be predominant in the clone libraries constructed in the first stage of nutrient substance and exogenous microorganisms addition in June and July, 2012, whereas Methanosaetaceae-like clones within the order Methanosarcinales were more abundant in libraries established in nest stage in August and September, 2012. This dynamic shift in methanogenic populations from $CO_2$-reducing to acetoclastic suggests that different carbon fixation mechanisms might have been involved. These findings provide some instructions on promoting substantial value of deployment of $CO_2$ capture and storage (CCS). Keywords: $CO_2$ fixation, methanogenesis, functional genes, microbial communities, oil reservoir, real-time quantitative PCR.

**Characterization of Stimulated Microbial Communities and Changes on Heteroatoms of Crude Oil in an Indigenous Microbial Enhanced Oil Recovery Culture**

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Microbial communities in both anaerobic and aerobic cultures in laboratory were analyzed based on 16Sr DNA clone library technique to provide pictures of stimulated microbial groups in near well region and deep well region for a coming indigenous microbial enhanced oil recovery (MEOR) process. Most of the stimulated bacteria were reported to have the ability to produce desired products for MEOR according to previous studies. The analysis of stimulated microbial communities and their possible uses could act as a new strategy to confirm the feasibility of indigenous MEOR in the study reservoir. In addition, heteroatoms in crude oil and two treated oils coincubated in the anaerobic and aerobic cultures were detected by electrospray ionization (ESI) coupled to high-field Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Results revealed that heteroatoms in treated oils were subjected to biodegradation and micro bial alteration mainly occured in alkyl side chains of these polar compounds. Convertion of long alkyl side chains to short ones also was a desired microbial activity for the application of MEOR. In general, all analysis further indicated that an indigenous MEOR field trial could be carried out in the study oil reservoir.

**Ecophysiology of Anaerobic Syntrophic Hydrocarbon-Degrading Microorganisms**

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The microbiological degradation of hydrocarbons is a complex process performed by aerobic and anaerobic microorganisms. Bacterial oxidation of hydrocarbons with dioxygen, sulfate and nitrate as the terminal electron acceptors very often is complete to carbon dioxide. However, methanogenic degradation of hydrocarbons is only possible via syntrophic interaction of microorganisms. It is thermodynamically feasible only if the dihydrogen partial pressure is maintained low. Methanogenic degradation of naturally occurring organic compounds is well researched and understood in the case of non-hydrocarbons. In the case of hydrocarbons, research is mostly done with complex enrichment cultures and the fine interaction of key microorganisms is not well understood. We studied several highly enriched methanogenic syntrophic cocultures of microorganisms degrading various aliphatic and aromatic hydrocarbons. Methanogenic degradation of alkanes differed from the degradation of aromatic hydrocarbons. In the first case, hydrocarbon-degrading bacteria were not able to degrade the substrate completely and in addition to dihydrogen produced acetate. Acetate degradation by methanogens was necessary for the complete alkanes mineralization. However, syntrophic toluene-degrading bacteria oxidized this aromatic hydrocarbon completely to dihydrogen and carbon dioxide and methane formation was only due to dihydrogen utilization. Methanogenic hydrocarbons degradation in natural environments including oil fields will be discussed.

**Toward the Design of Future Fuel Formulations: Relative Biodegradability of *n*- and iso-alkanes**

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Renewable or 'green' fuels are gaining in importance as alternatives to traditional petro-diesel fuels. Two of the most promising alternative fuels are Fischer-Tropsch (FT) and hydrotreated diesels. While different in production detail, both processes convert renewable feedstocks to hydrocarbon mixtures that include a tailored mixture of branched and straight-chain alkanes. Although the resulting fuels are energy-dense and well suited for mixture with petro-diesel, the relative susceptibility of *iso*- and *n*-alkanes to microbial degradation is not well documented. We therefore tested pure cultures and environmental enrichments for their ability to biodegrade pure alkanes, as well as gasoline, under defined anaerobic conditions. The pure cultures *Desulfoglaeba alkanexedens* strain ALDC and *Desulfatibacillum alkenivorans* strain AK-01 metabolized *n*-alkanes within a narrow metabolic range, but did not metabolize the corresponding *iso*-alkanes. Marine consortia degraded short- and long-chain iso-alkane mixtures at a significantly slower rate than the n-alkane counterparts. Similarly, a sediment enrichment from a hydrocarbon-contaminated aquifer incubated with gasoline could metabolize C5-C9 branched alkanes, but degraded the corresponding *n*-alkanes at a faster rate. Overall, our results indicate that iso-alkanes are amenable to anaerobic biodegradation, but they are more recalcitrant than n-alkanes. Understanding the factors that govern alkane recalcitrance may enable the design of better alternative fuels that resist decay yet biodegrade in the likely event these substrates reach the environment.

**Ozone Pre-treatment of Synthesized Naphthenic Acid Compounds Improves Biodegradation**

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The oil sands industry, located in North-Eastern Alberta, has become an integral part of Canada's economy. Over 170 billion barrels of crude oil are currently recoverable from oil sands deposits. Shallow deposits are processed by surface mining and caustic hot water extraction, generating 200 million litres per day of tailings (i.e. mixtures of sand, fines and oil sands process-affected water (OSPW)) every day. A significant challenge facing the oil sands industry is treating acutely toxic organic compounds found in OSPW, historically called "naphthenic acids (NAs)". Biodegradation is a cost-effective treatment option, but recalcitrance of NAs to degradation by microorganisms has been described previously. Pre-treatment via chemical oxidation was explored to increase NA bioavailability. Three synthesized model NA compounds were exposed to ozone for 0, 30, and 60 s prior to aerobic incubation for ten days. Growth was monitored by optical density and NA degradation by GC-MS. Forty percent of adamantane-1-carboxylic acid was biodegraded following 60 s ozone treatment, while no change was seen in the unozonated sample. Biodegradation of *cis,cis*-decahydro-2-naphthenic acid increased by 10% following 60s ozonation. Light ozonation did not enable biodegradation of 1,2,3-tetrahydro-2-naphthenic acid. In conclusion, this study demonstrated that a low dosage chemical oxidation, such as ozone treatment, is a potential pre-treatment step to improve biodegradability of recalcitrant NAs in OSPW.

**Phylogenetic Diversity of Bacterial Communities Associated with Bioremediation of Crude Oil in Microcosms**

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Bioremediation, mainly by indigenous bacteria, has been regarded as an effective way to clean up oil pollution after oil spill. In order to obtain a systematic understanding of succession of bacterial communities associated with oil bioremediation, sediments collected from Penglai 19-3 oil platform were co-incubated with crude oil. Oil biodegradation was assessed on the basis of changes in oil composition monitored by GC-MS. Changes in the bacterial community structure were detected by two 16S rRNA gene based culture-independent methods, denaturing gradient gel electrophoresis (DGGE) and clone library. The results suggested that crude oil was rapidly degraded during 30 days' bioremediation period. Bacteria affiliated into the genus *Pseudomonas* dominated all the three clone libraries. But dramatic changes were also detected in the process of biodegradation of crude oil. The "professional hydrocarbonocastic bacteria" (e.g. *Alcanivorax*) became abundant in the two samples executed in the bioremediation. Meanwhile, Delta-proteobacteria was only detected in the two samples. Information on bacterial community revealed in this study will be useful to develop *in situ* strategies for the bioremediation of Penglai 19-3 oil spills.

**Effect of Naphthenic Acids on Marine and Freshwater Algae**

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Naphthenic acids (NAs) occur naturally in petroleum deposits and can enter freshwater and marine environments through natural and anthropogenic sources. Despite this, little is known about the toxic effects of NAs on the photosynthetic processes in marine/freshwater phytoplankton. We investigated the effects of two different NAs: (4'-*n*-butylphenyl)-4-butanoic acid (*n*-BPBA); (4'-*tert*-butylphenyl)-4-butanoic acid (*tert*-BPBA) and natural NAs (i.e. NAs extracted from oil sands process waters) on maximum photosynthetic efficiency ($F_V/F_M$) and cellular growth in *Emiliania huxleyi* and *Chlorella vulgaris*. The ability of *E. huxleyi* and *C. vulgaris* to biodegrade NAs was also evaluated. *n*-BPBA (at 10 mg $L^{-1}$) and *t*-BPBA (at 50 mg $L^{-1}$) were highly toxic to *E. huxleyi*, causing significant decreases in $F_V/F_M$ and cell abundance after eight days. By contrast, only the higher concentration of 100 mg $L^{-1}$ of *n*-BPBA and *t*-BPBA affected cell abundance in *C. vulgaris* after eight days. The natural NAs had no deleterious affects on either $F_V/F_M$ or cell abundance with *E. huxleyi* and *C. vulgaris.* In conclusion, we demonstrated that at concentrations as low as 10 mg $L^{-1}$, some NAs are highly toxic to aquatic phytoplankton causing deleterious effects upon photosynthesis and growth. We also provide some evidence that $F_V/F_M$ could be applied to monitor NA detoxification in aquatic environments.

**Microbial Conversion of Crude Oil to Methane in Column Systems Simulating Marginal Oil Fields**

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Recent findings have shown that microorganisms can convert crude oil hydrocarbons to methane in shallow and subsurface oil-containing environments. This metabolic ability suggests the technological potential for recovering crude oil in the form of methane from marginal oilfields

where conventional extraction techniques are not longer economical. We assessed the feasibility of bioconverting residual crude oil to methane using sandstone-packed column systems simulating residual oil fields. Columns were amended with methanogenic, oil-degrading cultures enriched from either oil reservoir production waters or oil-contaminated sediments. Inoculated columns, and uninoculated or oil-free controls were incubated under anaerobic conditions for over a year. Methane and carbon dioxide production were monitored over time. Crude oil in the columns was extracted and analyzed by gas chromatography. In addition, microbial community analysis of the enrichments and columns was done by 454 pyrosequencing. The highest level of methane production (up to 173 µmol) was measured in a column amended with an oil production water-culture. In the same column, 50 to 70% of *n*-alkanes ($C_7$ to $C_{18}$) were depleted relative to controls. The microbial community structure of the inoculated column shifted compared to the original enrichment culture used for the inoculation. The number of archaeal reads, mainly methanogens, was enriched in the inoculated microbial community by 47%. Bacteria that were enhanced included members of the genus *Pseudomonas, Clostridium, Desulfovibrio* and *Sedimentibacter*. Our results showed that the simulated oil reservoir columns can be used to examine the prospect for oil-to-methane bioconversion to study methane production, oil biodegradation, and key microbial community members.

**BIOSURFACTANTS FROM YEAST: Production, Characteristics and Application**

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*University of Mumbai, India*

Biosurfactants or surface-active compounds, usually extracellular, are produced by microorganisms (bacteria, yeast and fungi).The molecules reduce surface tension of both aqueous solutions and hydrocarbon mixtures.Research on biological surfactant production has grown significantly due to the advantages they present over synthetic compounds such as biodegrability,low toxicity,diversity of applications and functionality under extreme conditions. Although majority of microbial surfactant have been reported in bacteria, the pathogenic nature of some producers restricts the wide application of these compounds; thus biosurfactant production using yeast is advantageous due to its GRAS status (generally regarded as safe) which allows it application in not only in bioremediation of oil-polluted soil and water,use in detergent industry,oil recovery enhancement but also in food and pharmaceutical industry. In this study, the yeast spp. were isolated from hydrocarbon contaminated soil by measuring the surface tension (ST) of culture broth using du nouy ring method.15 isolates were obtained from which 4 were found to be positive, out of which 2 of the best strain were used for further studies named Bio1 and Bio6 which reduced the ST of broth from 48 mN/m to 36 mN/m and 37 mN/m respectively,and was found to be highly efficient when compared to commercial surfactant like tween 80-39mN/m and Sodium dedecyl sulphate-36mN/m.On identification the yeast spps where found to be *Candida tropicalis* and *Rhodotorula mucilaginosa*.Further optimal conditions for biosurfactant activity with respect to different parameters-carbon source(almond oil,sunflower oil,paraffin oil,groundnut oil,mustard oil,coconut oil ,gingly oil(til).Nitogen source-(yeast extract ,peptone,tryptone,beef extract,ammonium sulphate,ammonium nitrate,sodium nitrate).pH-(4,4.5,5,5.5,6,6.5,7) were optimized for production,fr om which for Bio1 (yeast extract (1%)+almond oil-pH-5) and for Bio6(peptone (1%)+gingly oil-pH-5.5) results were obtained by performing emulsification assay(E24).The crude biosurfactant produced from Bio1 and Bio6 was obtained by extract with chloroform and proved to be mixture of protein-carbohydrate complex and protein-carbohydrate-lipid complex respectively by thin layer chromatography(TLC).Further protein:carbohydrate content was determined and found to be for Bio1(12.45 mg/ml:58 mg/ml) and Bio6(15 mg/ml:66 mg/ml).The characterization was confirmed by caring out FTIR analysis.The effect of different pH,salinity,temperature on stability of biosurfactant were also evaluated by performing emulsification assay.The cell hydrophobocity assay was also carried out to determine the affinity of the organism towards the hydrocarbon and was found to be for Bio1- 94.9% and Bio6 -99%.The sugars and amino-acids present in it

were identified by means of chromatography.The biosurf actant exhibited antimicrobial activity towards *B. subtilis, S. aureus*, and *E. coli*; and antifungal activity against *C. albicans*.It also has anti- adhesive property. Thus the biosurfactant produce from the *Candida tropicalis* and *Rhodotorula mucilaginosa* have potent biomedical applications.

**A Comparative Study On Biodegradation Of Diesel By Single Bacterial Isolates And Consortia In Liquid media (Flask Studies)**

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Petroleum hydrocarbons such as diesel fuel have been known to be a serious environmental problem. Concerns have been raised by government and environmental authorities in different countries about these problems. Different methods have been used to treat contaminated sites such as excavation and incineration. These methods are costly and they alter the general ecosystem in soils and water. Biodegradation, a cost effective method which involves the use of microorganisms to degrade these pollutants have been looked into in this study. Biodegradation of diesel in liquid media was carried out in the laboratory using single bacterial isolates and consortia which were isolated from a diesel contaminated soil. Pure isolates were enriched in diesel media and stock cultures were stored at 20°C until when needed. The degradation of diesel with single bacterium isolates was compared to that of different consortia. Results obtained showed that diesel was effectively degraded by both single bacterium strains and consortia. However, it took 12 days for single isolates to degrade 400mg/L of diesel while 800mg/L of diesel was degraded by the various consortia within 5 days. An average of 5.80±0.15 and 6.90±0.12 mg/L of low and high molecular weight polycyclic aromatic hydrocarbons was degraded daily by single isolates. On the other hand, an average of 32.30±0.11 and 20.02± 0.22 mg/L of low and high molecular weight polycyclic aromatic hydrocarbons was degraded daily by the different consortia. Diesel degradation was gre atly enhanced by stabilizing various factors linked to the environment such as temperature, pH, and nutrient availability. Biodegradation of diesel and other petroleum hydrocarbons can be achieved within a short space of time by using bacterial consortia as inocula.

**Study of the Biosurfactants Production by Degrading Hydrocarbons Marine Bacteria**

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Biosurfactants, naturally produced by microorganisms when grown on specific substrates, can be widely used in bioremediation of hydrocarbon contaminated marine ecosystems. In this work, wild strains of *Bacillus* sp. RFA, *Bacillus* sp. F9S, and *Pseudomonas* sp. B10, isolated from a marine environment, and with known degradation capabilities, were cultivated in three different growth media (MMM, YPG, and salt medium enriched with glycerol) to evaluate their ability to produce biosurfactant in each medium. The superficial and interfacial tension were measured and the kinetic of the surfactant synthesis were determined to each of the bacterial strain grown on independently on each medium. The results showed that the highest interfacial tension values were observed when the strains were grown on MMM medium. In particular, *Bacillus* sp. RFA, produced significantly high interfacial tension when 10 and 20% of biosurfactants were produced (6.2 and 5.4 $dyn.cm^{-1}$, respectively). Similarly, *Bacillus* sp. F9S produced 20% biosurfactants with 5.4 $dyn.cm^{-1}$ of interfacial tension. Isolates grown on YPG medium did not show significant differences ($p<0.05$) of interfacial and superficial tension values with the exception of *Bacillus* sp. F9S. On the other hand, significant differences were observed when the strains were grown on gycerol ($p>0.05$). The kinetic study revealed that after 48 hours

*Pseudomonas* sp. B10 showed the lowest tension values. In conclusion, the three strains studied are able to produce biosurfactant with the concomitant reduction of superficial and interfacial tensions. Therefore, either the bacterial strains or the biosurfactants could be used in future marine bioremediation procedures.

**Study of the Biosurfactants Production by Degrading Hydrocarbons Marine Bacteria**

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*Cuban Petroleum Research Center (CEINPET), Cuba*

Biosurfactants, naturally produced by microorganisms when grown on specific substrates, can be widely used in bioremediation of hydrocarbon contaminated marine ecosystems. In this work, wild strains of *Bacillus* sp*.* RFA, *Bacillus* sp*.* F9S, and *Pseudomonas* sp*.* B10, isolated from a marine environment, and with known degradation capabilities, were cultivated in three different growth media (MMM, YPG, and salt medium enriched with glycerol) to evaluate their ability to produce biosurfactant in each medium. The superficial and interfacial tension were measured and the kinetic of the surfactant synthesis were determined to each of the bacterial strain grown on independently on each medium. The results showed that the highest interfacial tension values were observed when the strains were grown on MMM medium. In particular, *Bacillus* sp. RFA, produced significantly high interfacial tension when 10 and 20% of biosurfactants were produced (6.2 and 5.4 $dyn.cm^{-1}$, respectively). Similarly, *Bacillus* sp*.* F9S produced 20% biosurfactants with 5.4 $dyn.cm^{-1}$ of interfacial tension. Isolates grown on YPG medium did not show significant differences ($p<0.05$) of interfacial and superficial tension values with the exception of *Bacillus* sp*.* F9S. On the other hand, significant differences were observed when the strains were grown on gycerol ($p>0.05$). The kinetic study revealed that after 48 hours *Pseudomonas* sp. B10 showed the lowest tension values. In conclusion, the three strains studied are able to produce biosurfactant with the concomitant reduction of superficial and interfacial tensions. Therefore, either the bacterial strains or the biosurfactants could be used in future marine bioremediation procedures.

**Respiratory activity of microorganisms decomposing petroleum**

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The great interest in efficient and cost-effective processes for bioremediation of petroleum-contaminated soil has pointed to the need to better understand the process. This study sought to quantify the specific capacity of microorganisms isolated in an assay of phytoremediation to degrade oil. The tests consisted of a system of $CO_2$-free air circulation in portions of oil-contaminated with substrate inoculated with each individual microorganism. The $CO_2$ given off was collected in a trap. The elapsed time was 180 h, until the trap is removed and the amount of $CO_2$. Tests were also conducted with added oil and glucose and with addition of glucose only, as a control. 10 isolates were tested, where 3 showed synergistic effect of addition of glucose with the oil, 3 showed higher activity only with glucose, 2 were similar both with oil as with glucose and 2 showed activity with oil greater than with glucose. Respiratory profiles of each isolated reflect the microbial diversity found in the soil, showing a difference in intensity of respiratory activity. The respiratory test can be a tool to evaluate efficiency in selection of isolated microorganisms for bioremediation projects. The study points to the need to work with "pools" of best-isolated performance.

**Entrapment of a metagenomic clone originated from oil reservoir in chitosan beads for application in bioremediation**

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Oil spills, whether accidental or due to human activities, are a frequent environmental problem that has been reported worldwide with huge economical impacts. Bioremediation process uses biological agents or their enzymes to mineralize or complex organic and inorganic pollutant compounds, transforming them into inert or non-toxic compounds. In this work, the biodegradation potential of clones from metagenomic libraries assembled from oil samples (Potiguar Basin, RN) was evaluated in microcosms scale aiming at a future application in bioremediation process. Microcosms experiments were carried out using artificial seawater, petroleum sample and vitamin solution in Erlenmeyer flasks. Chromatographic analyses were carried out in order to monitor the degradation of petroleum every seven days, for 21 days. The results demonstrated that the metagenomic clones were capable of degrading most of the compounds present in the oil sample. One of the metagenomic clones, named 2B, was selected as the best degradation-performer, and further submitted to entrapment in chitosan beads. Chitosan represents a source of ecofriendly material, with some interesting properties like biodegradability and lack of toxicity and the entrapment can offer a selective advantage to microorganisms under environmental changes. A 3% chitosan gel was prepared and the microbial cells were mixed into the gel. Cell counting by spread plate method was carried out onto ATGE plates before and after the immobilization process. Results showed that the immobilization process was successful. Further tests will be conducted using microcosms and the clone entrapped in chitosan beads in order to compare the degradation efficiency with the assays utilizing free cells.

**Microbial community composition by illumina hiseq sequencing during storage of blend (b10) contaminated with an uncharacterized inoculum, with and without mbo (50%)**

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During the diesel and biodiesel blends storage, microbial biofilms are formed at the oil/water interface in on the tanks, resulting in operational problems. The use of biocide should be evaluated to determine whether its use is appropriate to control the microbial contamination. This study utilized a B10 blend diesel-biodiesel (an inoculum uncharacterized was used as ASTM E1259-10 determinations), which was incubated with a product MBO (50%) at 0ppm (Control), 500ppm and 1000ppm, during 60 days. The biofilms formed at interface oil-water were collected for isolation of the genomic DNA, followed by amplification, purification, and Illumina HiSeq sequencing of the 16SrRNA gene. In total, 338,145 sequences were obtained, whereas according the RDP database, only 40,6% of those were classified (137,222 genera) while 59,4% were not classified (200,923 genera). The prevalent classified genera on the inoculum were *Burkholderia* (16,4%), *Comamonas* (10,5%), *Dysgonomonas* (5,9%), and *Hylemonella* (4,7%); and in the treatment without biocide (control-0ppm) at 28 days were *Pseudomonas* (12,1%), *Comamonas* (10,7%), *Burkholderia* (7,1%), and *Dysgonomonas* (4,6%). On the control treatment at 60 days the prevalent genera identified were the same as at 28 days. The treatment with 500ppm of MBO(50%) biocide reduced 5.2 fold the richness in the samples after 28 days of incubation, and the prevalent identified genera were *Comamonas* (12,0%), *Coprococcus* (7,5%) and *Pseudomonas* (4,2%). After 60 days, the formed biomass in

the treatments with 1000 ppm, 500 ppm and 0 ppm of MBO 50%, were similar. The monitoring of the microbial community composition can inform which genera are more resistant to MBO (50%) biocide addition after different times.

**Degradation of phenol by hydrocarbon assimilating *Candida* isolates**

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Phenols are occurring industrial pollutants and are often found as co-contaminants in the environment. The present study aims to evaluate the biosorption of phenol Candida isolates from different sources and select candidates to be used as biosorption and bioreduction of degradable aromatic hydrocarbons in water contaminated with hydrocarbons. Forty one (41) *Candida* strains (12 *Candida tropicalis*, 13 *Candida lipolytica*, 6 *Candida maltosa*, 10 *Candida parasopilis*) from Petrol Stations, Sludge and Wastewater Plants were screened for their phenol removing potential. The in vitro tests included survival and activity of these strains for phenol removing at different pH and temperatures regimes. Four (4) *C. tropicalis*, 3 *C. lipolytica*, 2 *C. maltose* and 6 *C. parasopilis* strains demonstrated the highest final population (>7 log cfu/ml) after 3 h of exposure at low pH and temperature. The majority of the tested strains was resistant and exhibited partial phenol degrading up to 700 µg/ml. Scanning electron microscopy of Candida indicated that no damaged of the cells or not signs of irregular wrinkled outer surface of cells when treated with phenol compared to the control. These strains are good candidates for further investigation within different wastewater treatment plan systems to elucidate their potential biosorption and bioreduction of hazard benefits in the system used for treating wastewater contaminated with hydrocarbons.

**Phytoremediation of petroleum hydrocarbons and bacterial structure from the rhizosphere**

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Nowadays sections of several areas have been observing with great interest the crescent number of pollutant existent all over the world. For that reason the interest for remediation techniques increases. Several techniques are studied and the phytoremediation has a prominence place due to its efficiency in the decontamination of the soils and also due to its cost to be more accessible than other techniques. The aim of this study was to analyze the capacity of sunflower (Helianthus annuus) to remove both hydrocarbons from a weathered soil from a landfarming system of a refinery area, and compare the bacteria structure from the rhizosphere of plants by molecular tools. The commercial sunflower cultivar, proper to biodiesel fuel production, was tested in order to permit the hydrocarbons to return to the fuel supply chain. At 45-day cultivation, the percent removals of total petroleum hydrocarbons (TPH) indicated that phytoremediatio n with sunflower may be a promising technology for treatment of petroleum-contaminated soil. For the cultivar tested, it was reached a 14% decrease of TPH concentration in soil. The greatest percentage of reduction of heavy metals was Nickel (approximately 87%) for all trials tested. In this study, the biosurfactant reduced the whole plant growth and root development in 16,5% and 23%, respectively. However the results showed that the presence of the plant increases removal of hydrocarbons and lead. Traditional tools of cultivation revealed that the biosurfactant stimulated the oil-degrading microorganisms population in soil and PCR-DGGE analysis showed that the biosurfactant caused variation in the rhizobacterial structure, diversity and predominant

**Diesel Bioremediation on soils subjected to drought.**

L. SantaCruz, J.I. Vilchez, I. Julca, G.A. Silva-Castro, C. Calvo, **M. Manzanera**.

*University of Granada, Spain*

Bioremediation of hydrocarbon-polluted soils requires a minimum of 20% humidity for an optimal microbial activity. Therefore humidity is a limiting factor in the bioremediation processes of arid and semiarid soils (Calvo et al. 2009). We have previously shown that at 20% humidity or over, soil microorganisms remove 68,5 ppm Total Petroleum Hydrocarbons per day (TPH/day). However, at 10% humidity TPH removal is dramatically reduced to 0,6 ppm TPH/day. To improve bioremediation of diesel-polluted arid and semiarid soils, we isolated a collection of 15 desiccation-tolerant microorganisms with the ability to grow in mineral media with Polycyclic Aromatic Hydrocarbons (PAHs) as the sole carbon source (Vilchez and Manzanera, 2011). In addition, some of these strains were shown to protect plants from drought to improve the rhizoremeditaion of diesel-polluted soils under water stress. Combination of PAH-degrading strains with plants and desiccation tolerant strains showed to be a more efficient treatment for the removal of diesel from soil under water stress (Narvaez-Reinaldo et al., 2011).

## Prospects for MEOR/ Biodesulfurization/biofuels

**MSP microorganisms in Oil Reservoirs: Microbial Selective Plugging Technology for Enhanced Oil Recovery.**

**K. Boccadoro**, S. Ramanand, E. Austerheim, A.V. Krøvel, A. Mehrabi, A. Stavland, T. Hildebrand-Habel.

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This work concerns a new Microbial Selective Plugging technology for Enhanced Oil Recovery (EOR). The aim of this project is to investigate whether anaerobic thermophilic MSP microorganisms could be grown in reservoirs to selectively plug water-rich oil depleted areas, diverting injection water flow into more oil saturated areas and thereby contributing to increased tail-end oil production. These bacteria are able to thrive in temperature, pressure, pH and salinity conditions of water-flooded areas of many oil reservoirs. They produce ethanol, which reduces the viscosity of crude oil and can in itself enhance oil recovery. Strain MSP1 has an interesting propensity to grow in low hydrocarbon environments and will reduce the permeability of porous sandstone media through the formation of a microbial plug in predominantly aqueous zones. Core flooding experiments conducted at pressures over 200 bar have shown that the plug´s size and metabolic activity can be controlled through changes in flow and nutrients in the injection water. The microbial plugging behaviour in standstone cores will divert water injection sweeps to lower permeability reservoir zones, causing EOR.

**Mechanistic Modeling and Advanced Monitoring of Microbially Enhanced Oil Recovery and Souring Processes**

**S. Hubbard**, V. Surasani, C. Hubbard, Y. Wu, J. Ajo-Franklin, J. Coates

*Lawrence Berkeley National Laboratory, USA*

Souring processes and microbially enhanced hydrocarbon recovery (MEHR) strategies are influenced by reservoir microbial, geochemical, hydrological and geological properties that occur over a range of scales. Our objective is to develop mechanistic reactive transport modeling and advanced geophysical approaches that can simulate and monitor the interplay of

these factors, respectively. We use the developed methods to explore MEHR strategies that strive to alter reservoir permeability through biopolymer clogging as well as strategies that strive to mitigate souring by favorably poising the reservoir redox state. For both objectives, laboratory studies were first conducted to quantify system responses to various treatments. Column pore fluid analysis permitted refinement of the reaction networks used in the mechanistic reactive transport models, and column time-lapse geophysical monitoring permitted identification of the geophysical signatures of induced biogeochemical processes. Synthetic reactive transport and geophysical monitoring studies were then performed in representative reservoirs using knowledge gained from the laboratory experiments. For the MEHR bioclogging study, we demonstrate the use of modeling to quantify key factors that lead to enhanced reservoir sweep and the ability to monitor bioclogging using seismic and complex electrical approaches. For the souring studies, we demonstrate use of a developed model to accurately predict the onset of biosouring, to assess souring treatment strategies, and to assess the ability to remotely monitor induced redox transformations using galvanic potential methods. Collectively, we show that a combination of advanced monitoring and modeling approaches has the potential to aid in the translation of laboratory process understanding into successful field scale practices.

**Are Biodiesel and its Blends More Susceptible to Microbial Growth than Conventional Hydrocarbon Diesel?**

**E. McFarlane**

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There is growing pressure to increase use of bio-components to develop renewable energy and reduce carbon dioxide emissions. However, there is some evidence from both field and laboratory studies to suggest that biodiesel and its blends are more susceptible to microbial growth than conventional hydrocarbon diesel. This growth can cause deterioration of product quality, filter blockage and corrosion problems. Then again, contradictory conclusions have been drawn from other studies that have demonstrated either no difference or even decreased susceptibility to microbial growth with biodiesel and its blends; this is clearly a complex area worthy of further investigation. A recent study found that higher biodiesel blends may inhibit or reduce microbial growth; water scavenging by biodiesel is cited as a possible explanation [1]. Further industry work to investigate this phenomenon is currently being undertaken. In this study, microbiological experiments have been performed over two months, in two-phase systems containing water and fuels with varying bio-component. Mixed bacterial and fungal genera were used as aqueous media assay organisms and growth assessed by standard plate count and IP385; increased biomass was estimated qualitatively by visual rating and quantitatively by IP416 . Results show a decreased susceptibility to microbial growth with some biodiesels. If these results are supported by conclusions from the industry investigation, this presents a very positive message for fuel supply and distribution, potentially leading to a reduced risk of operational issues. Reference 1. Microbial growth in diesels and other fuels containing fatty acid methyl esters (FAME), Technical Bulletin May 2011. www.energyinst.org

**Combined Application of Chemical and Molecular Approaches for the Detection of Signature Metabolites and Functional Microorganisms Implicated in the Anaerobic Degradation of Oil Hydrocarbons in Petroleum Reservoirs**

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Recent investigations of petroleum reservoirs have indicated the large occurrence of anaerobic microorganisms and the depletion of petroleum reserves. Studies conducted with axenic

cultures have indicated that anaerobic metabolism of oil hydrocarbons can proceed via fumarate addition, carboxylation and other alternative mechanisms. To date, the detection of signature metabolites and functional microbes in oil reservoir fluids have only been scarcely reported. Here, we show the application of chemical and molecular methods to characterize the production fluids from different oil bearing horizons in China. First, signature metabolites (benzylsuccinate, methyloctylsuccinate, methylpentadecylsuccinate, cyclohexylsuccinate, ethylsuccinate etc.) derived from addition to fumarate were chemically synthesized and characterized by means of GC-MS to serve as authentic standards for the identification of similar metabolites in environmental samples. Derivatized organic extracts from production fluids were then subjected to gas chromatography-mass spectrometry (GC-MS) for MS fragment ions indicative of similar anaerobic metabolites. MS profiles suggested the presence of metabolites derived from the anaerobic degradation of oil hydrocarbons (alkylsuccinate, alkanoic acids, monoaromatic acids and naphtoic acids). Functional microorganisms were detected by using a combination of functional genes for fumarate addition and genes implicated in the subsequent degradation of monoaromatic hydrocarbons via the benzoate pathway. The currently established approach is of fundamental interest for the understanding of metabolic pathway and microbial communities involved in the anaerobic degradation of oil hydrocarbons. These biomarkers can also be applied for evaluation of their potential in-situ anaerobic degradation and the likely environmental and ecological constraints on the kinetics and thermodynamics of microbial growth.

**Subsurface Bio-Electrochemical Conversion of Carbon Dioxide into Methane by using Indigenous Microorganisms**

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As potential $CO_2$ geological storage site in CCS, the use of depleted oil-gas reservoirs and aquifer has been proposed. The long-term aim of this research is to establish a biotechnological system to microbiologically convert geologically stored $CO_2$ into methane, as energy resource. To develop a means for the conversion, we focus on technological application of bio-electrochemical reaction using microbially catalyzed electrode. On the electrode surface, methanogenic microorganisms (as biocatalysts) utilize electrical current (electrons) to reduce $CO_2$ ($CO_2$ + 8H+ + 8e- → $CH_4$ + $2H_2O$). For such system, recruitment of microorganisms indigenous to the reservoir as the biocatalysts will be a better choice, as they are more likely to maintain the activity within the reservoir. Toward technological application of the bio-electrochemical system in $CO_2$ geological reservoir, we examined bio-electromethanogenic activity of subsurface microbial consortium for the first time. Indigenous microorganisms originated from a formation water of a domestic depleted oil reservoir were inoculated into electrochemical-cultivation reactors. Upon application of constant voltage of -0.75 V, the reactor inoculated with reservoir-indigenous microorganisms produced methane at a rate of 386 mmol day-1m-2 (cathode surface area), which was the highest electromethanogenic production rate so far documented. Moreover, current-to-methane conversion efficiency of the reaction was as high as approx. 98%. Thus, we concluded that microorganisms indigenous to the subsurface reservoir are highly capable of electromethanogenic reduction of $CO_2$. Electrochemical and microbial analyses suggested a reaction mechanism, in which electron-releasing bacteria mediated electron transfer from the electrode to methanogenic archaea.

**Comparative Analysis of Microbial Communities in Water Samples from Oil Fields ever Subjected to CO2-flooding and thoroughly Water-flooding using 16S rRNA gene**

**J-F. Liu**, X-B. Sun, G-C. Yang, S-Z. Yang, D-J. Gu, B-Z. Mu

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Carbon dioxide ($CO_2$), when dissolve in formation water, will result in the acidification of the brine, which leads to alterations of mineral phase sand increase in concentration of inorganic components. This alteration may hence affect microbial activities, microbial attachment and biofilm formation. Also as a matter of fact, $CO_2$ is a potential source of carbon and energy of some methanogens and acetogenic bacteria. The injection of $CO_2$ will notably influence the microbial structure and their activity *in situ*. Aiming to evaluate the potential impact of injected $CO_2$ on microbial communities in oil reservoirs, a molecular survey based on 16S rRNA gene cloning has been performed on microbial communities inhabiting the production waters from high temperature oil-bearing beds ever subjected to CO2 flooding and only water flooding. Results show that the microbial community in water flooded oil reservoir was predominantly composed by se quences affiliated to members of the Thermodesulfobacteria, Proteobacteria and thermophilic $CO_2$-reducing methanogens (*Methanothermobacter* spp). Sequences clustering with the Firmicutes, Thermotoga, Nitrospira, and Bacteroidetes and archaeal sequences affiliated with Archaeoglobales were also detected in minor proportions. In comparison, the microbial community composition in which $CO_2$ was injected indicated the predominance occurrence of members of the Proteobacteria, Firmicutes and $CO_2$-reducing methanogens (*Methanothermobacter* spp). Minor proportions of sequences affiliated with Thermodesulfobacteria, Bacteroidetes, acetoclastic and methylotrophic methanogens were also detected. These results provide information to understand how injected $CO_2$ impact microorganisms in subsurface high temperature oil reservoirs.

**Sequencing as a Diagnostic Tool in Microbiological Enhanced Oil Recovery (MEOR) and Characterization of Bacterial Communities in Oil fields**

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Microbiological Enhanced Oil Recovery processes may provide access to previously uneconomical oil reserves or increase oil recovery rates in oil production. However, more quantitative assessments of how different MEOR strategies affect microbial communities will be needed before MEOR technologies gain widespread acceptance. We investigated how the bacterial communities develop when seawater is injected into the reservoir during oil production, and how the microbial communities in produced water from the same oil reservoir develop when incubated with different energy sources and nitrate. The bacterial communities present in samples from 6 producing wells at the Siri and Dan fields in the North Sea and in laboratory scale incubations of produced water from Dan were examined by qPCR and pyrosequencing of 16S rRNA genes in order to address these questions. The Dan wells included wells without seawater breakthrough, representing origi nal reservoir conditions, as well as wells with seawater breakthrough through fractures or the sediment matrix, representing post-injection reservoir conditions. The results indicated that the original populations in the reservoirs at Siri and Dan are highly dissimilar. Samples from the Siri field yielded sequences affiliated with the gram-positive, halophilic order Halanaerobiales, and sequences affiliated with the Marinobacter. Samples from the Dan field yielded a higher diversity of bacterial phylotypes, including sequences affiliated with the "Firmicutes", "Proteobacteria", "Bacteroidetes", Spirochaetes, Thermotogae, and "Synergistetes". The phylogenetic diversity was higher at wells with seawater breakthrough than in wells without seawater breakthrough, and during the incubation of the produced water from the Dan field, the bacterial diversity was markedly altered.

**Effect of the Addition of Tween 80 in the Production of Xanthan Gum by *Xanthomonas campestris* Growing on Oil Industry Residues**

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Xanthan gum is a biopolymer produced by bacteria of the *Xanthomonas* genus that has been used in advanced recovery of oil, mainly due to its ability to form viscous solutions at low concentrations. When dispersed in injected water, xanthan gum increases its viscosity, resulting in improved oil/water wettability, an increase in oil production, and a decrease in produced water from the wells. The present work evaluated the effect of adding tween 80, a nonionic surfactant, to the growth medium of xanthan gum formulated with produced water from an oil producing well in the Recôncavo Basin, in Brazil. The experiments were conducted in Erlenmeyer flasks of 250 mL containing 50 mL of production media at 30ºC, 180 rpm, 72 h. Testing of apparent viscosity was done in aqueous solutions of gum at 1% and 25ºC, with shearing of 2.64 $s^{-1}$. The molecular structure was evaluated with Infrared Absorption Spectroscopy. Gum production without surfacta nt resulted in a maximum production of 4.85 g $L^{-1}$, but with the addition of the tween 80 the maximum production was 8.31 g $L^{-1}$, an increase of 71% in xanthan production. The biosurfactant probably conferred detergent properties to the medium, helping remove xanthan gum from the cellular walls of *Xanthomonas*. Testing of the gum produced without surfactant indicated an apparent viscosity of 232 cP, while with the tween 80 it was more than double, at 492 cP. The molecular structure of the gums produced was similar to the commercial grade gum.

## Exopolysaccharide Yields obtained from Growing *Enterobacter* sp on Glycerine Resultant from the Biodiesel Transesterification

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*Enterobacter* sp can produce a biopolymer rich of rare sugars. These are often used by the cosmetic and/or pharmaceutical industry. On the other hand, it is the petrol industry, which is using significant amounts of such substances. They are used for moving oil from the rock formation. The production of oil from mature wells can be enhanced by 10-40% with the introduction of biopolymer at concentrations higher than 500 ppm and with a viscosity of 40 cP. In order to reduce costs with the production of biopolymer it is necessary the identification and the physiological characterization of new isolates. In addition, it is also advantageous to test the use of cheap sources of carbon for support bacterial biopolymer production. Therefore, the aim of this research was to test biopolymer production by *Enterobacter* sp at distinct concentrations of glycerine resultant from the biodiesel transesterification. Tests were carried out using (i) 2% sucrose, (ii) 2% glycerine pa., (iii) 2% industrial glycerine and (iv) 1:1% glycerine and sucrose, respectively. The respective biopolymer yields in 72 hours were: 4.4, 4.8, 3.8, 3.9 g L-1. Thus, 2% glycerine was identified as the best culturing condition for the production of biopolymer. Statistical analysis suggested that conditions (iii) and (iv) are not significantly different.

## The Effect of Detergent Amendments upon Biopolymer Producing Bacteria: Product Yields and the use of Industrial Glycerine as a Carbon Source

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The petrol industry is being substantially impacted by the novel and innovative biological strategies for recuperating matured wells of low productivity. The strategy known as MEOR is based either on the use of microbial products or upon the injection of microbial cells directly. The most commonly used microbial products are detergents and biopolymers (xanthan-like

substances). The first are used for detaching and/or emulsifying the oil and the latter is used for producing oil from the formation. The aim of this research was to characterize biopolymer productivity at different detergent concentrations and carbon ratios for cultivating biopolymer-producing bacteria. Industrial glycerine leftover (IG) and sucrose (SU) were used in different ratios as the main sources of carbon. Treatments were: (I) 2:2%, IG:SU; (II) 1:1%, IG:SU; (III) 4:0% IG:SU; (IV) 0:4% IG:SU and (V) 1:1% IG:SU without detergent amendment. The corresponding biop olymer production was of 3.88, 7.72, 3.48, 5.27 and 4.67 g l-1, respectively. The statistical analysis showed a positive correlation for the interaction of co-culture of biopolymer-producing bacteria and detergent. Industrial glycerine showed to be adequate for growing the respective strains and for the production of biopolymer as a co-producing strategy.

**Possible Impact of Carbon Capture and Storage on the Methanogenic Activity and Pathway in a High-Temperature Petroleum Reservoir**

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Deep subsurface petroleum reservoirs are candidate sites for carbon capture and storage (CCS). The feasibility of CCS has been mainly studied from a geological perspective. However, little is known about the effects of $CO_2$ storage on microbes inhabiting the reservoirs. In this study, we investigated the effects of the elevated $CO_2$ concentration on the methanogenic microbial community and function in a high-temperature petroleum reservoir by high-pressure incubation experiments mimicking the in situ reservoir (55°C, 5 MPa) or $CO_2$ storage conditions. The microcosms were constructed using the production water and crude oil, pressurized with either $N_2$ or $N_2$+ $CO_2$ (90:10) at 5 MPa and then incubated at 55°C. Methane production was observed with the decrease of acetate included in the production water under both high and low CO2 conditions. However, the stable isotope tracer experiments and molecular biological analyses for both microcos ms showed that the major methanogenic pathway under the *in situ* reservoir condition was acetate oxidation coupled with hydrogenotrophic methanogenesis, whereas acetoclastic methanogenesis occurred under the $CO_2$ storage condition. Based on thermodynamic calculations, the change to acetoclastic methanogenesis by the increase in $CO_2$ partial pressure was energetically more favorable than acetate oxidation. These results clearly indicated that $CO_2$ storage into a high-temperature petroleum reservoir would cause a drastic change in the methanogenic pathways. Importantly, the elevated $CO_2$ concentration invokes the faster and more favorable methanogenic pathway (acetoclastic methanogenesis) for crude oil biodegradation. Our study presents a possibility of CCS for enhanced microbial production of natural gas in high-temperature petroleum reservoirs.

**Laboratory Study and Field Trial of Enhanced Oil Recovery By Selectively Stimulating Indigenous Microbial Community In A Low Temperature Oil Reservoir, China**

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The performance and dynamics of microbial community were investigated in a low-temperature (20°C) petroleum reservoir, China. Culture-dependent and culture-independent approaches were used to examine the response of microbial community to the nutrients, and the gas production, acid production and displacement of oil in core-flooding test, were studied in

laboratory. Then field trials were proceeding with nutrients stimulation from August 2010 to April 2012. The results of field trial indicated that, among the 7 production wells, 6 wells showed a positive response to the stimulation. Until December 2012, the cumulative incremental oil production about 3500 tons was obtained. Microbial community analysis revealed that the bacteria were mainly clustered within three phyla: Actinobacteria, Proteobacteria and Firmicutes. However, the microbial structure and the stimulated bacteria differed widely in different production wells. In T90 and T89 well, the Alcaligenaceae and Pseudomonadaceae were selectively activated and became dominant consortium, while in T95 well, Pseudomonadaceae decreased from 20.03% to 18.24% and 2%, and no Alcaligenaceae were detected. The results showed the feasibility of IMEOR in low temperature reservoir.

**Investigating Microbial Enhanced oil Recovery on Unconventional Resources in Replicated Model Systems using Molecular Techniques**

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Interest in microbial enhanced oil recovery (MEOR) has had renewed interest over the years with the potential to increase oil recovery that cannot be economically retrieved under secondary oil recovery. The advance in modern molecular techniques has allowed a greater understanding of microbial diversity present within formations, with diverse groups of microorganisms being identified that have a wide range of physiological and metabolic activities. Therefore oil reservoirs harbour the ability to sustain indigenous microbial communities that can be utilised for MEOR. In this ongoing study, bacteria have been isolated from core and formation water samples. A 16s metgenomic study on the MiSeq platform has been employed to gain insight into diversity without culture bias. Isolated bacterial species have undergone screening for MEOR potential. Under this study the ability of indigenous microorganisms to form biofilms, biosurfactants and alter contact angles has been investigated. A number of formation specific model systems were constructed to test the ability of isolated microorganisms to increase secondary oil recovery. These involved the oil flooding of sand packed pressurised vessels and subsequent recovery using chemically replicated formation water. Test microorganisms are then introduced with specific nutrient media and locked in to allow incubation for growth and metabolite production. Once these microorganisms have incubated, formation specific water recovery is resumed and the subsequent oil recovered is measured and compared to control recoveries performed on the same core. This study has been studying two concepts of MEOR including the ability of microorganisms to produce plugging biofilms and biosurfactants. Presented will be experiences and results to date on the MEOR project undertaken on a heavy oil field.

**Enabling MEOR: Microbial Community Analysis of a Carbonate Petroleum Reservoir**

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About half of the world's oil occurs in carbonate formations. While existing techniques recover 60-70% of the geological oil from terrigenous formations, recovery from carbonate reservoirs typically does not exceed 20%. Developing enhanced oil recovery methods could significantly increase oil production from known reservoirs. Carbonate reservoirs at biologically-compatible temperatures can contain microorganisms capable of altering oil mobility by producing

polymers, detergents, gases, organic acids, and solvents. Understanding these microbial populations can enable effective, low-impact microbial enhanced oil recovery (MEOR) methods. This study was undertaken to characterize the abundance, phylogenetic diversity and activity of microorganisms in formation waters of a US-based carbonate petroleum reservoir. Formation water in the studied reservoir was 22°C, with pH of 6.8-8.2, low mineral content (8-13 g/l) and low dissolved organic acid content (<12 ppm C). Sulfate content was 0.4-1.7 g/l and $H_2S$ was detected in all samples. The dominant metabolic process in formation water alone was sulfate reduction; methanogenesis was a minor process. Sulfate-reducing and fermentative prokaryotes predominated in enrichment cultures, while aerobic organotrophic microorganisms were scarce or not detected. In carbon-amended media, fermentative and methanogenic enrichments produced gases and low-molecular-weight organic acids and alcohols. Phylogenetic diversity was characterized by Bacterial and Archaeal 16S rRNA gene clone libraries constructed from DNA extracted from formation water. Analysis results of the 16S rRNA gene clone libraries obtained will be reported. The work was supported by CRDF (RUB1-30028-MO-12).

### $CaCO_3$ Biomineralization During Reactive Transport and Implications for Pore Space Occlusion

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Calcium carbonate ($CaCO_3$) is amongst the most abundant and reactive minerals comprising the < 5km deep subsurface of the Earth's crust. Precipitation and dissolution reactions have been linked to enhanced oil recovery sites, and are implicated in pore space alteration and process efficacy. In this study, microbial $CaCO_3$ biomineralization in quartz sandstone reservoirs is evaluated using a silicon-etched microfluidic reactor (i.e., GeoBioCell) that has a uniform pore network. Acetate and nitrate are mixed transverse to flow in the pore network, which promotes growth of a Pseudomonas stutzeri st. DCP-Ps1 inoculum. Acetate and nitrate are the coupled electron donor and acceptor pair, respectively, where their biological degradation produces alkalinity that raises the pH from 6.7 (influent) to a maximum of ~7.2 (in the pore network). After sufficient biomass growth occurs in the pore network, elevated $Ca^{2+}$ is introduced with va rying influent conditions to evaluate if carbonate precipitation occurs and can be attributed to the increase in pH, microbial nucleation sites, unknown factors (e.g., enzyme production) linked to biological activity, or some combination of these factors. Results from these experiments suggest that several mechanisms contribute to $CaCO_3$ precipitation, all of which have direct implications for directly analogous porous and permeable sandstone reservoirs currently targeted for enhanced oil recovery projects around the world.

### Effect of the Addition of *Pseudomonas aeruginosa* Lineage on the Production of Xanthan Gum Grown in Oil Industry Residues

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Xanthan gum is a biopolymer produced by bacteria of the *Xanthomonas* genus that has been used in advanced recovery of oil, mainly due to its ability to form viscous solutions at low concentrations. When dispersed in injected water, xanthan gum increases its viscosity, resulting in improved oil/water wettability, an increase in oil production, and a decrease in produced

water from the wells. The present work evaluated the effect of adding *Pseudomonas aeruginosa* lineage, a biosurfactant producer, to the growth medium of xanthan gum formulated with produced water from an oil-producing well in the Recôncavo Basin, in Brazil. The experiments were conducted in Erlenmeyer flasks of 250 mL containing 50 mL of production media at 30ºC, 180 rpm, 72 h. Testing of apparent viscosity was done in aqueous solutions of gum at 1% and 25ºC, with shearing of 2.64s-1. The molecular structure was evaluated with Infrared Absorption Spectroscopy. Gum pr oduction with only the *Xanthomonas* inocula resulted in a maximum production of 4.85 g $L^{-1}$, but with the addition of the *Pseudomonas aeruginosa* inocula the maximum production was 8.31 g $L^{-1}$, an increase of 71% in xanthan production. The biosurfactant probably conferred detergent properties to the medium, helping remove xanthan gum from the cellular walls of *Xanthomonas*. Testing of the gum produced with only the *Xanthomonas* lineage indicated an apparent viscosity of 232 cP, while with the bacterial consortia it was more than double, at 492 cP. The molecular structure of the gums produced was similar to the commercial grade gum. Key words: Xanthan gum, advanced recovery, biosurfactant.

**Optimization of Surfactant Biomolecule Production with Potential Applications in the Oil Industry**

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Biomolecules with surfactant properties (SBM) produced by microorganisms may change the surface tension (ST) and interfacial (IT). These biomolecules have the advantage of being biodegradable, less toxic and can be synthesized by microorganisms from economical substrates like wastes. The SBM production is affected by different factors such as: specific medium formulation and operating conditions (temperature, salinity, pH, agitation, aeration). The SBM can be applied in oil industry, it can reduce ST and IT, and it can improve the oil rheological properties, and viscosity, which allows the oil release and displacement into the reservoir and pipelines. The aim of this work was to optimize the SBM production with IMP-X strain. The bacteria was isolated from a hydrocarbon-contaminated soil and identified with 16S rRNA. The effect of substrate, concentration, and agitation by Taguchi L9 experimental design was evaluated. The substrates were: soybean oil, hexadecane, and sucrose at concentrations of 5, 10 and 15 mL/L and 100, 150 and 200 rpm. Statistical analysis was performed with STATISTICA V.6 software. The IMP-X nucleotide sequence was deposited in GenBank under accession number HQ686060, corresponding *Serratia marcescens*. The best system for SBM production had soybean oil at a concentration 10 mL/L and 100 rpm. That system decreased ST to 26.5 mN/m with a CMC of 275 mg/L. The variable that had the greatest effect was substrate, followed by its concentration. The SBM production was 33.3 g/L. The results indicate the high potential production of SBM in laboratory level for application in the oil industry.

**Biosurfactant-Producing Bacteria in Hydrocarbon-Enriched Waters From Brazilian Coastal Aquatic Environments**

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Biosurfactants are active compounds produced by microorganisms that modify the surface tension between liquids. These compounds have been increasingly used in bioremediation of accidental oil spills to remove oil from the contaminated surface, enhance the dispersal rate of the oil and stimulate biodegradation by indigenous microorganisms. Biosurfactants have also

been employed for tanks and pipelines cleaning, oil transportation and enhanced oil recovery by microorganisms. In this study, we aimed to isolate bacterial strains able to produce efficient surface active compounds from hydrocarbon-enriched water samples collected at Massambaba beach, Jacarepia lagoon and Vermelha lagoon (located at Saquarema, RJ). Bacterial isolates were screened for biosurfactant production using different detection methodologies such as emulsification activity, oil displacement test, drop collapse test, surface tension measurement, hemolytic assay an d CTAB plate test. From 132 isolates, 22 strains produced biosurfactant using at least one of the six methodologies. Pseudomonas was the predominant genus among the biosurfactant producing strains, although strains belonging to Acinetobacter, Alcaligenes, Achromobacter, Bacillus, Cobetia, Ensifer, Marinobacter and Vibrio genera were also found. An efficient biosurfactant producer, isolated from a naphthalene-enriched water sample from Jacarepia lagoon and identified as Pseudomonas aeruginosa JNB6, was selected for further characterization. It showed a great emulsification activity (53%), a clear halo of 35 mm in the oil displacement test, a flat drop in the drop collapse test and a surface tension of 31.7 mN/m. This strain also showed hemolytic activity. The positive CTAB plate test suggested that the biosurfactant produced by strain JNB6 belongs to the rhamnolipid class.

**The use of Microbial Enhanced Oil Recovery Techniques to Reduce Residual Oil Saturations in Carbonate Reservoirs**

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Selective plugging by microbial biomass is one of the proposed mechanisms for improving reservoir sweep efficiency in fractured reservoirs. In this study, the potential of Bacillus licheniformis strains isolated from oil contaminated soil from Oman was tested for their ability to grow in induced fractures in carbonate rocks and to divert subsequent injection water to the unswept matrix zones. Three Bacillus licheniformis strains were tested with name codes; B29, B17 and W16. Their growth behavior using different nitrogen and carbon sources was investigated. Carbon/nitrogen ratios were optimized where it was found that sucrose was the carbon source that maximized bacterial growth at 2% concentration and yeast extract was the selected nitrogen source with concentration of 0.1%. The combination of B. licheniformis strain W16 in a minimal medium containing sucrose was the optimum condition for maximum cell growth within 10-12 hours of incubation. Indiana limestone core plugs were used for coreflooding experiments where a fracture was simulated by slicing the cores vertically into two sections. The bacterial cells were injected into the cores and the ability of the microbes to grow and plug the fracture was examined. Scanning electron microscopy was used to prove the growth of the microbial cells in the fracture after the experiment. Coreflooding experiments showed promising results where enhancement of oil recovery was observed after bacterial injection. A total of 27-30% of the residual oil was produced after 11 hours of incubation. This shows the high potential of using microbial biomass for selective plugging in fractured reservoirs.

**Roles and Contribution of Chromatographic Data Interpretation while Drilling to Evaluate Low Thickness Hydrocarbon Bearing Sand to Detect Low Thickness Pay Zones and Maximized the Productivity. Ras Fanar Field. Gulf of Suez, Egypt (case study)**

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Ras Fanar oil field located on the western margin of the Gulf of Suez. It is situated Offshore at a depth of ~115 ft. The distance from Ras Gharib shoreline is approximately 3.5 Km East. Ras Fanar field is producing from Belayim Nullipore (local name for Reefal limestones of Ras Fanar

and Ras Gharib oil fields) carbonate reservoir. Zeit Sand member is a secondary target which containing accumulation of oil discovered in a thin sand body in the basal part of the Zeit Formation, (Middle Miocene). This thin sand body tested oil by one well only (31° API). This paper shows how we can Evaluate Zeit sand by using chromatographic data because we have a little logging data covered this interval (most wells without logging in this interval also logging tools which covered this interval were old thus we get inaccurate measurements). We are using new equations for gas reading (chromatographic data) to determine Hydrocarbon type and quality, with satisfactory results. To determine the accuracy of this method this paper shows the result of chromatographic data correlated by test results of Exploratory well test (tested 350 BOPD from Zeit Sand) and sample description, and is in agreement.

**Sophorolipids Production by *Candida bombicola* ATCC 22214 and its Possible Application in Enhancing Oil Recovery**

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Sophorolipids biosurfactant production by *Candida bombicola* ATCC 22214 and its possible applications in enhancing oil recovery were studied at laboratory scale. The seed media and the production media were standardized for optimal growth and biosurfactant production. The production media were tested with different carbon sources: glucose, corn oil, glucose and corn oil. The samples were harvested at 24h interval up to 120h, and checked for growth (OD660), biosurfactant production (ST, IFT) and pH changes. The medium with both glucose and corn oil gave better biosurfactant production and reduced surface tension (ST) and interfacial tension (IFT) to 28.56 $\pm$ 0.42 and 2.13 $\pm$ 0.09, respectively in 72h incubation. The biosurfactant was quite stable at 13-15% salinity, pH range of 2-12, and up to 100 °C temperatures. The biosurfactant gave stable emulsions ($\%E_{24}$) with different hydrocarbons (*n*-Pentane, Hexane, Heptane, *n*-Tridecane, *n*-Tetradecane, *n*-Hexadecane, 1-Methyl naphthalene, 2,2,4,4,6,8-Heptamethylnonane, light crude oil and heavy crude oil). The sophorolipids were extracted by ethyl acetate and will be partially characterized using TLC, FTIR and HPLC. The potential of sophorolipids in enhancing oil recovery will be tested using core-flooding experiments.

**Indigenous Microbes in Oil Formation and Their Potential for MEOR**

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The goal of this study was to simultaneously assess the number, geochemical activity and phylogenetic diversity of microorganisms in the Daqing oil field and to determine whether microbial populations capable to produce compounds useful for MEOR were present in this oil field, Potential of indigenous microorganisms for MEOR also was studied. The 16S rDNA-based molecular approach and analytical, microbiological and radioisotope methods were used for comparative studies, and a micro glass plate model was used to conduct microbial-flooding experiments. It was shown that the number of most studied groups of microorganisms and diversity of prokaryotes in formation waters from the lot subjected to polymer flooding was higher than in waters from the water-flooded lot. Physical modeling of the growth of aerobic oil-oxidizing and anaerobic methanogenic microorganisms in porous matrix shows that a key component of the oil mobilization m echanism is the capability of microorganisms to produce localized high concentrations of biosurfactants or gases, respectively at an oil-water interface. The stimulation of the biosurfactants producers in the reservoir may affect the flow of fluids. The formation of biofilm at the oil water interface changes the rheology of the interface, and may provide a useful mechanism to control mobility and area sweep in reservoirs. The method of

microbial enhancement of oil recovery based on activation of the indigenous microorganisms by injection of water-air mixture and nitrogen and phosphorous salts could be tested at the both studied lots of the Daqing oil field.

### Field Tests of Active Endogenous Microbial Flooding in the Reservoir after Polymer Flooding

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Polymer flooding has become an effective method to enhance oil recovery in many countries especially in China.How to continue recovery oil is a key problem for the reservoir after polymer flooding. Microbial flooding is one of the selected methods to enhance oil recovery. A nutrition system to activate endogenous microbes was studied in laboratory. A microbial flooding pilot test with one injection well and four production wells was carried out through activating reservoir endogenous microbes in a typical reservoir after polymer flooding at the South-East of Sa Nan, Daqing oil field, China. Total 0.03Pv nutrition fluid was injected into formation. Several key parameter tests shown good responses, that the incremental oil was 3068.13t in the trial block, water cut of production fluid decreased by1.5%, the number of total microbes increased by 10000 times, and the oil quality also was improved clearly. Results of this field trial showed MEOR by activating reservoir endogenous microbes is a potential and perspective method to enhance oil recovery for the reservoir of after polymer flooding, MEOR should be given more attention in oil industry.

### Identifying Microbes For a MEOR Application: Comparing a Surface And Subsurface Sampling Strategy

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Wintershall and BASF initiated a research project with the aim to gain a better understanding of the underlying mechanisms that lead to enhanced oil recovery by microbes (MEOR: microbial enhanced oil recovery) and to develop a nutrient feeding strategy that is suited to achieve this aim. Since the research is based upon the oil samples as starting material it is crucial to obtain non-contaminated oil samples containing active microbes. Therefore experiments were performed to identify the best sampling strategy. A surface (at wellhead) and subsurface sampling (at approx. 700 m depth) were organized at the same oil well to be able to compare the microbial communities found in the samples. The subsurface sample was taken under reservoir pressure and slowly released in the lab. Surface and subsurface samples were compared in terms of cell number, identity of microbes using molecular biology techniques (454 sequencing) and the potential for microbial oil degradation. Since substantial differences were observed between the surface and subsurface fluids in terms of bacterial activity, the selection of the sampling strategy should further be considered for MEOR research and industrial application. The oil samples also served as starting material for anaerobic, microbial enrichment cultures on various substrates. Obtained enrichment cultures were screened for the production of metabolites that might have a positive effect on oil recovery e.g. gases, acids, emulsifiers, biopolymers and biosurfactants. To determine if cultured microbes have an effect on oil recovery, model systems like microfluidics, sandpacked columns and core floodings are used.

### Microbial Lipopeptides: Structural Characterization and Their Potential Application in Enhanced Oil Recovery

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Lipopeptide synthesized by microorganisms is one of the five major biosurfactants, and it has received much attention from scientific and industrial communities due to its powerful interfacial and biological activity. The lipopeptide is a series of structural analogues of different families, such as surfactin, lychenysin, fengycin, iturins, etc. 23 families covering over 90 kinds of lipopeptides have been reported in last two decades. This paper presents the producing strains, isolation and purification, structural analysis of the surfactin, one of the 23 families of lipopeptides. In particular, two novel surfactin derivatives isolated from cell-free broth of Bacillus subtilis HSO121 in our laboratory are introduced. In addition, a pseudo ternary system (alkaline/ surfactant/ polymer) containing lipopeptides for oil recovery and its potential application in petroleum industry is also discussed in this paper.

## Characterization of Stimulated Microbial Communities and Changes on Heteroatoms of Crude Oil in an Indigenous Microbial Enhanced Oil Recovery Culture

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Analyzing stimulated microbial communities and their potential functions for MEOR in aerobic and anaerobic culture conditions before a field trial may be essential to providing a reference guide for field trial of MEOR.Microbial communities in both anaerobic and aerobic cultures in laboratory were analyzed based on 16Sr DNA clone library technique to provide pictures of stimulated microbial groups in near well region and deep well region for a coming indigenous microbial enhanced oil recovery (MEOR) process. Most of the stimulated bacteria were reported to have the ability to produce desired products for MEOR according to previous studies. The analysis of stimulated microbial communities and their possible uses could act as a new strategy to confirm the feasibility of indigenous MEOR in the study reservoir. In addition, heteroatoms in crude oil and two treated oils coincubated in the anaerobic and aerobic cultures were detected b y electrospray ionization (ESI) coupled to high-field Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Results revealed that heteroatoms in treated oils were subjected to biodegradation and microbial alteration mainly occured in alkyl side chains of these polar compounds. Convertion of long alkyl side chains to short ones also was a desired microbial activity for the application of MEOR. In general, all analysis further indicated that an indigenous MEOR field trial could be carried out in the study oil reservoir.

## MEOR Methods in Oil Production for Conditions of Kazakhstan

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Now at use of traditional ways about half of geological oil stocks is taken from deposits. Use of biotechnological methods allows increasing the reservoirs recovery. It can be reached by various ways. Gasifier microorganisms form gases (hydrogen, methane, nitrogen, carbonic acid) due to this gas pressure in formation liquid can increase for several atmospheres. Microorganisms capable to form the surface-active substances and capable to reduce surface tension in border between oil and water displacing it also can be used. This approach also results to extension of oil recovery. Creation of biotechnology of oil viscosity reduction and also improvement of oil and mineral oil properties can be the following steps in the researches

directed to increase of oil recovery. With use of biotechnological ways it is possible to carry out oil dewaxing. Biotechnological methods also allow developing methods of removal from crude oil of sulfur compounds that litter environment with toxic substances. We have developed the basic circuit of laboratory installation for modeling and optimization of biotechnology of oil recovery increase. Biotechnological methods of increase of oil recovery are safe for environment as the microflora developing in the oil pool does not contain in its structure pathogenic and (or) toxic microorganisms. And for carrying out of technological actions permission of the sanitary oversight agency is not required. Thus, secondary oil recovery can be increased due to allocation of acids by bacteria (particular in carbonic acids), which increase the interstices of calcareous collectors due to formation of gases. Gases being dissolved in oil, increase its mobility, therefore bacteria take active participation in oxidation and transformation of oil and gas deposits. Increase of oil recovery at the developed deposits equivalently to opening of new deposits, therefore the given problem is actual for all oil-producing countries of the world and especially for Kazakhstan.

**Progress on Pilot Tests of Microbial Enhanced Oil Recovery in Daqing Oilfield**

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Microbial enhanced oil recovery (MEOR) is a technology that utilizes bio-gas, bio-surfactants, bio-polymers and degradation produced by underground fermentation of bacteria for petroleum exploitation. In this study, it is introduced that progress in pilot tests of MEOR in Daqing Oilfield in recent decades, which analyzes suitable reservoir conditions and application characteristics. By the end of 2012, cumulative incremental oil production reached to $12\times10^4$ t, including 518 wells by single-well microbial huff-and-puff with cumulative incremental oil production of $6.3\times10^4$ t, and 10 projects (45 well patterns) by microbial flooding and profile modification with cumulative incremental oil of $5.7\times10^4$ t. This technology plays an important role in stabilizing production of Daqing Oilfield.

***Ex Situ* Evaluation of the Effects on Crude Oil Recovery by Bacterial Consortia Isolated from a Mexican Oilfield**

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Microbial Enhanced Oil recovery (MEOR) can be highly complex. In general, the mechanisms of MEOR´s action are most probably due to multiple effects of the bacteria on the oil well and oil. The aim of this work was to investigate the utilization of bacterial consortia IMP-100 and IMP-200 (isolated from a Mexican oilfield) in MEOR and the possible mechanisms involved in crude oil detachment. Consortia were grown in the SS1-M medium, static cultures were performed at 70°C under anaerobic conditions through 120h of fermentation. By the Lowry method we found that consortia were able to grow under environmental conditions tested in this work. By gas chromatography techniques the production of gasses, organic acids and alcohols was found. Cell surface hydrophobicity was tested with the BATH proof; results showed that cells of both consortia presented 17-19% of adherence to hexadecane. Hydrocarbon droplets were observed under light microscope (LM) using epifluorescence. Micrographs confirmed the presence of cells attached to hexadecane droplets. Cultures in presence of calcite rocks (impregnated with heavy crude oil) were carried out. Data showed that ~10% of the crude oil was detached from the rocks. SEM images and EDXS analysis confirmed the presence of 2-µm length rods immersed in porous region areas where oil has been removed. Bioproducts LC-1 and LAF-1 (obtained from both consortia) were able to act as crude-oil dispersants and reduce the

viscosity of heavy crude oil. Results demonstrated the potential of IMP-100 and IMP-200 consortia and LC-1, LAF-1 for utilization in MEOR.

**Evaluation and Characterization of Biosurfactants Produced by Microorganisms Isolated from Brazilian Oils**

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Surface-active agents or surfactants are amphiphilic molecules that comprise both hydrophilic and hydrophobic moieties, allowing the reduction of the surface and interfacial tensions, as well as the formation of oil in water or water in oil emulsions. Due to their interesting properties, surfactants are widely used by petroleum industries to reduce the capillary forces that retain the oil inside the reservoir. However, since chemical surfactants present some limitations related to environmental issues and restrictive laws, the biosurfactants constitute a reliable alternative due to their lower toxicity, higher biodegradability and effectiveness at extreme temperature, salinity and pH conditions. In this work, different biosurfactant-producing microorganisms under reservoir conditions were isolated from Brazilian oils. Biosurfactant production was evaluated by measuring surface tensions, interfacial oil-water tensions and emulsifi cation activities. Among the isolated microorganisms, two Pseudomonas and three *Bacillus* strains were able to grow and produce extracellular biosurfactants at 40ºC. Furthermore, the biosurfactants were characterized using different spectroscopic techniques, namely FTIR, 1H NMR, ESI/MS and MS/MS. Structural characterization of these molecules is important to understand their surface-active properties, as well as the formation of molecular aggregates. Biosurfactants produced by *Pseudomonas* and *Bacillus* strains were found to be rhamnolipids and surfactins, respectively. The results obtained show that it is important to characterize the biosurfactants in order to optimize their application in bioremediation with crude oil, or in microbial enhanced oil recovery processes.

**A Biosurfactant-producing and Oil-degrading *Bacilus Subtilis* Strain Enhances Oil Recovery under Simulated Reservoir Conditions**

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Microbial Enhanced Oil Recovery (MEOR) is potentially useful to increment oil recovery from reservoirs beyond primary and secondary recovery operations using microorganisms and their metabolites. *In situ* stimulation of microorganisms that produce biosurfactants and degrade heavy oil fractions reduces the capillary forces that retain the oil inside the reservoir and decreases oil viscosity, thus promoting its flow and increasing oil production. *Bacillus subtilis* #573, isolated from crude oil samples obtained from a Brazilian oil field with a moderate temperature (40ºC), was selected for further use in MEOR. This isolate can grow at temperatures up to 55ºC and salinities up to 100 g/l, and produces extracellular biosurfactants under both aerobic and anaerobic conditions in the presence of hydrocarbons. The biosurfactants produced reduced the surface tension to 30 mN/m, decrease the interfacial tension oil-water and exhibited a high emulsifying activity, as well as thermo- and salt-tolerance. The microbial isolate also showed the ability of degrading long-chain n-alkanes under aerobic and anaerobic conditions. Mobilization of heavy crude oil by this isolate was evaluated using sand-pack columns at 40ºC. Growing *in situ B. subtilis* #573 for 14 days allowed a 17% recovery of the entrapped crude oil. The recovered crude oil showed a decrease in the

percentage of *n*-alkanes higher than $C_{25}$ and its viscosity was reduced about 32%, which contributed to enhance its mobility. A core flooding equipment was used for a better simulation of the oil reservoir conditions (40ºC and 32.4 bars). Preliminary results showed an additional oil recovery of 4%. The results obtained demonstrated that the selected isolate can be useful to recover residual oil from mature reservoirs.

**Bioconversion of Extraheavy Oil from the Orinoco Oil Belt, Venezuela**

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Currently, oil industry shows an interest on biotechnologies applied to the recovery and upgrading of heavy oils and other fuels, since microorganisms have potential in several processes such as: upgrading, biopolymers production for water control and removal of paraffin, sulphur, nickel and vanadium. The Orinoco Oil Belt (FPO) as the most important reservoir of heavy oil the World has a strategic importance in the economic and social development of our Country. On the other hand, as environmentally safe technologies, bioprocess could be considered as an option for oil upgrading in order to facilitate transport processes due to a bacterial reduction of viscosity without chemicals that could produce environment damages. A bioconversion process of extra heavy oil from the Carabobo Area in the Orinoco Oil Belt, using Venezuelan bacterial strains is study in this work. SARA analysis and simulated distillation curves of treated oils s how transformations made by the bacterial action that facilitates an increase of the saturated fraction and a descent in the boiling point.

**Anaerobic microbial communities potential to enhance oil recovery in a low-temperature heavy oil reservoir**

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Anaerobic microorganisms with potential physiological and metabolic abilities to enhance oil recovery gain attention from researchers. In this study, microbial communities in samples collected directly from wellheads in a low-temperature heavy oil reservoir and in five anaerobic enrichments were analyzed based on construction of 16S rRNA gene clone libraries. The five enrichment cultures included hydrocarbon-degrading bacteria (HDB), fermentative bacteria (FB), nitrate-reducing bacteria (NRB), sulfate-reducing bacteria (SRB) and methane-producing bacteria (MPB). The results showed that dominant microbes in original sample and the five enrichment cultures were different. Additionally, unclassified bacteria detected in all samples may be new species in the oil reservoir. While, Spirochaetes was determined in all the five samples, this genus of which may be indigenous microorganisms in oil reservoirs. The analysis of anaerobic micro bial communities in heavy oil reservoir of Xinjiang Oilfield could provide some value for activating selectively beneficial microorganisms (HDB, FB, and NRB) and inhibiting the detrimental ones (SRB) in the reservoir, and may be fatherly applied to MEOR. Moreover, Spirochaetes could be paid more attention to further study because of its diverse metabolic abilities.

**Molasses injection as a MEOR strategy: Enrichment incubations of brine/oil from a North Sea Oil Field**

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The concept of microbial enhanced oil recovery (MEOR) is not new. However, little is known about the microbes and the mechanisms involved. In this study, anaerobic enrichment tests were conducted by using brine and crude oil from the North Sea oil field for 28 days at 55°C. Three different conditions were carried out: (A) control - no nutrient addition, (B) with addition of 2% molasses and (C) with addition of 2% molasses and 2.5 mM nitrate. Analyses were conducted to study metabolite production and their effects on the crude oil-brine system. The microbial population was monitored by qPCR and pyrosequencing. The results revealed that sugar was hydrolyzed and acetate was produced in incubations containing molasses, but not in control incubation. In condition C, nitrate was consumed continuously. Oil emulsion and gas production were observed during incubation in condition B and C. Furthermore, in both conditions the interfacial t ension (IFT) was reduced from 18 mN/m to 2mN/m when microbes were present in the oil-water interphase. When microbes were removed from the interphase, there was no reduction of IFT. The presence of microbes in the interphase was confirmed by phase contrast microscopy. The qPCR results revealed that microbial growth was stimulated in condition B and C. Further investigation by pyrosequencing showed that addition of molasses stimulated enrichment of Anaerobaculum, Petrotoga and methanogenic archaea. Addition of both molasses and nitrate seemed to favor the growth of Petrotoga over Anaerobaculum. These findings give indication of potential MEOR application by IFT reduction when molasses is added.

**Self-Cycling Fermentation of Rhamnolipid Production For Enhancing Oil Recovery**

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Rhamnolipid biosurfactants were continuously produced with Pseudomonas aeruginosa PAO1 by self-cycling fermentation on a laboratory scale plant. The fermentation was established with 1.0% inoculums of the pure culture, and followed by removal and supplement of three fourths volume every 120 h for each cycle. Unsteriled soybean oil and mineral salts medium were used for the total 30 cycles and a biosurfactant concentration of approximately 25 g/L was achieved in 5th, 10th, 15th, 20th, 30th cycle, respectively. Considering the sundry bacteria contamination, the most probable number was employed to numerate the microbial concentration of Pseudomonas aeruginosa and total bacteria of the cultural broth. The crude biosurfactant obtained was able to lower the interfacial tension (diesel) to 1.0 mN/m and achieved a CMC value of 75 mg/L. Core flooding experiments showed an enhancement from 6.45% to 21.86% of total oil recovery by the harv ested broth dilutions. The present work describes the performance of a new biosurfactant producing method based on the continuous self-cycling fermentation, which might be further scheduled in the oilfield for enhancing oil recovery application.

**Production of biosurfctant utilizing raw glycerin, a sustainable biotechnological alternative**

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Biosurfactants have been the object of studies in various biotechnology areas, since they have properties applicable in many industries especially in oil industries. Among the glycolipids, the most studied are the rhamnolipids produced by bacteria of the *Pseudomonas* genera. Alternatives to improve their large scale production and lower costs would be to use recycled

carbon sources, such as raw glycerin, which is a byproduct of biofuels manufacturing, and whose market price is ever more depressed. The use of glycerin was tested as an alternative carbon source (CS) for growing two strains of Pseudomonas aeruginosa. They were grown in a saline mineral medium (SM) with commercial (CG) and raw glycerin (RG) at 2% as the CS. Analysis of the surface tension (ST) and stability of the Biosurfactants produced were performed. The results showed that for strain CCMICS 106 there were no significant differences among production media, but for strain CCMICS 109 there was a higher production of rhaminolipids (RL) in the 2% CG SM. In the 2% RG SM, the CCMICS 106 and 109 strains produced 2.87 g/L and 2.31 g/L, respectively, while in the 2% CG SM, they produced 2.47 g/L and 3.96 g/L. Chromatographic analysis suggests that a type of mono-rhamnolipid was produced. RL achieved a reduction in ST from 60.02mN/m to 26-30mN/m, and also exhibited good stability in relation to temperature, salinity and pressure. The results demonstrate the viability of using raw glycerin as a substrate and low cost alternative CS.

## Microbes in Extreme Operating Conditions

### Calm and Frenzy, Feast and Famine of Marine PAH-Degrader *Cycloclasticus* sp. ME7 as Revealed by Physiological Studies and Genome/Proteome Analyses.

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In many petroleum contaminated marine ecosystems all over the world including shallow and deep-sea water and sediments, marine bacteria of genus *Cycloclasticus* are recognized as the predominant players in aerobic breakdown of polycyclic aromatic hydrocarbon compounds (PAH). The genus name refers to ‘‘ring-breaking’’ activity, *i.e*. to the capability to degrade the PAHs consisting of up to four-five condensed rings. Based on comprehensive study included physiology, modern molecular biology and bioinformatics approaches, we demonstrated the life style of *Cycloclasticus*, highlighting the environmental factors governing its prosperity. The work was performed with *Cycloclasticus* sp. 7ME recently isolated from tar residues collected in Mediterranean Sea at tanker Haven’s wreck. This accident, happened 22 years ago and released > 40,000,000 gallons of crude oil, is considered as one of top-ten oil spills in the human history. One of remarkable features of *Cycloclasticus* is its narrow substrate range delimiting by an uptake of almost exclusively PAHs, alkyl-PAHs, nitrogen- and sulphur-containing PAHs. The genome of *Cycloclasticus* sp. ME7 contains three large operons with more than fifteen different enzymes belonging to four different classes of ring-cleavage dioxygenases. Subtractive proteome analysis revealed that transcriptional activation of various operons depends on the amount of condensed rings in PAHs. This finding explained how these bacteria regulate the pathways when high-molecular weight PAHs compounds are present together. *Cycloclasticus* sp. ME7 does not produced siderophores and is highly specialized to uptake the iron from the environment applying the “cheating” strategy, which might explain the difficulties in its cultivation in a pure culture.

### Bioremediation of Oil-polluted Sites under Extreme Environmental Conditions: a Case Study from the Sultanate of Oman

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Although there is a substantial database concerning the types of bacteria capable of degrading hydrocarbons and ways to accelerate their degradation rates, this knowledge cannot be

extrapolated to arid regions without keeping in mind the significant differences in environmental conditions. For example, the soils of Oman are characterized by temperatures >55°C and coastal sediments experience salinities up to 15%. Therefore, bioremediation of polluted sites in Oman is challenging and strategies relying on indigenous bacteria are required. We studied different polluted sites from Oman for their microbial community composition using culture-dependent and 16S rRNA-based approaches as well as for their ability to degrade hydrocarbons at elevated salinities and temperatures. The efficiency of different biostimualtion and bioaugmentation approaches was also tested. More than 120 halophilic and thermophilic hydrocarbon-degrading bacteria were isolated. Most of the isolates and the obtained 16S rRNA sequences belonged to *Marinobacter, Alcanivorax, Holomonas* and *Psuedomonas*. Experiments with intact samples showed their ability to degrade pristine, phenanthrene, dibenzothiophene and n-octadecane at salinities between 5 and 12% (w/v NaCl) and at temperatures up to 45°C, however degradation rates decreased at higher salinities and temperatures. While bioremediation of the polluted soils by the addition of oil-degrading consortia was partially successful, biostimulation using different N:P ratios (5:1, 20:1 and 100:1) and non-ionic surfactants (Tween80,Triton X100 and Brij35) showed different degrees of success, depending on the soil type and the amount of nutrients. We conclude that Omani soils are rich in extremophilic hydrocarbon-degrading bacteria that can be utilized for bioremediation under harsh environmental conditions.

**Study of Microbial Diversity in Hyper Saline Environment**

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The pre-salt layer is an area of oil reserves found under a deep layer of salt, which forms one of the various rock layers in the seabed. The total depth of these rocks can reach over 7000 meters, which makes this place an environment with unique characteristics. The extreme conditions for life can hinder the study of organisms that live in this environment. In order to better understand these microorganisms, this work aimed to study the microbial diversity in a sample of injection water from a well near the pre-salt layer since it is not yet possible to obtain samples from the exact location of the pre-salt. Microbiological and molecular biology techniques were employed since the majority of microorganisms are no able to grow under laboratory conditions. Microbial quantification, isolation, and identification of the main microbial groups were performed using conventional and alternative culture media. The results of the experime nts using alternative culture media have demonstrated a higher number of aerobic and anaerobic bacteria when compared to conventional media. The absence of sulfate-reducing bacteria was detected in both culture media. Chromohalobacter salexigens and Marinobacter gudaonensis were among the strains that were identified. These microorganisms are characteristic of highly saline environments and oil industries. This work will help understanding these sites, and will demonstrate that the use of alternative culture media can serve as a strategy in cultivating these extremes microorganisms.

**Extreme Heat Resistance of Spore-forming Thermophilic Sulfate-reducing Bacteria in Cold Sediments**

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Thermophilic sulfate-reducing bacterial endospores (thermo-spores) have been found in cold marine sediments that do not support their activity or growth. Estimates from endospore germination experiments suggest that 108 spores per square meter per year are being added to Arctic sediment. This high dispersal rate may be explained by a warm anaerobic source habitat associated with significant fluid flow creating a warm-to-cold microbe dispersal mechanism, e.g., connecting the deep biosphere with the cold ocean. Genomic analyses reveal that many closely related bacteria have been detected in oil reservoir samples, leading to the hypothesis that seabed hydrocarbon seepage transports thermophiles out of deep oil reservoirs and up into the ocean. As such strategic possibilities may exist for mapping or characterizing subsurface petroleum deposits based on microbiological investigations of surface sediments, motivating investigations of thermo-spore biogeography and physiology. Detailed investigation of sediment from the Tyne estuary (UK) revealed the presence of thermo-spores with a remarkable heat resistance. Despite triple autoclaving at 121°C, subsequent incubation at 50°C promoted rapid and reproducible sulfate reduction rates in sediment microcosms. Different Desulfotomaculum species were enriched when pre-autoclaved sediment was incubated at 50, 60 and 70°C, and also when the autoclaving temperature was increased to 130°C. Closely related Desulfotomaculum 16S rRNA gene sequences have been reported from deep biosphere habitats including oil reservoirs. Differences in heat resistance and temperature optima suggest Desulfotmaculum thermo-spores could have originated in warm habitats with slightly different features, and/or possibly in hostile subsurface environments that promote their endosporulation.

**Biological (Per)chlorate Reduction In Oil Reservoirs**

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The physiology of microorganisms indigenous to the subsurface is of great interest. Extended knowledge about the metabolism of microbes thriving in oil fields increases the possibilities for controlling or stimulating growth or particular metabolic characteristics, which is highly demanded by the oil business. Here we show that *Archaeoglobus fulgidus*, a thermophilic archaeon that is abundant in oil fields is capable of reducing perchlorate and chlorate (Liebensteiner et al., 2013). Thus far *A. fulgidus* has only been known as a sulfate-reducing microorganism that contributes to the formation of sulfide in oil fields under elevated temperatures, causing problems like reservoir souring. Our results prove that the continuous cultivation on perchlorate is even leading to a partial loss of the organism's sulfate-reduction capability. Genomic and proteomic analyses show that A. fulgidus utilizes molybdo-enzymes for the reduction of perchlorate that are similar to the ones described from bacteria. In contrast to its mesophilic bacterial counterparts, the reactive chlorine intermediates that are formed during the reduction of perchlorate by A. fulgidus are chemically oxidizing sulfide. This study demonstrates that that the group of perchlorate-reducing microorganisms is probably much bigger than estimated so far and also present in the deep subsurface. From a more applied viewpoint perchlorate and chlorate seem to be interesting agents for the upstream business. The results suggest a mitigating effect on reservoir souring and might therefore be of interest for a similar use like nitrate during oil recovery.

**Monitoring of Germination, Growth and Sporulation of Physiologically Diverse Thermophilic Bacterial Populations from a Cold Marine Sediment**

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Anaerobic endospore-forming members of the bacterial phylum Firmicutes are frequently detected in oil reservoirs. This group includes fermentative and sulphate-reducing thermophilic bacteria that may be involved in hydrocarbon degradation and souring in warm deep oil fields. However, their functional diversity and abundance are prone to underestimation, since endospores are not easily detected by nucleic acid-based cultivation independent approaches. By applying molecular methods and biogeochemical process measurements to incubation experiments, we assessed populations of anaerobic thermophilic Firmicutes present in cold marine sediments that grew exponentially within a few hours of sediment heating. Next generation Ion Torrent sequencing of 16S rRNA enriched in sediment incubated at 50°C revealed successional growth of diverse thermophilic fermentative and sulphate-reducing bacteria closely related to Tepidibacter, Caloramator a nd Desulfotomaculum species found in North Sea oil reservoirs, geothermal waters and oil contaminated soils. Germination and growth of fermentative Firmicutes was detected in less than 6 h at 50°C. Within 18 h of incubation, Firmicutes increased from 10^6 to 10^7 cells/g sediment, as revealed by qPCR of 16S rRNA genes. A concomitant increase in endospores, from $10^7$ to $10^8$/g sediment, was revealed by measuring dipicolinic acid (found in all bacterial endospores). Sulphate reduction rates indicated an increase from $<10^4$ to $10^6$ sulphate-reducing cells/g sediment. These results indicate that rare populations of endospores, while sometimes overlooked, can rapidly proliferate within a few hours if favourable conditions are encountered and highlight the importance of monitoring microbial communities in industrial systems.

## Novel *Thermoanaerobacter* spp. from Hot Water Produced from the Thar Jaht Oil-Field in South Sudan

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Two thermophilic bacterial isolates, termed S1.1 and S3.1 were recovered from oil-well production water from a deep oil-well in the Thar Jaht oil-field in South Sudan and characterized. Based on their 16S rRNA gene sequences and physiological properties they belong to the *Thermoanaerobacter* genus, possibly representing novel species. Both are strict anaerobes, grow optimally at 65 - 70$^o$C at neutral pH and with ~0.3% NaCl, and can use a large variety of carbohydrates, proteinaceous compounds and organic acids as growth substrates. Strain S1.1 differs from strain S3.1 in its ability to grow on xylane and D-ribose, and S3.1 from S1.1 in capability to grow on acetate, arabinose, cellulose and lactate. Both strains displayed a fermentative metabolism, producing acetate, ethanol, carbon dioxide and hydrogen as fermentation products, but growth was stimulated by thiosulfate. Strain S1.1 produced typical endospores. Cultures of both strains survived autoclaving at 121$^o$C for 40 mins, indicating the formation of extremely heat-resistant endospores.

## Response of Archaeal Community to Simulated Petroleum Derived-hydrocarbon Contamination in Marine and Hypersaline Ecosystems

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In extreme habitats, such as those with high salt concentration, Archaea may play an important role as an indicator of petroleum contamination. To achieve a better understanding of their

ecological significance, we examined the composition of archaeal communities in marine and hypersaline ecosystems and their response to simulated petroleum contamination. For this purpose, marine and hypersaline water samples were collected in Saquarema, Rio de Janeiro, Brazil, and different microcosms were prepared in triplicate, as follows: 20 ml of each water sample was enriched with 1% of the contaminant (v/v) in 50 ml-glass flasks. The contaminants used were heptadecane (as a model for aliphatic hydrocarbons), naphthalene (as a model for aromatic hydrocarbons) and crude oil (as a complex mixture of different aliphatic and aromatic hydrocarbons). Temporal analyses (0, 4, 12 & 32 days) were performed based on DNA and RNA extractions followed by PCR-DGGE analysis of Archaea-specific 16S rRNA. The results showed that different archaeal OTUs were enriched only in hypersaline microcosms after hydrocarbon contaminations. The dendrogram analysis of the DGGE showed that the samples clustered according to the kind of contaminant and also to the time of hydrocarbon contamination exposure. Redundancy Analysis of PCR-DGGE patterns corroborated the different response of archaeal communities to heptadecane, naphthalene and crude oil. In addition, the results showed an increase of specific bands after 32 days of contamination. The further identification of these selected archaeal bands is essential as they can represent important biomarkers for petroleum hydrocarbon contaminated environments.

**Impact of heavy metals and NaCl on the growth and ability to volatilize methylmercury by *Pseudomonas putida* V1 isolated from petrochemical sludges**

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Petroleum extraction and coal mining comprises two main forms of mercury introduced into the environment. Among various forms of mercury, methylmercury is the greatest cause of concern due to it high toxicity and easy accumulation in live organisms. Despite this, some microorganisms have developed mechanisms to adapt these contaminants. This study aimed to determine the influence of different concentrations of heavy metals (lead, copper, chromium and nickel) on the growth and in vitro methylmercury removal capacity of *P. putida* V1. *P. putida* V1 was isolated from soil taken from a land farming site on a petrochemical base located in the State of Rio Grande do Sul, Brazil. The effects of the presence of copper, nickel, chromium, lead and NaCl on the growth and methylmercury removal capacity of *P. putida* V1 were determined using LB broth. The culture medium contained concentrations of 100, 300 and 600 µmol $L^{-1}$ of each metal and 0.5%, 1%, 3%, 5%, 10% and 20% of NaCl. The growth of *P. putida* V1 was determined through measurement of optical density ($OD_{600}$ nm). Analysis of mercury was done through cold steam generation in an atomic absorption spectrophotometer. The heavy metals studied influenced the growth and removal of methylmercury by *P. putida* V1. Nonetheless, the concentration of the methylmercury was the determinant factor for the survival of *P. putida* V1. The results of the present study suggest *P. putida* V1 as a promising candidate in the bioremediation of ecosystems contaminated with methylmercury and presenting high salinity (up to 10%).